Feeding Habits of Bigeye Tuna (*Thunnus obesus*) in the Western Indian Ocean Reveal a Size-Related Shift in Its Fine-Scale Piscivorous Diet

Chien-Hsiang Lin[1], Jing-Siang Lin[2], Kuo-Shu Chen[3], Meng-Hsien Chen[3,4], Chiee-Young Chen[5] and Chih-Wei Chang[3,6,7]*

[1] Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, [2] Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan, [3] Department of Oceanography, and Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung, Taiwan, [4] Institute of Marine Ecology and Conservation, National Sun Yat-sen University, Kaohsiung, Taiwan, [5] Department of Marine Environmental Engineering, National Kaohsiung University of Science and Technology, Kaohsiung, Taiwan, [6] Marine Ecology and Conservation Research Center, National Academy of Marine Research, Kaohsiung, Taiwan, [7] National Museum of Marine Biology and Aquarium, Pingtung, Taiwan

This study analyzed the piscivorous diet of bigeye tuna (*Thunnus obesus*) through species identification of both fish and otolith remains in stomachs of 183 bigeye tuna collected in the western Indian Ocean. A total of 642 fish remains and 1,021 fish otoliths were examined. Prey items identified in the fish and otolith remains were not completely consistent. Although 12 items out of the 53 identified taxa were found in both remains, 20 items of fish remains were not found in otolith remains, and 21 items were added only from the otoliths. The main fish remains were Alepisauridae, which accounted for 13.9%. Most of the otoliths belonged to Myctophidae (49.5%) and Scopelarchidae (21.4%). Three prey items, namely *Valenciennellus tripunctulatus*, *Evermannella* sp., and *Zenion* sp., were recorded for the first time in the diet of bigeye tuna from the region. The otolith remains substantially enhanced the taxonomic resolution of the diet. Bigeye tuna stomach contents were independent of location, depth, and time of catch but varied with tuna size. The proportion of dominant Myctophidae prey items decreased markedly as the tuna size increased, whereas the proportion of Macrouridae increased with size. In addition, larger bigeye tuna were found feeding on larger prey (*Electrona risso* and *Scopelarchus analis*), demonstrating that diet changes in both prey composition and size are related to the ontogeny of the fish.

Keywords: otolith, feeding ecology, dietary change, prey size distribution, Myctophidae

INTRODUCTION

The bigeye tuna [*Thunnus obesus* (Lowe, 1839)] is an economically important fish that typically inhabits tropical and subtropical waters, between approximately 40°N and 40°S in the Pacific, Atlantic, and Indian Oceans (Hanamoto, 1987). The maximum fork length (FL) of the fish in the Indian Ocean was estimated to be approximately 170 cm in fish older than 6 years

(Stéquert and Conand, 2004). The vertical distribution of the epi- and mesopelagic bigeye tuna ranges from the surface to 400 m, occasionally reaching depths over 1,000 m. This depth varies substantially with day–night shifts, seasonal changes, and changes in water temperature (Shannon, 1987; Dagorn et al., 2000; Musyl et al., 2003; Matsumoto et al., 2013; Lam et al., 2014). As an economically valuable species, bigeye tuna has been caught using industrial purse seine and longline fisheries in the past and using gillnet fisheries in the Indian Ocean in recent years. Landings peaked in the late 1990s before diminishing across all oceans (Nootmorn, 2004; IOTC, 2005; Pillai and Satheeshkumar, 2012). The International Union for Conservation of Nature Red List of Threatened Species classifies bigeye tuna as a vulnerable species, indicating that ensuring a theoretically optimal catch is essential.

Bigeye tuna are top predators with an opportunistic feeding strategy (Ménard et al., 2006; Olson et al., 2016). Therefore, its prey composition can be an indicator of the food web and marine ecosystem conditions of the ocean in which they live (Polovina et al., 2009). Furthermore, changes in the fish components of the bigeye tuna diet may be attributable to the effect of intensive commercial fishing on either the top predator or its prey (IOTC, 2003). Determining the components of the diet of bigeye tuna is necessary for delineating the potential trophic level change in the marine food web as well as for developing effective fishery management strategies.

Diet analysis using the stomach content of bigeye tuna has been an efficient approach and widely investigated for samples from the western Indian Ocean (Kornilova, 1980; Bashmakov et al., 1991; Potier et al., 2004), southern Africa (Shannon, 1987), the tropical Atlantic (Ménard et al., 2000; Vaske-Jr et al., 2012; da Silva et al., 2019), the central Pacific (Grubbs et al., 2001; Ménard et al., 2006), the west Pacific (Ohe and Moriguchi, 2009), and eastern Australia (Young et al., 2010). These studies have indicated that teleosts, cephalopods, and crustaceans are the three main diet groups consumed, and teleosts have been consistently reported to account for the highest proportion of the diet. However, these studies have identified teleosts with different taxonomic resolutions, and to our best knowledge, relevant studies from the Indian Ocean have been scant.

Focusing on teleost prey items by using both fish body remains and otoliths, we analyzed the piscivorous diet of bigeye tuna in the western Indian Ocean. Spatiotemporal and biological dynamics of the feeding habits were further explored.

MATERIALS AND METHODS

Sampling Design
Samples of bigeye tuna stomachs were collected by scientific observers aboard three Taiwanese longline vessels. The observers were trained by the government-commissioned Overseas Fisheries Development Council of the Republic of China (Huang, 2011). The samples were collected between April and December 2006 in the main fishing ground of bigeye tuna in the western Indian Ocean (between 6°45′S–4°05′N and 40°16′E–70°55′E; **Figure 1**). The specific catch sites and fish FL (in cm) were recorded on board. The stomach of each sample

was preserved in a sealed and labeled plastic bag and frozen at −20°C for subsequent analysis in the lab. Depth of capture was estimated using an 8-bit minitag device (Minilog TDR; Vemco Ltd., Nova Scotia, Canada) placed on a randomly selected short line attaching the hook (snood) (Wang, 2010).

Each stomach sample was weighed and photographed after being thawed. Stomachs that contained only fluid or mucus were considered empty. The stomach contents were sorted into three major groups: teleosts, molluscs, and crustaceans. Scombrids, carangids, and *Chanos chanos* (Chanidae), which are used as baitfish by longline fisheries, were excluded from the analysis. Among the 183 bigeye tuna stomach samples, 44 were empty, and 139 contained prey items. Preliminary sorting led to the identification of 642 teleosts, 854 crustaceans, and 201 molluscs. The crustaceans and molluscs were briefly identified and listed but are not further analyzed here (**Table 1**).

In addition to the 642 fish remains, 78 out of the 139 stomach samples yielded 1,021 otoliths. These were further analyzed for a fine-scale examination of the piscivorous diet of bigeye tuna. The FL of bigeye tuna with fish-containing stomachs ranged from 88 to 190 cm, and they were captured at depths ranging from 51 m to approximately 283 m between April and September 2006. For bigeye tuna with otolith-containing stomachs, their FLs were 91–177 cm, capture depths were 60–280 m, and capture months were between April and September 2006.

To evaluate sample size coverage, we conducted sample-based rarefaction and extrapolation for both fish remains and otoliths. Unidentified remains were excluded, leaving 94 and 77 stomach samples in the analysis for fish remains and otoliths, respectively. The analysis was calculated using EstimateS ver. 9.1.0 (Colwell, 2013).

Otolith Examination
Only sagittal otoliths were analyzed; asterisci and lapilli were excluded. The otoliths were pretreated with a solution of 3% potassium hydroxide, washed with distilled water, and air-dried. Otoliths collected from a single stomach were stored together in a 20-mL vial. Images were obtained for each identified taxon by using a stereomicroscope (Discovery V20, Zeiss), and the maximal otolith length (OL; in micrometers) was measured using the imaging software package AxioVs40 V4.7.2.0 (Zeiss).

Otoliths were identified based on the descriptions, identification keys, and images provided by Nolf (1985, 2013), Smale et al. (1995), and Rivaton and Bourret (1999) as well as the authors' reference materials (Lin and Chang, 2012). Otoliths were identified at least at the family level and to lower taxa whenever possible. Subsequent analyses were based on the family level.

Prey Composition Analysis
The index of relative importance (IRI) is a standardized measure for prey occurrence (%O), number (%N), and weight (%W) when analyzing stomach contents (Pinkas et al., 1971; Hyslop, 1980; Cortés, 1997, 1998, 1999; Liao et al., 2001). Hence, the following metrics were calculated for fish remains: percentage of occurrence %O = (number of stomachs containing the prey item/total number of otolith-containing stomachs) × 100,
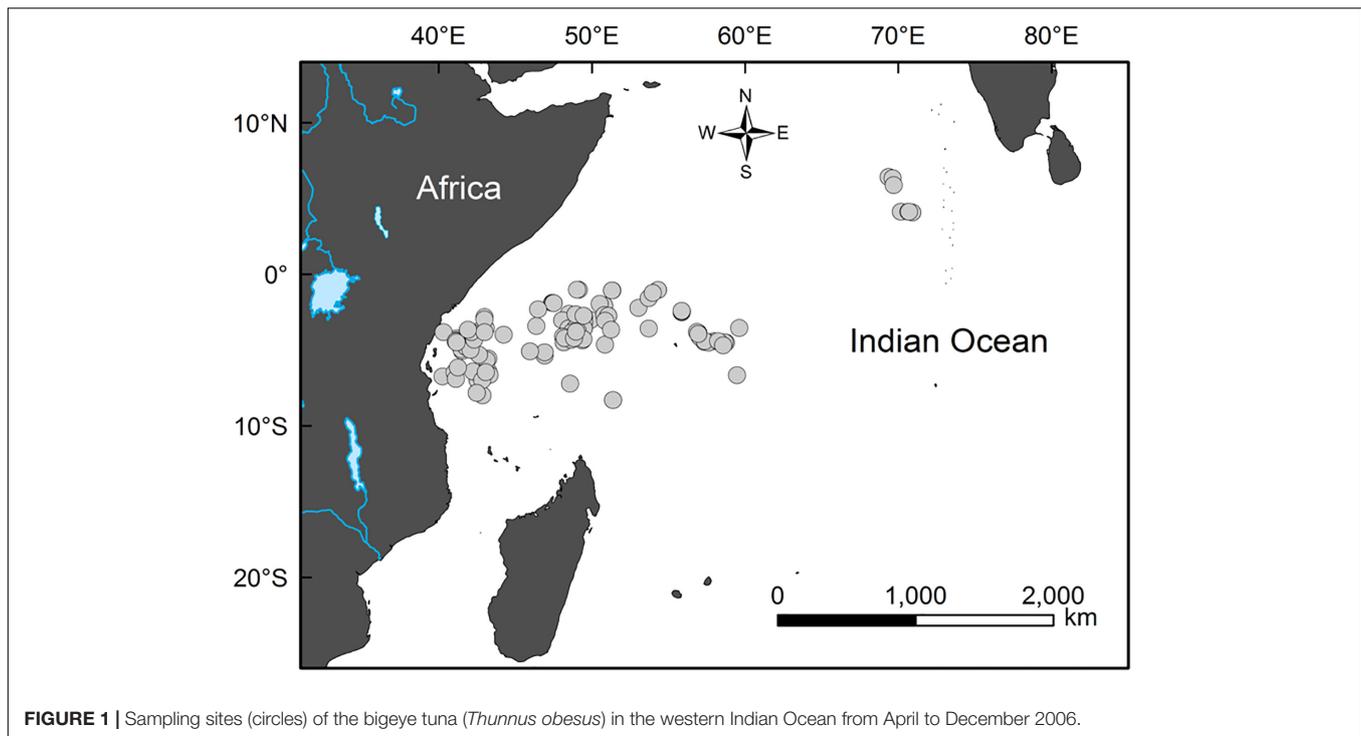
FIGURE 1 | Sampling sites (circles) of the bigeye tuna (*Thunnus obesus*) in the western Indian Ocean from April to December 2006.

numerical percentage %N = (number of prey items/total number of prey items) × 100, and weight percentage %W = (wet weight of the prey item/total wet weight of prey items) × 100. The IRI = (%N + %W) × %O. As for otolith remains, the IRI cannot be used without estimating the prey weight by determining the relationship between the OL and fish weight. To date, the OL–fish weight relationships among Indian Ocean fishes are yet to be fully elaborated, and additional studies are necessary to determine said relationships. Therefore, in this study, %O and %N were used to reveal the prey preferences by otolith remains (irrespective of the left or right side) among the bigeye tuna. A chance existed that some individuals might be counted only once due to the loss (broken or digested) of left or right otoliths; however, we assumed that all the otoliths were included because the digestion of the fish in the stomach was fixed and the number of broken and unidentified otoliths was low. Furthermore, because our subsequent analyses were based on proportions, no differences would exist between the use of both sides (left and right) or only a single side of otoliths (Fitch and Brownell, 1968; Potier et al., 2004).

The relationships between bigeye tuna diets from both the fish- and otolith-containing stomachs (MN%, mean proportion by individual) and the following four variables were structured: capture site (spatial), depth (spatial), month (temporal), and fish FL (biological). Capture sites were grouped by longitude as <45°E, 45–52°E, 53–60°E, and >60°E. Capture depths were grouped as <100 m, 100–200 m, and >200 m. Capture dates were grouped into 2-month intervals, beginning from the first sample caught in April and ending with the final sample caught in September. These grouping criteria were determined on the basis of the acquired data and an arbitrary stratum. Fish FLs

were grouped by size at sexual maturity (Zhu et al., 2011) and age (Stéquert and Conand, 2004): <111 cm FL (immature, <3 year), 111–127 cm FL (mature, 3+ year), 128–138 cm FL (mature, 4+ year), 139–147 cm FL (mature, 5+ year), and >147 cm FL (mature, >6 year). The Kruskal–Wallis one-way analysis-of-variance-by-ranks test was applied to measure the statistical significance of differences in percent number for each prey family (Potier et al., 2007, 2008).

The allometric relationships between otolith size and fish length have been widely supported (Smale et al., 1995; Granadeiro and Silva, 2000; Harvey et al., 2000; Battaglia et al., 2010). The size distribution of prey otoliths has been considered a suitable and independent indicator for prey fish size distribution (Granadeiro and Silva, 2000). OLs of dominant prey species were compared against each variable as mentioned above by using one-way analysis of variance and a *post-hoc* Tukey multiple range test (Siegel, 1956). In addition, the relationships between prey otolith size and fish length were studied using both least-squares and quantile regressions, of which the latter is well-adapted for estimating the lower (5th quantile) and upper (95th quantile) boundaries of prey size changes by predator size (Scharf et al., 2000; Ménard et al., 2006).

RESULTS

Prey Composition Based on Fish and Otolith Remains

Among the 1,021 fish otoliths, 33 taxa belonging to 17 families that range from 690 μm (Alepisauridae) to 5,730 μm (Microstomatidae) in OL were identified (**Figure 2**). **Table 2**

TABLE 1 | Number of stomachs containing the prey item (O), percentage of occurrence (%O), number of prey items (N), numerical percentage (%N), weight of prey items (W), weight percentage (%W), and index of relative importance (IRI) of bigeye tuna *Thunnus obesus*.

Prey item	O	%O	N	%N	W (g)	%W	IRI
Teleosts	**94**	**67.6**	**642**	**37.8**	**7291.7**	**67.2**	**7098.0**
Alepisauridae	35	25.2	89	5.2	2838.2	26.2	790.4
Anoplogastridae	1	0.7	1	0.1	0.6	0.0	0.0
Bothidae	1	0.7	1	0.1	0.5	0.0	0.0
Callionymidae	1	0.7	1	0.1	0.6	0.0	0.0
Chiasmodontidae	16	11.5	21	1.2	72.5	0.7	21.9
Diretmidae	3	2.2	4	0.2	31.3	0.3	1.1
Gempylidae	3	2.2	3	0.2	12.3	0.1	0.6
Myctophidae	8	5.8	18	1.1	40.9	0.4	8.3
Nemichthyidae	4	2.9	7	0.4	3.4	0.0	1.3
Nomeidae	2	1.4	2	0.1	0.6	0.0	0.2
Ophidiidae	1	0.7	1	0.1	0.9	0.0	0.0
Ostracoberycidae	2	1.4	2	0.1	2.0	0.0	0.2
Paralepididae	19	13.7	42	2.5	1013.9	9.3	161.5
Scombridae	1	0.7	8	0.5	318.8	2.9	2.5
Scopelarchidae	8	5.8	16	0.9	86.0	0.8	10.0
Sternoptychidae	17	12.2	35	2.1	117.1	1.1	38.4
Trachichthyidae	1	0.7	2	0.1	1.2	0.0	0.1
Zeniontidae	14	10.1	32	1.9	44.8	0.4	23.1
Unidentified fish	83	59.7	357	21.0	2706.2	24.9	2702.3
Crustaceans	**93**	**66.9**	**854**	**50.3**	**990.2**	**9.1**	**3973.9**
Amphipoda	2	1.4	3	0.2	0.2	0.0	0.3
Caridea	18	12.9	30	1.8	35.0	0.3	27.0
Crab larva	2	1.4	3	0.2	0.1	0.0	0.3
Isopoda	2	1.4	2	0.1	0.2	0.0	0.2
Palinuridae	3	2.2	4	0.2	5.3	0.0	0.6
Platyscelidae	3	2.2	4	0.2	0.5	0.0	0.5
Polychelidae	1	0.7	1	0.1	2.1	0.0	0.1
Portunidae	16	11.5	150	8.8	607.5	5.6	166.1
Oplophoridae	80	57.6	650	38.3	333.3	3.1	2378.6
Squillidae	4	2.9	7	0.4	6.0	0.1	1.3
Molluscs	**64**	**46.0**	**201**	**11.8**	**2569.7**	**23.7**	**1633.0**
Conch	1	0.7	1	0.1	0.1	0.0	0.0
Enoploteuthidae	3	2.2	3	0.2	139.5	1.3	3.2
Octopodidae	6	4.3	8	0.5	390.1	3.6	17.5
Oegopsida	1	0.7	1	0.1	40.2	0.4	0.3
Ommastrephidae	5	3.6	22	1.3	226.1	2.1	12.2
Unidentified cephalopods	64	46.0	166	9.8	1773.7	16.3	1202.3
Other invertebrate	**2**	**1.4**	**2**	**0.1**	**1.4**	**0.0**	**0.1**
Jellyfish	2	1.4	2	0.1	1.4	0.0	0.1
Total	139	100.0	1,699	100.0	10,853	100.0	12705.0

The bold fonts and numbers indicate the category title and values for the groups such as fish, crustacean, mollusc, and others.

summarizes the frequency of occurrence and number by prey items recovered from the 78 otolith remain-containing stomach samples compared with those from the 94 fish remain-containing samples. Identifiability for fish remains was 96 items (15.0%) at the species level, 105 (16.4%) at the genus level, and 84 (13.1%) at the family level. However, much of the otolith remains were assigned to lower taxa, among which 625 items (61.2%), 295 (28.9%), and 81 (7.9%) were at species, genus, and family levels, respectively. The majority of 357 (55.6%) fish remains were

unidentified because of the advanced state of digestion, whereas only 20 (2.0%) otoliths, including six unknown and 14 otoliths, could not be identified.

Prey items identified in the fish and otolith remains were not completely consistent. Among the 53 assigned taxa, 12 items were found in both fish and otolith remains: *Alepisaurus ferox* (Alepisauridae); *Pseudoscopelus* (Chiasmodontidae); *Diretmichthys parini* and *D. argenteus* (Diretmidae); Myctophidae indet.; *Lestidiops*, *Magnisudis atlantica*, and

TABLE 2 | Number of stomachs containing the prey item (O), percentage of occurrence (%O), number of prey items (N), and numerical percentage (%N) for fish and otolith remains of bigeye tuna *Thunnus obesus*.

Prey item	Fish remain				Otolith remain			
	O	%O	N	%N	O	%O	N	%N
Alepisauridae	**35**	**37.2**	**89**	**13.9**	**1**	**1.3**	**1**	**0.1**
Alepisaurus ferox	25	26.6	61	9.5	1	1.3	1	0.1
Alepisaurus spp.	10	10.6	17	2.6	–	–	–	–
Omosudis lowii	1	1.1	1	0.2	–	–	–	–
Alepisauridae indet.	7	7.4	10	1.6	–	–	–	–
Anoplogastridae	**1**	**1.1**	**1**	**0.2**	**–**	**–**	**–**	**–**
Anoplogaster sp.	1	1.1	1	0.2	–	–	–	–
Bothidae	**1**	**1.1**	**1**	**0.2**	**–**	**–**	**–**	**–**
Callionymidae	**1**	**1.1**	**1**	**0.2**	**–**	**–**	**–**	**–**
Cepolidae	**–**	**–**	**–**	**–**	**1**	**1.3**	**1**	**0.1**
Owstonia sp.	–	–	–	–	1	1.3	1	0.1
Chiasmodontidae	**16**	**17.0**	**21**	**3.3**	**3**	**3.8**	**4**	**0.4**
Pseudoscopelus spp.	16	17.0	21	3.3	3	3.8	4	0.4
Diretmidae	**3**	**3.2**	**4**	**0.6**	**28**	**35.9**	**77**	**7.5**
Diretmichthys parini	1	1.1	1	0.2	10	12.8	16	1.6
Diretmus argenteus	2	2.1	3	0.5	20	25.6	61	6.0
Evermannellidae	**–**	**–**	**–**	**–**	**2**	**2.6**	**2**	**0.2**
Evermannella spp.	–	–	–	–	2	2.6	2	0.2
Exocoetidae	**–**	**–**	**–**	**–**	**2**	**2.6**	**3**	**0.3**
Gempylidae	**3**	**3.2**	**3**	**0.5**	**–**	**–**	**–**	**–**
Gempylus sp.	1	1.1	1	0.2	–	–	–	–
Nesiarchus nasutus	1	1.1	1	0.2	–	–	–	–
Gempylidae indet.	1	1.1	1	0.2	–	–	–	–
Gonostomatidae	**–**	**–**	**–**	**–**	**1**	**1.3**	**1**	**0.1**
Macrouridae	**–**	**–**	**–**	**–**	**25**	**32.1**	**47**	**4.6**
Coelorinchus spp.	–	–	–	–	13	16.7	28	2.7
Ventrifossa sp.	–	–	–	–	1	1.3	1	0.1
Macrouridae indet.	–	–	–	–	14	17.9	18	1.8
Microstomatidae	**–**	**–**	**–**	**–**	**22**	**28.2**	**56**	**5.5**
Nansenia spp.	–	–	–	–	22	28.2	56	5.5
Myctophidae	**8**	**8.5**	**18**	**2.8**	**51**	**65.4**	**505**	**49.5**
Diaphus meadi	–	–	–	–	15	19.2	42	4.1
Diaphus spp.	–	–	–	–	23	29.5	133	13.0
Diaphus watasei	–	–	–	–	1	1.3	1	0.1
Electrona risso	–	–	–	–	31	39.7	290	28.4
Gymnoscopelus sp.	–	–	–	–	1	1.3	1	0.1
Myctophum sp.	1	1.1	1	0.2	–	–	–	–
Myctophidae indet.	8	8.5	17	2.6	14	17.9	38	3.7
Nemichthyidae	**4**	**4.3**	**7**	**1.1**	**–**	**–**	**–**	**–**
Nemichthys scolopaceus	4	4.3	7	1.1	–	–	–	–
Nomeidae	**2**	**2.1**	**2**	**0.3**	**3**	**3.8**	**4**	**0.4**
Cubiceps spp.	–	–	–	–	3	3.8	4	0.4
Psenes spp.	2	2.1	2	0.3	–	–	–	–
Ophidiidae	**1**	**1.1**	**1**	**0.2**	**–**	**–**	**–**	**–**
Brotula sp.	1	1.1	1	0.2	–	–	–	–
Opisthoproctidae	**–**	**–**	**–**	**–**	**2**	**2.6**	**3**	**0.3**
Dolichopteroides binocularis	–	–	–	–	2	2.6	3	0.3
Ostracoberycidae	**2**	**2.1**	**2**	**0.3**	**–**	**–**	**–**	**–**
Ostracoberyx dorygenys	2	2.1	2	0.3	–	–	–	–

(Continued)

TABLE 2 | Continued

Prey item	Fish remain				Otolith remain			
	O	%O	N	%N	O	%O	N	%N
Paralepididae	**19**	**20.2**	**42**	**6.5**	**30**	**38.5**	**56**	**5.5**
Dolichosudis fuliginosa	1	1.1	1	0.2	–	–	–	–
Lestidiops spp.	1	1.1	1	0.2	2	2.6	4	0.4
Lestrolepis sp.	–	–	–	–	1	1.3	1	0.1
Magnisudis atlantica	4	4.3	7	1.1	4	5.1	8	0.8
Magnisudis spp.	–	–	–	–	9	11.5	18	1.8
Paralepis spp.	–	–	–	–	13	16.7	15	1.5
Sudis spp.	–	–	–	–	5	6.4	6	0.6
Paralepididae indet.	14	14.9	33	5.1	4	5.1	4	0.4
Polymixiidae	**–**	**–**	**–**	**–**	**1**	**1.3**	**1**	**0.1**
Polymixia sp.	–	–	–	–	1	1.3	1	0.1
Scombridae	**1**	**1.1**	**8**	**1.2**	**–**	**–**	**–**	**–**
Scopelarchidae	**8**	**8.5**	**16**	**2.5**	**51**	**65.4**	**219**	**21.4**
Scopelarchus analis	7	7.4	10	1.6	49	62.8	202	19.8
Scopelarchidae indet.	1	1.1	6	0.9	12	15.4	17	1.7
Sternoptychidae	**17**	**18.1**	**35**	**5.5**	**9**	**11.5**	**12**	**1.2**
Argyropelecus spp.	10	10.6	26	4.0	–	–	–	–
Polyipnus spp.	1	1.1	1	0.2	8	10.3	11	1.1
Sternoptyx sp.	1	1.1	1	0.2	–	–	–	–
Valenciennellus tripunctulatus	–	–	–	–	1	1.3	1	0.1
Sternoptychidae indet.	6	6.4	7	1.1	–	–	–	–
Trachichthyidae	**1**	**1.1**	**2**	**0.3**	**–**	**–**	**–**	**–**
Gephyroberyx darwinii	1	1.1	2	0.3	–	–	–	–
Zeniontidae	**14**	**14.9**	**32**	**5.0**	**8**	**10.3**	**9**	**0.9**
Zenion spp.	14	14.9	32	5.0	8	10.3	9	0.9
Unidentified fish	**83**	**88.3**	**357**	**55.6**	**–**	**–**	**–**	**–**
Unidentified otoliths	**–**	**–**	**–**	**–**	**4**	**5.1**	**6**	**0.6**
Incomplete otoliths	**–**	**–**	**–**	**–**	**6**	**7.7**	**14**	**1.4**
Total	94	100.0	642	100.0	78	100.0	1,021	100.0

The bold fonts and numbers indicate the category title and values for the groups such as fish, crustacean, mollusc, each fish family and others.

Paralepididae indet.; *Scopelarchus analis* and Scopelarchidae indet.; *Polyipnus* (Sternoptychidae); and *Zenion* (Zeniontidae). Twenty fish remain items were not found in otolith remains. By contrast, 21 items were added only from the otolith remains, including *Owstonia* (Cepolidae); *Evermannella* (Evermannellidae); Exocoetidae indet.; Gonostomatidae indet.; *Coelorinchus* and *Ventrifossa* (Macrouridae); *Nansenia* (Microstomatidae); *Diaphus meadi*, *D. watasei*, *Electrona risso*, and *Gymnoscopelus* (Myctophidae); *Cubiceps* (Nomeidae); *Dolichopteroides binocularis* (Opisthoproctidae); *Lestrolepis*, *Paralepis*, and *Sudis* (Paralepididae); *Polymixia* (Polymixiidae); and *Valenciennellus tripunctulatus* (Sternoptychidae) (**Table 2**). This indicated that the otoliths support a finer-scale prey composition. Rarefaction curves calculated from both fish remains and otolith samples reached close to asymptotes. Through extrapolation, we estimated that the number of taxa at the family level would not increase substantially with greater sampling, indicating our collection provided solid sampling coverage (**Figure 3**).

Contribution of Important Prey Items to Diet Composition

The contribution of prey items to stomach content composition was also inconsistent between fish and otolith remains. Excluding unidentified fish remains, Alepisauridae was the most important prey item, accounting for 13.9% (%N) of fish remains, followed by Paralepididae (6.5%), Sternoptychidae (5.5%), Zeniontidae (5.0%), Chiasmodontidae (3.3%), Myctophidae (2.8%), Scopelarchidae (2.5%), and others (all < 1.1%). The top seven dominant fish remain items contributed only approximately 40% of the accumulated %N. Different in either species composition or rank of dominant items, the top six otolith taxa contributed approximately 94% of %N. These were Myctophidae (49.5%), Scopelarchidae (21.4%), Diretmidae (7.5%), Microstomatidae (5.5%), Paralepididae (5.5%), and Macrouridae (4.6%). All other minor families were less than 1.2% (**Figure 4A** and **Table 2**).

Regarding the prey item percentage of occurrence (%O) for the 94 fish remain-containing stomachs, it was 37.2%

FIGURE 2 | Otoliths recovered from stomach contents of the bigeye tuna (*Thunnus obesus*). Scale bar = 1 mm.

for Alepisauridae, followed by Paralepididae (20.2%), Sternoptychidae (18.1%), Chiasmodontidae (17.0%), Zeniontidae (14.9%), Myctophidae and Scopelarchidae (both 8.5%), and others (all < 4.3%). The %O for the 78 otolith remain-containing stomachs was also different from those of fish remains: Myctophidae (65.4%), Scopelarchidae (65.4%), Paralepididae (38.5%), Diretmidae (35.9%), Macrouridae (32.1%), Microstomatidae (28.2%), Sternoptychidae (11.5%), Zeniontidae (10.3%), and others (all <3.8%) (**Figure 4B** and **Table 2**). The values of %N and %O in the present study
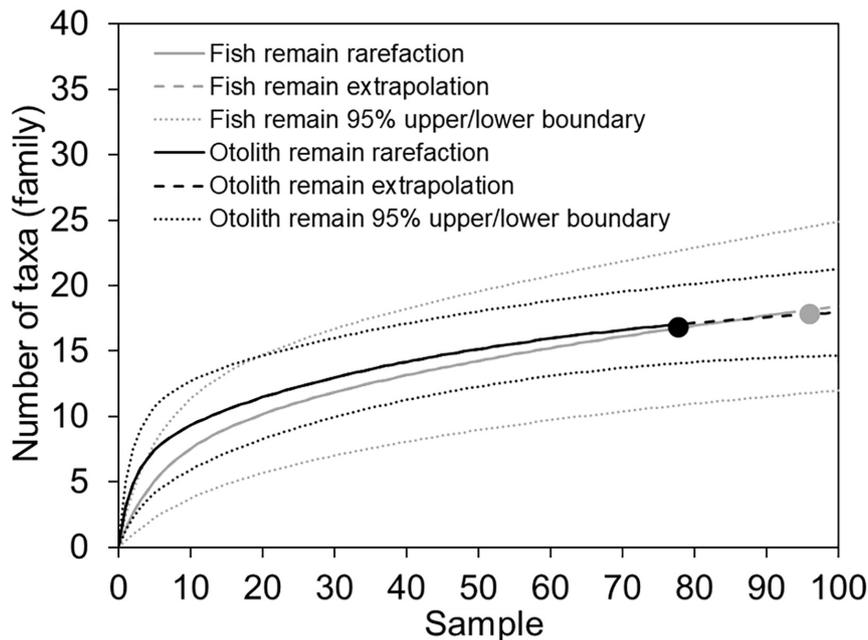
FIGURE 3 | Sample-based rarefaction and extrapolation curves with 95% confidence intervals. Prey taxa are counted at family level. There were 94 and 77 reference samples for fish and otolith remains, respectively.

presented a similar pattern (**Figure 4**), thus preventing potential conflicts between the two indices.

Spatiotemporal and Biological Factors in Relation to Prey Composition

Figures 5, **6** present a comprehensive structure of prey composition (MN% by individual stomach) for both fish and otolith remains in relation to the four spatiotemporal and biological factors. Regarding the 10 important prey items in both remains, the MN% of the families Alepisauridae, Paralepididae, Sternoptychidae, Zeniontidae, Diretmidae, and Microstomatidae did not differ significantly between prey composition and either capture site and depth, month of catch, or tuna size (Kruskal–Wallis test, all $P > 0.05$). However, the prey compositions of Myctophidae, Scopelarchidae, and Macrouridae significantly differed by tuna size, where Myctophidae prey decreased significantly as the tuna FL increased ($P = 0.004$). By contrast, the composition of Scopelarchidae was higher in tuna with an FL of 128–138 cm compared with others ($P = 0.004$), and that of Macrouridae prey increased as the tuna FL increased ($P = 0.005$) (**Figure 6**). The composition of Chiasmodontidae exhibited a significant site effect ($P = 0.004$), but this is likely caused by the relatively small sample size (**Figure 5**).

Size-Related Shift in Dominant Prey Items

The two most abundant prey species of otolith remains were *Electrona risso* ($n = 290$, Myctophidae) and *Scopelarchus analis* ($n = 202$, Scopelarchidae) (**Table 2**). The OL for *E. risso* ranged from 2386.2 to 4392.7 μm ($n = 277$), and it was in the range of

2306.0–5217.7 μm ($n = 195$) for *S. analis*. Changes in OLs by tuna capture months were not consistent for either prey species. Moreover, the OLs did not differ with respect to tuna capture depths (ANOVA, $P = 0.056$ and 0.138) but significantly differed by tuna capture sites ($P < 0.001$ and 0.05, respectively) and tuna size (both $P < 0.001$) for both prey species. The mean OLs of both *E. risso* (3,598–3,780 μm) and *S. analis* (3,686–4,077 μm) were significantly larger for larger tuna (>111 cm FL) than it was for the smallest tuna (3,480 μm for *E. risso* and 3,131 μm for *S. analis*). This size-related selective prey target indicated that larger bigeye tunas feed on larger individuals of the same prey species (**Figure 7**).

The least-squares regression between tuna size and prey OL revealed a significantly positive relationship for both *E. risso* and *S. analis* (both $P < 0.001$). However, because of great variations in prey otolith size consumed by tuna of specific sizes, the upper (95th quantile) and lower (5th quantile) boundaries of prey–predator sizes were only positively regressed for *E. risso* ($P = 0.040$) and *S. analis* ($P < 0.001$) (**Figure 8**). Nevertheless, these results again supported larger bigeye tuna feeding on larger prey.

DISCUSSION

Diet Composition of Bigeye Tuna in the Western Indian Ocean: Similarities and Differences

Overall, our results agreed well with those of relevant studies in that teleosts, cephalopods, and crustaceans are the top three
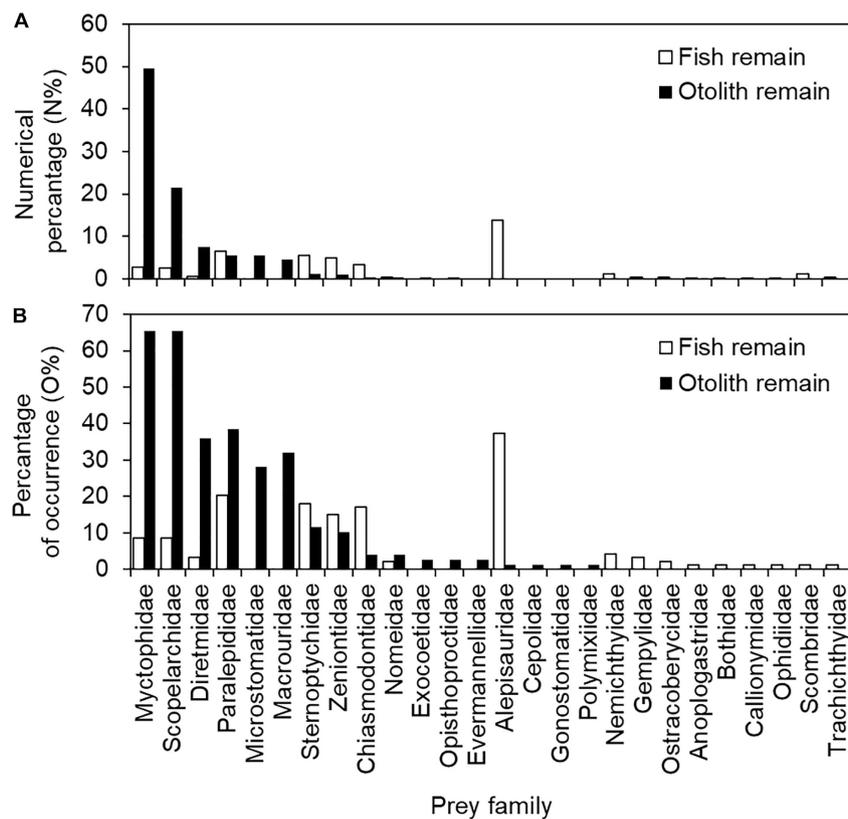
FIGURE 4 | Numerical percentage (%N) and percentage of occurrence (%O) in **(A)** 94 fish remain-containing and **(B)** 78 otolith remain-containing stomachs of bigeye tuna (*Thunnus obesus*).
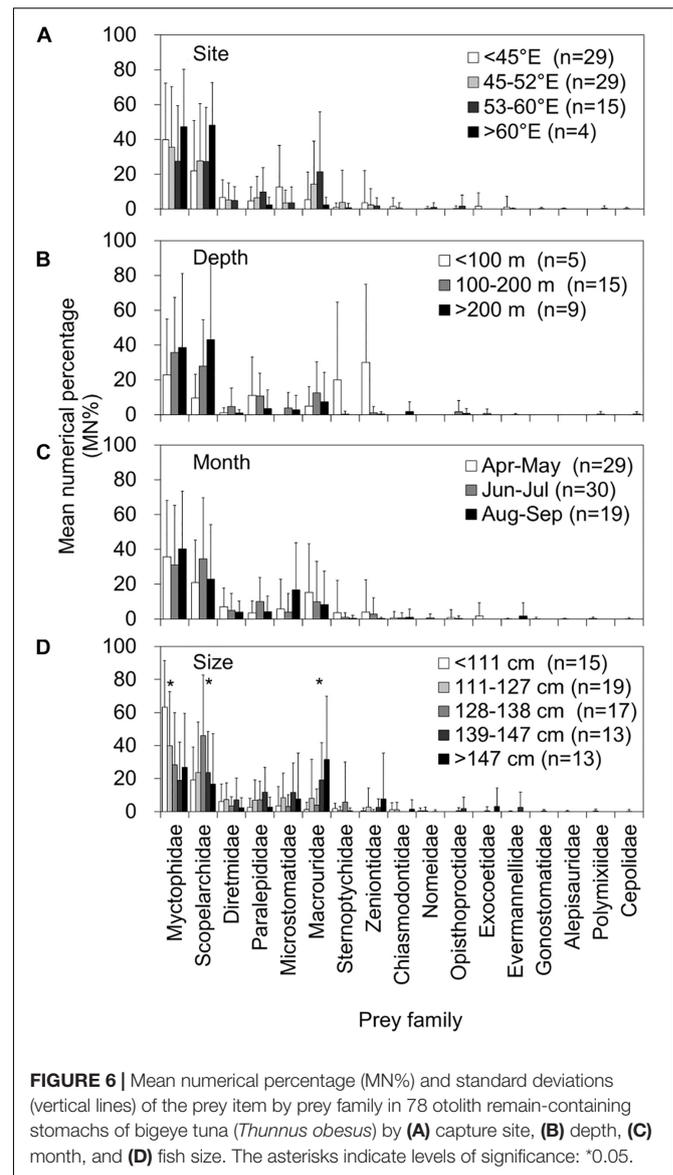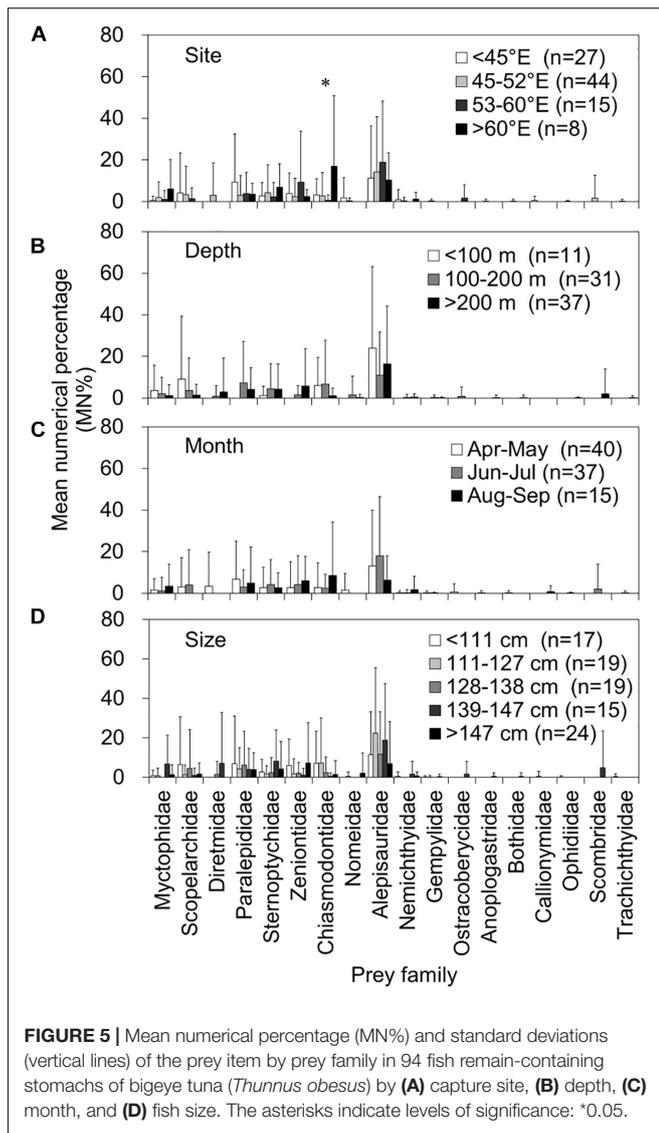
major categories consumed by the bigeye tuna. In particular, teleosts are their primary target in terms of %O, %W, and computed IRI (**Table 1**).

Our result of 56 fish taxa in 26 families (both fish and otolith remains) for the diet of bigeye tuna in the Indian Ocean can be regarded as significant in terms of taxonomic diversity. For example, three previous studies that did not examine otolith remains in stomachs yielded fish prey items of 42 taxa belonging to 29 families (Kornilova, 1980; n = 534 stomachs), 20 families (Bashmakov et al., 1991; n = 56), and eight taxa belonging to six families (Potier et al., 2004; n = 29). Our results are consistent with the findings of Potier et al. (2004) from the same region, where the occurrence of Scopelarchidae, Alepisauridae, Paralepididae, Myctophidae, Diretmidae, and Nomeidae were commonly recorded. By contrast, Kornilova (1980) and Bashmakov et al. (1991) have discovered more nearshore prey items, including Acanthuridae, Sparidae, and Serranidae, which were not reported elsewhere. Although the result of diet composition is primarily related to sample size, we were able to reveal additional prey taxa in fish and otolith remains (five and six families, respectively) as newly recorded food items of bigeye tuna. In addition, new lower taxa including *Omosudis lowii* (Alepisauridae); *Gempylus* and *Nesiarchus nasutus* (Gempylidae); *Dolichosudis fuliginosa* (Paralepididae), *Argyropelecus* (Sternoptychidae); and

Gephyroberyx darwinii (Trachichthyidae) in fish remains and *Diaphus meadi*, *D. watasei*, *Electrona risso*, and *Gymnoscopelus* (Myctophidae); *Lestrolepis* (Paralepididae); and *Valenciennellus tripunctulatus* (Sternoptychidae) in otolith remains were further recorded. These newly recorded taxa not found in previous studies mainly belong to deep-water mesopelagic species, and they constitute a marginal proportion of the total diet composition (**Table 1**). For newly recorded fish remains, since other similar taxa were recorded, no evidence existed that their carcasses are more rapidly digested and difficult to identify, and these were not observed in previous studies. Similarly, for the otoliths, these new taxa do not have particularly thick or large otoliths that are resistant to digestion with respect to other mesopelagic taxa. We therefore concluded that these new taxa reflect prey items not consumed historically and/or that were overlooked in previous studies that did not use otoliths.

Taxonomical work on fish otoliths essentially relies on the coverage of reference collections and regional otolith atlases (e.g., Smale et al., 1995). Along with the creation of more collections and references, a higher taxonomic resolution on stomach content diversity, either for fish and otolith remains, is expected to be revealed.

Through a detailed comparison of identifications of both fish and otolith remains and the subsequent independent prey content analyses, we further indicated that otoliths indeed
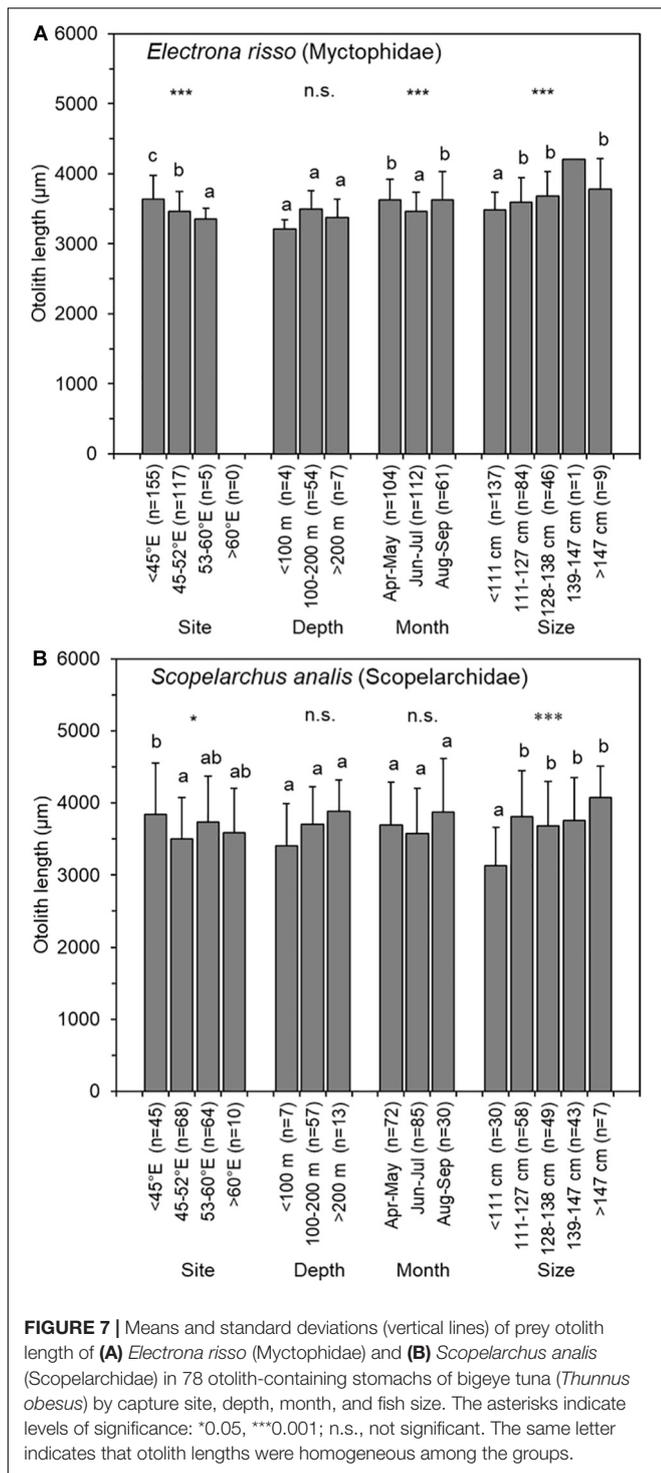
FIGURE 5 | Mean numerical percentage (MN%) and standard deviations (vertical lines) of the prey item by prey family in 94 fish remain-containing stomachs of bigeye tuna (*Thunnus obesus*) by **(A)** capture site, **(B)** depth, **(C)** month, and **(D)** fish size. The asterisks indicate levels of significance: *0.05.

FIGURE 6 | Mean numerical percentage (MN%) and standard deviations (vertical lines) of the prey item by prey family in 78 otolith remain-containing stomachs of bigeye tuna (*Thunnus obesus*) by **(A)** capture site, **(B)** depth, **(C)** month, and **(D)** fish size. The asterisks indicate levels of significance: *0.05.

provide a more diverse composition than do fish carcasses alone. This is, however, not only because of a potential higher taxonomical resolution in otoliths but also, to a lesser extent, a broader spectrum of taxonomic composition documenting more families.

The main difference in prey composition between fish and otolith remains is that large fish such as *Alepisaurus ferox* are more frequently found as carcasses. By contrast, much smaller mesopelagic taxa are only left as isolated otoliths. Such an effect of digestion could result in overestimation in specific prey taxa when using either remains as a single approach (see below).

Use of Otoliths in Stomach Content Analysis: Advantages and Caveats

Fish prey recovered from the alimentary canal of a piscivore can be identified based on fresh and accumulated remains; for example, teeth, otoliths, spines, and occasionally bones.

Otoliths, which are taxon-specific, are composed of hard tissue and enclosed by cranial bones that protect them from direct exposure to digestive acids. They are typically easier to recognize by well-trained specialists compared with half-digested fish carcasses, and they are less affected by acid erosion than other parts of a fish. They are thus extensively used to identify the stomach contents of cetaceans (Fitch and Brownell, 1968), seals (Pierce et al., 1991), sharks (Joyce et al., 2002), other large piscivores (Clarke et al., 1995; Watanabe et al., 2004), and sea birds (Neves et al., 2012). The rarefaction curves, extrapolation, and narrow range of 95% confidence intervals for both fish remains and otoliths suggest that the sampling effort was, on the whole, sufficient to characterize diet composition (**Figure 3**). When extrapolated, however, the diversity of otoliths would likely be slightly higher than that obtained from the fish remains.

FIGURE 7 | Means and standard deviations (vertical lines) of prey otolith length of **(A)** *Electrona risso* (Myctophidae) and **(B)** *Scopelarchus analis* (Scopelarchidae) in 78 otolith-containing stomachs of bigeye tuna (*Thunnus obesus*) by capture site, depth, month, and fish size. The asterisks indicate levels of significance: *0.05, ***0.001; n.s., not significant. The same letter indicates that otolith lengths were homogeneous among the groups.

FIGURE 8 | Relationship between fish length of bigeye tuna (*Thunnus obesus*) and prey otolith length of **(A)** *Electrona risso* (Myctophidae) and **(B)** *Scopelarchus analis* (Scopelarchidae). The solid lines indicate the least-squares regression and dotted lines the 95th and 5th quantile regressions.

Although otoliths have traditionally been used in studying piscivores' feeding ecology (Jobling and Breiby, 1986), they are often ignored. The primary reason is probably that few taxonomic specialists assist in their identification, which would otherwise be a challenging task to accomplish. However, several other limitations may still have influenced our results. First,
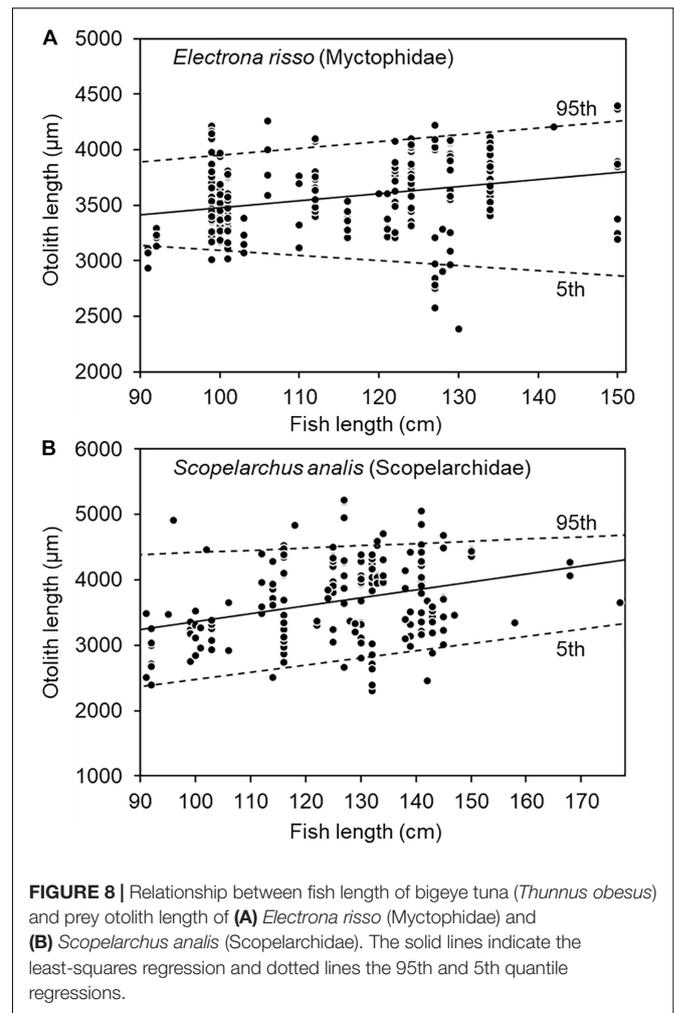
most studies have regarded otoliths as accumulated food items, similar to beaks, eye lens, mandibles, and parasphenoids, which were excluded from the analysis because they might have overemphasized the importance of certain prey items (Potier et al., 2007, 2008). Extremely tiny otoliths may have been easily digested, eroded, or lost during the preparation process. Lastly, the otoliths observed may have been the result of secondary predation.

Studies have shown that bigeye tuna forage at both the surface and at depths of 400–500 m (Dagorn et al., 2000; Potier et al., 2004; Ohshimo et al., 2018), implying that a longline catch–based dietary analysis alone might only reflect the food consumed at shallower depths (i.e., less than approximately 200 m), where longline gears operate. The prey items consumed in deeper waters may have been digested for an extended period; consequently, using otolith remains as an identification tool may provide comprehensive results indicating the food items naturally available at deeper depths. Therefore, using both fish and otolith remains would not overemphasize certain prey items in the case of bigeye tuna.

The size of the otoliths examined in this study ranged from 690 to 5,730 μm. Relevant studies have reported that *Alepisaurus*

ferox is a principal bigeye tuna prey item (Kornilova, 1980; Potier et al., 2004). However, only one *A. ferox* otolith specimen was observed in this study, which might be attributed to its extremely small size (690 μm). Furthermore, if many of the Alepisauridae remains were intact, there would be fewer free otoliths relative to prey with more rapidly digested soft tissue. Moreover, Tetraodontiformes have been reported as bigeye tuna prey items (Bashmakov et al., 1991; Young et al., 2010). This taxon was not observed in this study either because its otoliths are generally less than 1,000 μm in length (Nolf, 1985; Lin and Chang, 2012). However, in both cases, their fish remains would have been easy to recognize; *A. ferox* has a characteristic long-bodied form that is usually large in size, and tetraodontiforms have a solid body that is more resistant to digestive fluid.

In addition, the otoliths observed may have been consumed by piscivorous prey rather than by bigeye tuna. This condition is of particular concern regarding the consumption of Paralepididae, Gempylidae, and other medium-sized predators (Kornilova, 1980; Bashmakov et al., 1991; Ménard et al., 2000; Young et al., 2010). Cannibalism among *Alepisaurus* spp. is also common (Young et al., 2010) and may have affected the results. Although these isolated otoliths would likely experience advanced erosion on the surface and be excluded from the analysis, this secondary predation could result in major bias in the study.

Spatiotemporal and Biological Variations in Bigeye Tuna Foraging

Bigeye tuna prey item composition did not differ with site, depth, or time. This is likely because bigeye tuna are highly mobile and can move vertically in the water column; thus, the depth of capture would not affect the overall prey composition (Hanamoto, 1987; Dagorn et al., 2000; Bertrand et al., 2002; Musyl et al., 2003). Teleost assemblages may differ in response to seasonal changes, but because summer season samples were not acquired for analysis, this potential temporal factor did not affect prey composition. During the sampling period (April–September), the prey items did not differ substantially; instead, they presented ontogenetic changes.

A significant result of the present study was the revelation of a shift in both diet composition and prey size from small to large bigeye tuna. Myctophidae and Scopelarchidae are small prey and foraged abundantly by small bigeye tuna. By contrast, large prey, such as Macrouridae, are consumed by large bigeye tuna (**Figure 6**). More interestingly, evident by the dimension of otolith remains, larger bigeye tuna feed on larger prey of the same taxon (**Figures 7**, **8**), which agrees well with the size dependence in opportunistic predation in tuna reported by Ménard et al. (2006) and Ohshimo et al. (2018).

CONCLUSION

This study improves the taxonomic resolution of fish prey items, many of which were reported here for the first time. The study demonstrated that diet changes in both prey composition and size can be related to the ontogeny of bigeye tuna. Moreover, the result of the ontogenetic shift in prey item and size suggests a

rather rapid change in the trophic level during the life history of bigeye tuna; therefore, understanding the diet composition of this species alludes to a rough index of energetic flux in a regional marine ecosystem and remains for further investigation. Hence, the results provide a more precise scale and analysis of diversified content of the prey fish composition of bigeye tuna in the Indian Ocean.

Despite several caveats, the attempt of our study based on both fish and otolith remains led to a more comprehensive interpretation of the composition of fish prey. Our material provides a unique window into the feeding habits of bigeye tuna more than a decade ago. Multiple-year studies in the future would still be needed and beneficial for delineating whether the entire fish prey composition of bigeye tuna has changed over time under anthropogenic disturbance (cf. Duffy et al., 2017). Genetic barcode analysis could provide substantial improvements regarding the unidentifiable prey items (Jakubavièiûtë et al., 2017), and stable isotope analysis, including nitrogen stable isotopes, could help to elucidate trophic level shifts through ontogeny (Chung et al., 2019).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because ethical review and approval were not required for the animal study because the study is using the fisheries catch.

AUTHOR CONTRIBUTIONS

C-HL identified the otoliths and drafted the manuscript. J-SL identified the fish remains. K-SC processed the stomach samples. M-HC and C-YC designed and directed the project. C-WC led the work and analyzed the data. All authors contributed to the manuscript's preparation.

FUNDING

ACKNOWLEDGMENTS

otolith identification at the early stage of the study, and also to Ciao-Yun Hung of the Graduate Institute of Marine Biology, National Dong Hwa University, Taiwan for OL measurement.

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