



## Short communication

Feeding habits of juvenile yellowfin tuna (*Thunnus albacares*) in Ecuadorian waters assessed from stomach content and stable isotope analysisJosé Luis Varela<sup>a,b,c,\*</sup>, Ketty María Intriago<sup>a</sup>, Julio César Flores<sup>a</sup>, Cristhian Ronald Lucas-Pilozo<sup>a</sup><sup>a</sup> Departamento Central de Investigación, Universidad Laica Eloy Alfaro de Manabí, Av. de Circunvalación, Manta, Ecuador<sup>b</sup> Departamento de Biología, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Av. República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain<sup>c</sup> Biology Department, Acadia University, 33 Westwood Ave., B4P 2R6 Wolfville, Nova Scotia, Canada

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## ABSTRACT

Size-related shifts in feeding habits of juvenile yellowfin tuna (YFT), *Thunnus albacares* (Bonnaterre, 1788), in Ecuadorian waters were assessed using stomach content analysis (SCA) ( $n = 339$ ) and stable isotope analysis ( $n = 83$ ). In all size classes, fishes were the predominant prey group in the diet (Alimentary Index, %AI = 96.86) followed by cephalopods (%AI = 3.29) and crustaceans (%AI = 0.03). PERMANOVA analysis revealed significant variations in diet with body size: i) Class I YFT ( $\leq 50$  cm in fork length, FL) fed mainly on lanternfishes, family Myctophidae (%AI = 37.34), and jumbo squid, *Dosidicus gigas* (%AI = 36.06), Class II YFT (50–60 cm, FL) consumed bullet and frigate tunas, *Auxis* spp., unidentifiable fish and jumbo squid (%AI = 47.75, %AI = 25.06 and %AI = 21.67, respectively), and Class III YFT ( $\geq 60$  cm, FL) preyed almost exclusively on bullet and frigate tunas (%AI = 90.85). Mean  $\pm$  SD muscle isotope values were  $12.14 \pm 1.95\text{‰}$  for  $\delta^{15}\text{N}$  and  $-17.42 \pm 0.27$  for  $\delta^{13}\text{C}\text{‰}$ ; mean ( $\pm$  SD) liver values were  $11.30\text{‰}$  for  $\delta^{15}\text{N}$  and  $-17.42 \pm 0.27\text{‰}$  for  $\delta^{13}\text{C}$ . Overall, liver and muscle samples showed significant size-related differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Stable isotope Bayesian ellipses, SEAC, did not show trophic overlap among size classes. A significant positive linear correlation was observed between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  measured in liver and muscle tissues with body size, suggesting an increase of prey size as tuna grew. These results indicate that the observed size-related diet shifts are not only related to prey type, but also to prey size.

## 1. Introduction

The yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788), is an epipelagic species widely distributed in the tropical and sub-tropical waters of the world's major oceans (Collette and Nauen, 1983). For management purposes, two stocks are considered in the Pacific Ocean: the stock of the Eastern Pacific Ocean (EPO) and the stock of the Western-Central Pacific Ocean (WCPO), both of which have been subjected to intensive fishing over the last decades (ISSF, 2015). In Ecuador, this species represents one of the most important fishery resources, with a total landing of 253743 tons in 2013 (INP, 2015). In spite of its commercial importance, there are very few studies based on stomach content analysis (SCA) addressing the feeding habits of yellowfin tuna (YTF) in this region (Alverson, 1963; Baque-Menoscal et al., 2012). Alverson (1963) studied predation habits of YFT in the Gulf of Guayaquil and identified scombrid fishes as the most important resources, whereas Baque-Menoscal et al. (2012) documented the importance of squid *Dosidicus gigas* in the diet of YFT caught around the Galapagos Islands.

Although SCA is the most common method used to assess food habits and dietary composition of tunas, it may provide inaccurate results due to several factors, including fast digestion rates and regurgitation of gastric contents during capture events (Aloncle and Delaporte, 1970; Chase, 2002; Olson and Boggs, 1986). For these reasons, stable isotope analysis (SIA) has become a useful method to complement gut analyses as it provides information on consumed prey at longer time scales (Logan et al., 2006; Peterson and Fry, 1987). The carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) give information about dietary sources (Fry, 2006; Fry and Sherr, 1984), whereas nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) are used as indicators of the consumer's trophic level (Post et al., 2007). Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can provide trophic information over weeks or months, depending on the tissue turnover rate

(Gannes et al., 1997). In fishes, slow turnover tissues like muscle (Hesslein et al., 1993; MacAvoy et al., 2001) produce information trophic biology at mid-time scale (months), whereas tissues with faster metabolic rates such as liver (Guélinckx et al., 2007; Suzuki et al., 2005) give information at a shorter time scale (weeks) (Logan et al., 2006; MacNeil et al., 2006). Furthermore, stable isotopes are also good

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descriptors of trophic niches and the values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in predator tissues allow us to estimate trophic parameters such as niche width and overlap (Bearhop et al., 2004; Newsome et al., 2007; Syväranta et al., 2013).

In comparison with strictly poikilothermic fish species, tunas have high standard metabolic rates (Korsmeyer and Dewar, 2001), which may be particularly high in juvenile individuals (i.e. in rapid growth phase) and in individuals that perform long-distance migrations (Harden Jones, 1984). Their feeding ecology, therefore, has critical implications for life history features of growth and survival (Goñi et al., 2011).

Size-related shifts in the feeding patterns of juvenile YFT by SCA and SIA have been assessed in Sri Lankan, Hawaiian and Taiwanese waters (Graham et al., 2007; Maldeniya, 1996; Weng et al., 2015), but no similar studies have been undertaken in Ecuadorian waters. Hence, the present study was conducted to investigate size-related variations in dietary composition, trophic niche width and overlap of juvenile YFT in Ecuadorian waters by combining stomach content and stable isotope analysis.

## 2. Material and methods

### 2.1. Sampling and stomach-content analysis

A total number of 339 juvenile YFT, ranging from 41.1 to 75.8 cm in straight fork length (FL) and from 1.1 to 6.4 kg in body mass, were sampled from purse seine commercial vessels landing in Manta (Ecuador) (Fig. 1) from July 2014 through March 2015. The straight fork length (FL) was estimated from the curved fork length (CFL) using the equation proposed by Scida et al. (2001):  $\text{FL} = 0.8 + 0.96 \times \text{CFL}$ .

Whole stomachs were collected from all the sampled fish and stored at  $-20^\circ\text{C}$  until analysis. In the laboratory, they were dissected and their contents thoroughly examined under a stereoscopic microscope. Preys were grouped by taxon and their wet weight was recorded to the nearest 0.01 g. Partially digested fish were identified from otoliths (Harvey et al., 2000; García-Godos Naveda, 2001), whereas cephalopod prey were identified from mandible using the key of Clarke (1986). Stomachs containing only hard parts were excluded from SCA.

White muscle and liver samples were removed from 83 YFT and kept frozen at  $-20^\circ\text{C}$  before being treated for analysis. Once thawed, all tissue samples were rinsed with distilled water to remove blood remains and placed into glass tubes for cryodesiccation until total dryness. Then, they were ground to powder by pestle and mortar, packed into tin capsules and analysed for  $\delta^{15}\text{N}$ , carbon (%) and nitrogen (%). Prior to  $\delta^{13}\text{C}$  analysis, samples with high lipid content (C:N ratio  $> 3.5$ ; see Post et al. (2007)) were subjected to total lipid extraction by chloroform-methanol 2:1 (v/v) (see Varela et al., 2012, 2013). The relative abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$  ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) were measured by a continuous gas flow system using a Thermo Finnigan Elementary Analyzer Flash EA1112 coupled to a Finnigan MAT Delta Plus mass spectrometer. All carbon and nitrogen isotope data are reported in  $\delta$  notation according to the following equation:  $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  (Peterson and Fry, 1987). Standard materials are Vienna Pee Dee belemnite for carbon and atmospheric  $\text{N}_2$  for nitrogen and expressed as parts per thousand (‰) relative to standards (Peterson and Fry, 1987).

### 2.2. Data analysis

The dietary importance of each prey was assessed by three indices: (1) percent composition by weight (%Wi = weight of prey item  $i \times 100$ /total weight of all prey items), (2) frequency of occurrence (% Oi = number of stomachs containing prey item  $i \times 100$ /total number of non-empty stomachs), and (3) alimentary index expressed as percentage (%Ai =  $[(\% \text{Oi} \times \% \text{Wi})/(\sum \% \text{Wi} \times \% \text{Oi})] \times 100$ ) (Kawakami

and Vazzoler, 1980). Mean percent weight was calculated as  $\% \text{MW}_i = (1/P) \sum_{j=1}^P (S_{ij}/\sum_{i=1}^Q S_{ij}) \times 100$ , where P is the number of ABFT with non-empty stomachs, Q the number of prey types and  $S_i$  the total weight of prey  $i$  (Chipps and Garvey, 2007).

Size-related shifts in diet composition were evaluated by a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001). An experimental design with one fixed factor was considered: 'Size class' (with three levels, class I ( $\leq 50$  cm in FL), class II (50–60 cm in FL) and class III ( $\geq 60$  cm in FL)). The analysis was based on a Gower similarity matrix calculated from the total prey weight, after performing a fourth-root transformation. Significant terms were investigated using a *posteriori* pair-wise comparisons with the PERMANOVA test. The homogeneity of multivariate dispersion among size classes was tested by PERMDISP (Anderson 2006). Similarity percentages (SIMPER) (Clarke, 1993) were used to identify which dietary categories typified each size class. Multivariate analyses were performed using the software PRIMER v6.1.13 & PERMANOVA+ v1.0.3 statistical package (PRIMER-E Ltd, Plymouth, UK).

Feeding strategy, prey importance, and inter- and intra- individual components of the trophic niche were evaluated using the graphical method of Costello (1990) as modified by (Amundsen et al., 1996). In this procedure, prey-specific abundance is plotted against %Oi in order to obtain information about prey importance and feeding strategy of the predator. The prey-specific abundance is calculated as follows:  $\% \text{Pi} = (\sum \text{prey } i \text{ weight}/\sum \text{weight of all prey in the stomach containing prey } i) \times 100$ . As in Varela et al. (2017), prey species that only were found in one stomach were not considered in the analyses.

The dietary niche width of each size class was calculated by the standardized Levin's index expressed as:  $B_i = [1/(n-1)][(1/\sum P_{ij}^2) - 1]$ , where  $B_i$  is the measure of the Levin's niche breadth,  $n$  is the number of prey categories and  $P$  is the proportion of the AI (expressed as per unit) (Krebs, 1989). The standardized Levin's index ranges between 0 and 1, where low values indicate specialist feeding behaviour and high values indicate generalist feeding behaviour (Krebs, 1989).

To test differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among size classes in both muscle and liver tissues, a Kruskal-Wallis test was used. Then, differences between pairs of sample groups were assessed using Mann-Whitney  $U$  test. Significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between liver and muscle tissues were analysed by Student's  $t$ -test or Mann-Whitney  $U$  test. Simple regression analyses were used to evaluate the relationship between  $\delta^{15}\text{N}$  and LF, the strength of the correlation being determined by the Pearson's correlation coefficient,  $r$ . A significance level of  $\alpha = 0.05$  was considered for all statistical tests. Statistical analyses were performed using Statgraphics Centurion v16.2.04.

The isotopic niche width and overlap of the three size classes were also estimated by standard Bayesian ellipses adjusted for small sample size (SEAc) using the SIBER package (Jackson et al., 2011) of SIAR (Stable Isotope Analysis in R) (Parnell and Jackson, 2013). SEAc overlap values  $\geq 0.8$  were considered to be biologically significant (Cartes and Sardà, 1989).

## 3. Results

### 3.1. Stomach content analysis

The length frequency distribution of the sampled YFT is depicted in Fig. 2. Of the 339 stomachs analysed, 219 (64.60%) were empty, whereas the remaining 120 (35.40%) contained at least one prey item (Table 1). The diet comprised 16 prey taxa, including 11 fishes, 6 cephalopods and 2 crustaceans (Table 2). Teleost fishes were the predominant prey group (%AI = 96.68), *Auxis* spp. was the most important teleost (%AI = 90.85) followed by the family Myctophidae



Fig. 1. Map of the study area. The filled circle represents the sampling location.

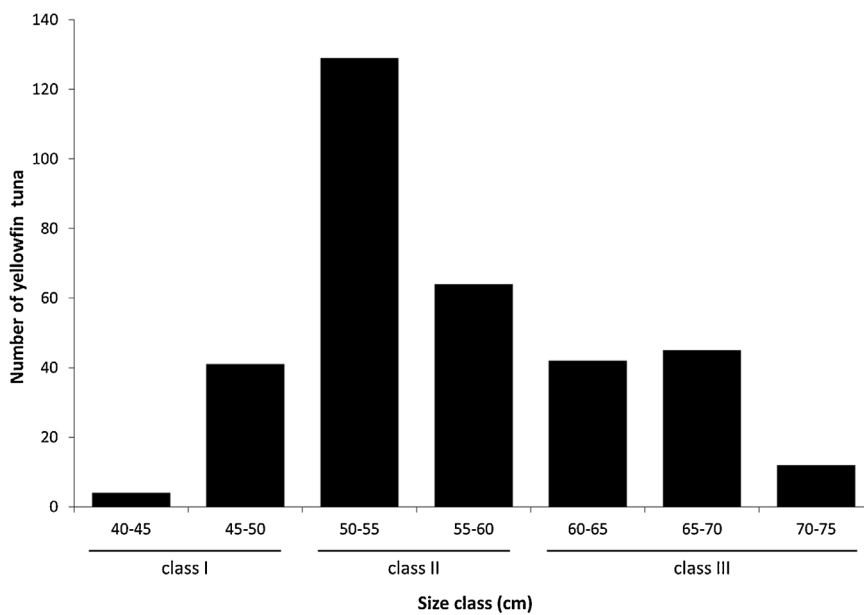


Fig. 2. Length frequency distribution of the yellowfin tuna sampled.

**Table 1**  
Data summary of the yellowfin tuna samples used in stomach content analysis.

Size class	Fork length (cm)		Body mass (kg)		n	Percentage of stomachs containing prey	Percentage of empty stomachs
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD			
I	41.10–50.00	47.66 $\pm$ 2.09	1.11–2.96	2.12 $\pm$ 0.35	81	46.91% (n = 38)	53.09% (n = 43)
II	50.24–59.84	53.60 $\pm$ 2.56	2.03–5.81	2.81 $\pm$ 0.53	169	28.40% (n = 48)	71.60% (n = 121)
III	60.22–75.78	64.84 $\pm$ 3.26	2.99–6.40	4.61 $\pm$ 0.73	89	38.20% (n = 34)	61.80% (n = 55)
Overall	41.10–75.78	55.13 $\pm$ 6.81	1.11–6.40	3.12 $\pm$ 1.09	339	35.40% (n = 120)	64.60% (n = 219)

(%AI = 2.93). The jumbo squid (*Dosidicus gigas*) was the most abundant invertebrate species (%AI = 3.93), whereas crustaceans were poorly represented in the stomach contents (%AI = 0.03) (Table 2).

The PERMANOVA analysis showed differences in dietary composition among size-classes (PERMANOVA,  $p = 0.001$ ). Posterior pair-wise PERMANOVA comparisons revealed highly significant differences between classes I and II ( $p = 0.005$ ), I and III ( $p = 0.001$ ), and II and III ( $p = 0.001$ ). The PERMDISP analysis yielded no significant differences (PERMDISP,  $p = 0.313$ ), suggesting that the differences obtained with PERMANOVA were not due to multivariate dispersion.

Teleost fishes were the predominant prey group in all size classes, but prey composition varied with body size. Class I YFT ( $\leq 50$  cm, FL) preyed mainly on myctophids and jumbo squid, which showed similar %AI values (37.34 and 36.06, respectively) (Table 2). Nevertheless, the former was the main contributor to the similarity (56.97%) within this size class (Fig. 3). The medium-sized YFT (50–60 cm, FL) mainly consumed bullet and frigate tunas, *Auxis* spp., unidentifiable fish and jumbo squid in terms of %AI (47.75, 25.06 and 21.67) and contribution to similarity (38.47, 22.19 and 32.56%, respectively) (Table 2, Fig. 3). The largest YFT ( $\geq 60$  cm, FL) fed almost exclusively on *Auxis* spp. (%AI = 90.85) as also indicated by the SIMPER analysis (contributing to 94.47% of the similarities) (Table 2, Fig. 3).

The Amundsen plot shows that ABFT in the SoG have a varying degree of specialisation on different prey (Fig. 4). Most prey species were located in the lower left corner of the graph or close to the vertical axis, suggesting that they were unimportant and rare. The teleost *Auxis* spp., which was consumed by a relatively large number of tuna (% O = 32.50), can be considered as the dominant prey.

The niche width estimated from Levin's index decreased with YFT size from 0.18 for small YFT to 0.15 for medium-sized YFT and  $< 0.01$  for large YFT (Table 2).

### 3.2. Stable isotope analysis

Mean isotopic values of YFT muscle and liver samples are shown in Table 3.  $\delta^{15}\text{N}$  values ranged from 8.32 to 15.53‰ for muscle and from 7.30 to 14.01‰ for liver.  $\delta^{13}\text{C}$  values ranged from  $-17.97$  to  $-16.88$ ‰ for muscle and from  $-19.12$  to  $-16.42$ ‰ for liver. Both muscle and liver samples showed significant isotopic differences among size classes (Kruskal–Wallis followed by Mann–Whitney  $U$  test,  $p < 0.05$ ), except for liver  $\delta^{13}\text{C}$  between size classes I and II ( $p > 0.05$ ). In class II and class III YFT, the  $\delta^{15}\text{N}$  values in muscle tissue were significantly higher than in liver (Mann–Whitney  $U$  test,  $p < 0.01$ , statistical analysis not shown in table), whereas no differences were observed in class III YFT (Mann–Whitney  $U$  test,  $p > 0.05$ ). Muscle samples also showed significantly higher  $\delta^{13}\text{C}$  values (Student's  $t$ -test or Mann–Whitney  $U$  test,  $p < 0.01$ ) in classes I and II YFT; however, no differences were detected in class III YFT (Mann–Whitney  $U$  test,  $p > 0.001$ ).

A moderate positive correlation was observed between  $\delta^{15}\text{N}$  and FL in muscle ( $r = 0.53$ ,  $p < 0.001$ ) and liver tissues ( $r = 0.57$ ,  $p < 0.001$ ).  $\delta^{13}\text{C}$  and FL were also moderately correlated in muscle tissue ( $r = 0.61$ ,  $p < 0.001$ ), whereas a relatively weak relationship was found between both variables in liver samples ( $r = 0.44$ ,  $p < 0.001$ ) (Fig. 5).

Table 4 and Fig. 6 show data on isotopic niche width and overlap, as measured by the standard ellipse corrected for sample size (SEAc). In both muscle and liver samples, class I YFT showed the narrowest trophic niche, whereas similar values of SEAc were found in size classes II and III. No significant isotopic overlap between size classes was identified in muscle or liver samples.

## 4. Discussion

### 4.1. Stomach content analysis

SCA showed that the diets of juvenile YFT caught in Ecuadorian waters consisted primarily of fishes. Within the fish prey group, according to all feeding indices, *Auxis* spp. were the most important prey items. This observation is in accordance with earlier studies carried out in all seas worldwide. Dragovich (1970) found that bullet and frigate tunas (*Auxis rochei* and *A. thazard*, respectively) were the most important prey-species by volume for YFT caught in the coastal waters off the middle Atlantic U.S. states, whereas Rawlins et al. (2007) and Perera et al. (2015) observed that the frigate tuna was the predominant prey by weight in the Caribbean Sea and Indian Ocean. In Ecuadorian waters, *Auxis* represents an important dietary component not only for YFT (Alverson, 1963), but also for sharks, billfishes and dolphinfishes (Galván-Magaña et al., 2013; Polo-Silva et al., 2013; Rosas-Luis et al., 2016; Varela et al., 2017). For instance, Polo-Silva et al. (2013) found that *Auxis* was a frequent prey (%O = 15.29) in the diet of thresher shark, while Rosas-Luis et al. (2016) observed that this species was the most important prey in terms of %W ( $> 38.50\%$ ) for billfish species, *Istiophorus platypterus*, *Makaira nigricans* and *Kajikia audax*. In a recent study, Varela et al. (2017) reported that *Auxis* was the second most important prey in terms of %AI (25.25%) for dolphinfish.

Bullet and frigate tunas are known to be epipelagic species, which suggests that juvenile YFT feed intensively in shallow waters. The presence of deep-water species, such as myctophids and jumbo squid, also suggests that juvenile YFT perform deep dives in search of potential preys. Nevertheless, electronic tagging data have shown that immature YFT spend most time swimming above 200 m (Holland et al., 1990; Matsumoto et al., 2013). Hence, these mesopelagic preys might also be ingested at night-time as they become available in the epipelagic zone (Alverson, 1961; Bazzino et al., 2010).

Multivariate analysis suggests two size-related shifts in feeding habits. Class I YFT preyed mainly on myctophids and jumbo squid, which are important components of food webs linking organisms located at low trophic levels to top predators (Gilly et al., 2006; Olivar et al., 2012). Similarly, Olson and Boggs (1986) observed that fishes and cephalopods were the most representative prey groups in the diet of YFT  $< 55$  cm (968–3255 g) caught in the eastern tropical Pacific. In contrast, studies carried out in Sri Lankan, Hawaiian and Taiwanese waters (Graham et al., 2007; Maldeniya, 1996; Weng et al., 2015, respectively) revealed that crustaceans, such as stomatopods or crab larvae, made up the diet of YFT  $< 50$  cm. Such differences among areas are probably related to geographical variations in the abundance and distribution of planktonic and nektonic preys. The first shift in the feeding habits was detected between the small- and the medium-sized YFT, and was caused by a sharp decrease in the consumption of

**Table 2**  
Diet composition of yellowfin tuna by size classes. Percentage of weight (%W), mean percent weight (%MW) ± SD, occurrence (%O) and alimentary index (%AI). The trophic niche width was calculated by the standardized Levin's index (B<sub>i</sub>).

Preys	Size class												Overall			
	class I (≤ 50 cm)				class II (50–60 cm)				class III (≥ 60 cm)				%W	%AI	%O	%AI
	%W	%MW	%O	%AI	%W	%MW	%O	%AI	%W	%MW	%O	%AI				
Fishes	66.88	56.57 ± 49.07	57.86	75.04	72.37	67.74 ± 45.34	70.83	83.39	98.44	84.32 ± 35.63	76.47	99.64	92.81	68.90 ± 45.05	72.50	96.68
Axius spp.	15.37	15.68 ± 36.70	15.79	17.40	39.83	27.70 ± 44.22	29.17	47.75	91.85	55.82 ± 50.34	55.88	98.76	79.89	31.86 ± 46.35	32.50	90.85
Mycetophidae	16.49	30.37 ± 45.47	31.58	37.34	4.64	7.95 ± 25.36	12.50	2.38	4.33	5.74 ± 23.32	5.88	0.49	5.03	14.42 ± 34.16	16.67	2.93
<i>Cubiceps pauciradiatus</i>	31.58	2.63 ± 16.22	2.63	5.96									1.71	0.83 ± 9.13	0.83	0.05
Exocoetidae	2.75	2.63 ± 16.22	2.63	0.52					1.53	10.90 ± 30.62	11.76	0.35	1.37	3.92 ± 19.04	4.17	0.20
<i>Hyporhamphus micropterus</i>					3.89	2.08 ± 14.43	6.25	1.00	0.42	2.94 ± 17.15	2.94	0.02	0.92	1.67 ± 12.86	1.67	0.05
Scombridae					4.13	3.13 ± 16.00	4.17	0.71					0.62	1.25 ± 10.17	1.67	0.04
<i>Selene peruviana</i>					0.37	1.46 ± 10.10	2.08	0.03					0.06	0.58 ± 6.39	0.83	< 0.01
<i>Mycotaphum</i> sp.									0.03	3.04 ± 17.14	5.88	< 0.01	0.02	0.86 ± 9.13	1.67	< 0.01
Unidentifiable fish	0.69	5.26 ± 22.63	5.26	0.26	19.51	25.42 ± 42.32	31.25	25.06	0.28	5.88 ± 23.88	5.88	0.03	3.19	13.50 ± 33.47	15.83	1.77
Cephalopods	32.20	29.99 ± 45.46	39.47	24.64	27.06	29.71 ± 44.70	37.50	16.51	1.55	12.39 ± 31.94	17.65	0.36	7.05	24.89 ± 42.15	32.50	3.29
<i>Dosidicus gigas</i>	27.30	14.53 ± 34.67	18.42	36.06	21.09	20.80 ± 38.84	25.00	21.67	1.53	6.81 ± 24.18	11.76	0.35	5.86	14.85 ± 34.12	19.17	3.93
<i>Argonauta</i> sp.	0.50	4.01 ± 17.93	7.89	0.28	2.61	3.46 ± 14.10	8.33	0.90					0.42	2.65 ± 13.47	5.83	0.09
Teuthidae	2.35	5.26 ± 22.63	5.26	0.89	1.07	2.08 ± 14.43	2.08	0.09					0.29	2.50 ± 15.68	2.50	0.03
<i>Histioteuthis</i> sp.	0.18	0.48 ± 2.97	2.63	0.03	1.43	2.08 ± 14.43	2.08	0.12					0.22	0.99 ± 9.27	1.67	0.01
Ommastrephidae	0.37	2.63 ± 16.22	2.63	0.07	0.83	0.76 ± 4.93	4.17	0.14	0.01	2.85 ± 16.60	2.94	< 0.01	0.15	1.94 ± 12.99	3.33	0.02
<i>Thysanoceuthis</i> sp.					0.02	0.52 ± 3.61	2.08	< 0.01					< 0.01	0.21 ± 2.28	0.83	< 0.01
Unidentifiable cephalopod	1.50	3.08 ± 16.38	5.26	0.57					0.02	2.74 ± 15.95	2.94	< 0.01	0.10	1.75 ± 12.49	2.50	0.01
Crustaceans	0.92	13.44 ± 34.18	18.42	0.33	0.58	2.55 ± 14.45	10.42	0.10	0.01	3.29 ± 17.15	8.82	< 0.01	0.14	6.21 ± 23.51	12.50	0.03
Porcellanidae larvae	0.75	7.96 ± 27.31	10.53	0.57	0.54	2.44 ± 14.55	6.25	0.14					0.12	3.50 ± 18.05	5.83	0.02
Stomatopoda larvae	0.02	2.66 ± 16.22	5.26	0.01					0.01	3.15 ± 17.16	5.88	< 0.01	0.01	1.73 ± 12.86	3.33	< 0.01
Unidentifiable crustacean	0.15	2.82 ± 16.23	5.26	0.06	0.04	0.11 ± 0.75	4.17	0.01	< 0.01	0.14 ± 0.82	2.94	< 0.01	0.01	0.98 ± 9.16	4.17	< 0.01
Trophic niche width (B <sub>i</sub> )				0.18				0.15				< 0.01				0.01



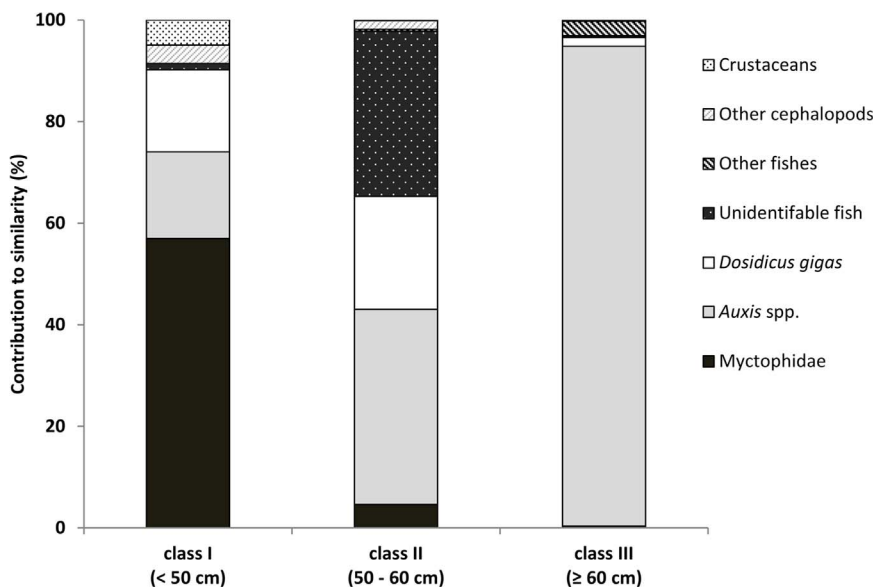


Fig. 3. Similarity percentage contribution given by the main prey types for each size class.

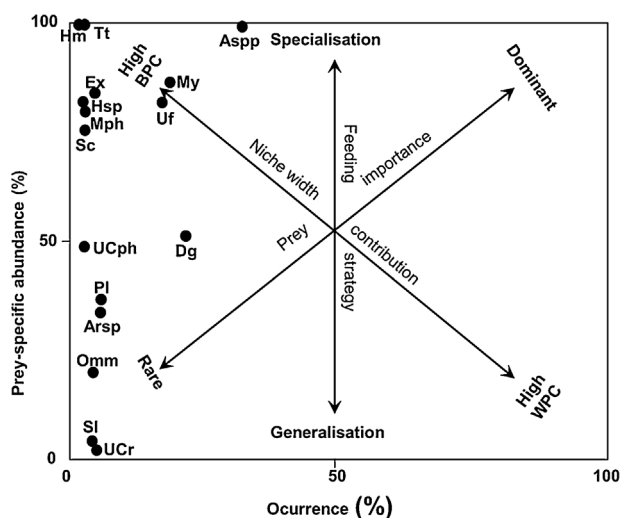


Fig. 4. Prey-specific abundance plotted against frequency of occurrence of prey species for yellowfin tuna from Ecuadorian waters. Explanatory axes for foraging patterns are those of Costello (1990) as modified from Amundsen et al. (1996). The two diagonal axes represent the importance of prey (dominant vs rare) and the contribution to the niche width (high between-phenotype vs high within-phenotype contribution); the vertical axis defines the predator feeding strategy (specialist vs generalist). Aspp, *Auxis* spp.; Arsp, *Argonauta* sp.; Dd, *Dosidicus gigas*; Ex, *Exocoetidae*; Hsp, *Histiotheuthis* sp.; Hm, *Hyporhamphus micropterus*; My, *Mycetophidae*; Mph, *Mycetophum* sp.; Omm; *Ommastrephidae*; Pl, *Porcellanidae* larvae; Sc, *Scombridae*; Sl, *Stomatopoda* larvae; Te, *Teuthidae*; Uf, *Unidentifiable fish*; UCph, *Unidentifiable cephalopod*; UCr, *Unidentifiable crustacean*.

myctophids and jumbo squids along with an increased proportion of *Auxis* spp. The second shift was observed around 60 cm, where YFT preyed almost exclusively on *Auxis* spp. Although class II and class III YFT primarily fed on these scombrids, the diet of the former group was

more varied.

Although the YFT has been widely defined as a generalist and opportunistic predator (Ménard et al., 2006; Potier et al., 2007; Young et al., 2010; Olson et al., 2014, 2016), the niche width estimated from the standardized Levin's index suggests that this species behaves as a specialist feeder in the Ecuadorian waters. A similar observation was reported for other large pelagic fishes, such as hammerhead sharks (*Sphyrna lewini*), thresher sharks (*Alopias pelagicus* and *Alopias superciliosus*) and dolphinfishes (*Coryphaena hippurus*) (Estupiñán-Montaño et al., 2009; Polo-Silva et al., 2007, 2009, 2013; Varela et al., 2017), caught around the same area. The narrow niche width found in these large pelagic fishes may be related to the fact that the food chain is mainly dominated by few species located at middle trophic levels. In fact, Olson and Waters (2003), based in Ecopath models, reported that cephalopods and *Auxis* spp. are the most influential species on Eastern tropical Pacific Ocean ecosystems. It is noteworthy that the trophic niche underwent a decrease with increasing body size, which might be caused by an increase of interspecific competition (Layman et al., 2007). In contrast, a positive relationship between body size and niche breadth has been recently reported for subadults and adults *Thunnus thynnus* (77–212 cm in FL) in the western Mediterranean Sea (Sorell et al., 2017).

4.2. Stable isotope analysis

$\delta^{15}N$  measured in muscle and liver tissues (12.14 and 11.30‰) showed higher values than those observed in YFT tuna  $\geq 45$  cm caught in Hawaiian Island (10.2 and 8.4‰; Graham et al., 2007). Such disparities could be due to several factors, including variation in the trophic level caused by differences either in the diet or in the isotopic baseline composition (Graham et al., 2010; Popp et al., 2007). Considering muscle  $\delta^{15}N$  values of 10.2‰ for Hawaiian YFT (Graham et al.,

Table 3  $\delta^{13}C$  and  $\delta^{15}N$  values (mean  $\pm$  SD) of YFT by size class. Values in the same row bearing different superscript letters are significantly different ( $p < 0.05$ ).

		class I ( $\leq 50$ cm) and n = 11	class II (50-60 cm) and n = 44	class III ( $\geq 60$ cm) and n = 28	Mean n = 83
$\delta^{15}N$	Muscle	9.73 $\pm$ 0.65 <sup>a</sup>	12.19 $\pm$ 1.78 <sup>b</sup>	13.02 $\pm$ 1.76 <sup>c</sup>	12.14 $\pm$ 1.95
	Liver	9.33 $\pm$ 1.30 <sup>a</sup>	11.21 $\pm$ 1.69 <sup>b</sup>	12.21 $\pm$ 0.98 <sup>c</sup>	11.30 $\pm$ 1.68
$\delta^{13}C$	Muscle	-17.68 $\pm$ 0.18 <sup>a</sup>	-17.47 $\pm$ 0.27 <sup>b</sup>	-17.23 $\pm$ 0.17 <sup>c</sup>	-17.42 $\pm$ 0.27
	Liver	-18.05 $\pm$ 0.28 <sup>a</sup>	-17.86 $\pm$ 0.41 <sup>a</sup>	-17.37 $\pm$ 0.65 <sup>b</sup>	-17.72 $\pm$ 0.55

Differences among size classes were analysed using Kruskal-Wallis test, followed by Mann-Whitney U test.

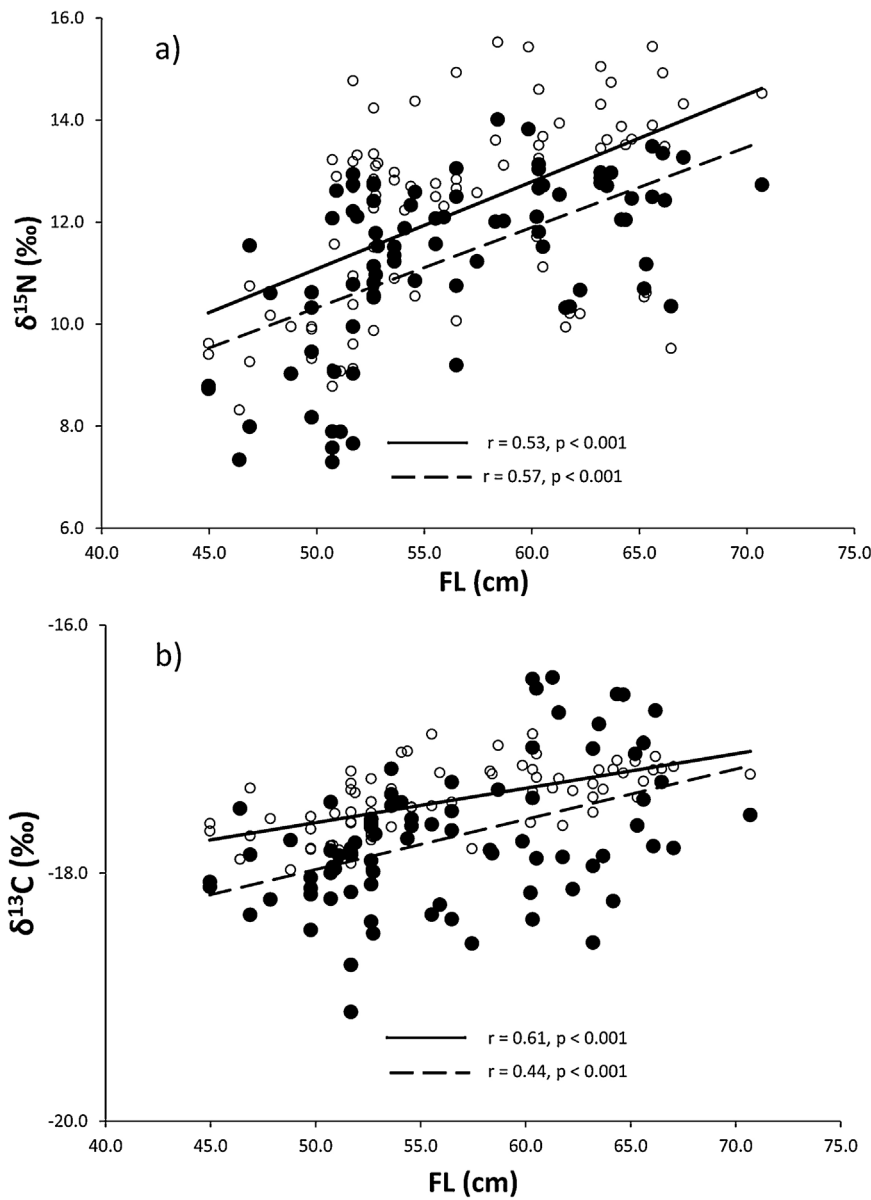


Fig. 5. Relationships between a)  $\delta^{15}\text{N}$  and b)  $\delta^{13}\text{C}$  and straight fork length for muscle and liver tissues. Filled circles, muscle data; open circles, liver data. Linear regressions are represented as solid lines for muscle and dashed lines for liver.

Table 4

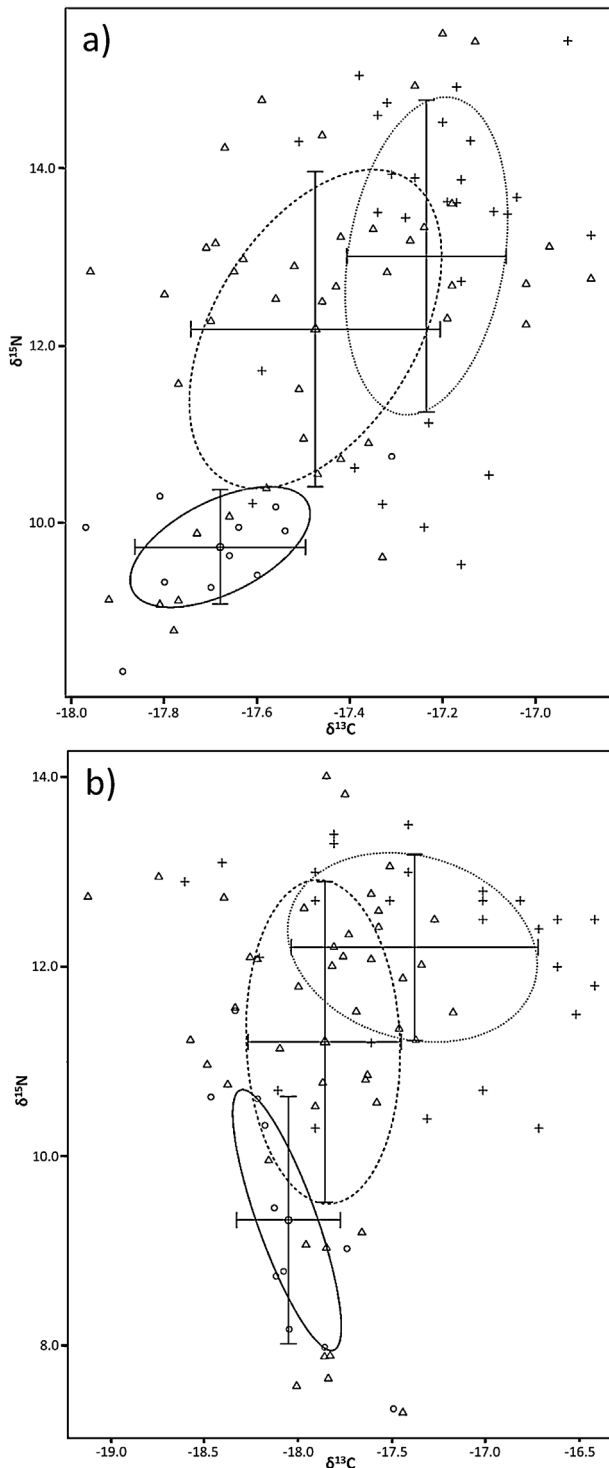
Trophic niche width and overlap of YFT by size class, as estimated by SIBER (Stable Isotope Bayesian Ellipses in R) analysis of muscle and liver isotopic values. SEAc, corrected standard ellipse area.

Group	SEAc	SEAc Overlap
Muscle		
class I	0.35	I vs II (< 0.01)
class II	1.37	I vs III (< 0.01)
class III	0.96	II vs III (0.43)
Liver		
class I	0.72	I vs II (0.09)
class II	2.21	I vs III (< 0.01)
class III	2.06	II vs III (0.62)

2007), and 12.1‰ for individuals sampled in Ecuador (present data), and a prey-muscle discrimination factor of 2.1‰ (Popp et al., 2007), we conclude that YFT occupy a similar trophic level (TL) in both areas (TL = 3.7 and TL = 4.0, respectively) (calculated from the equation proposed by Post et al. (2007); data of baseline species have been taken from Graham et al. (2007) and Polo-Silva et al. (2013)). These results, therefore, confirm that the isotopic differences between both areas are

mainly caused by differences in  $\delta^{15}\text{N}$  values at the base of the food webs. Comparing with other top predators co-occurring in the area, the  $\delta^{15}\text{N}$  values measured in muscle samples showed lower values than those reported in muscle of thresher (*A. pelagicus*; 13.6–14.0‰; Polo-Silva et al., 2013) and hammerhead (*S. lewini* and *S. zygaena*; 14.8–15.4‰; Loor-Andrade et al., 2015) sharks. These observations suggest that these elasmobranchs seemingly occupy higher trophic levels; in fact, Hunsicker et al. (2012) reported that YFT are significantly preyed upon by hammerhead sharks. Another plausible cause for such differences could be related to the low turnover rate of shark muscle (over one year) (Logan and Lutcavage, 2010; MacNeil et al., 2005), which may reflect isotopic values of preys from a variety of EPO oceanic ecosystems.

Because  $\delta^{15}\text{N}$  increases more sharply with trophic levels than  $\delta^{13}\text{C}$  (DeNiro and Epstein, 1981), the narrower  $\delta^{13}\text{C}$  range found in both muscle and liver samples was not unexpected. The range of muscle  $\delta^{13}\text{C}$  values observed in the present study (−16.97 to −16.88‰) was lower than those observed in the western Indian Ocean (−17.4 to −15.2‰; Ménard et al., 2007) (−17.2 to −15.7‰; Zudaire et al., 2015) and in the eastern Pacific Ocean (−17.8 to −15.4‰; Olson et al., 2010). In the present study, a slightly wider  $\delta^{13}\text{C}$  range (−19.12 to −16.42‰)



**Fig. 6.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plots for yellowfin tuna a) muscle and b) liver tissues. Circles, class I YFT; triangles, class II YFT; crosses, class III YFT. Ellipses represent the standard ellipse area (SEA) estimated for class I YFT (solid line), class II YFT (dashed line) and class III YFT (dotted line).

was observed in liver tissues than those reported by Zudaire et al. (2015) in the western Indian Ocean ( $-18.3$  to  $-16.1\text{‰}$ ). The narrow range of  $\delta^{13}\text{C}$  values found in both liver and muscle tissues apparently indicates that all sampled fish fed on the same area.

Complementary to SCA analysis, conventional statistical and SEAc/overlap analyses revealed two size-related shifts in feeding habits of juvenile YFT. Furthermore, the significant positive relationship between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  measured in liver and muscle with body size

suggests an increase of prey size as the predator grows (see Jennings et al., 2002; O'Farrell et al., 2014). In contrast, no relationship was found between YFT body length and  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the western Indian Ocean and eastern Pacific Ocean (Ménard et al., 2007; Olson et al., 2010). Our findings would indicate that the observed size-related diet shifts are not only related to prey type, but also to prey size, as was reported by Varghese and Somvanshi (2016) in YFT caught in the Arabian Sea.

The trophic diversity estimated by the Bayesian standard ellipse corrected areas (SEAc) (Jackson et al., 2011) showed that the diet of the smallest fish was less diverse than that of the medium-size and largest individuals, which is not consistent with the estimations based on SCA. These contrasting results may be due to a recent diet shift which is not reflected in the isotopic composition of muscle and liver tissues (Medina et al., 2015).

## 5. Conclusion

By combining stomach content (SCA) and stable isotope analysis (SIA), we provide new information regarding the feeding habits, trophic niche width and trophic overlap of juvenile yellowfin tuna (YFT) in Ecuadorian waters. Our findings indicate that this species is an epipelagic carnivorous predator which shows two size-related dietary shifts to occur at a FL  $\sim 50$  cm and FL  $\sim 60$  cm. The low number of muscle and liver samples, especially for the class I YFT, along with the lack of information on the accurate location of capture, did not allow us to study seasonal and/or spatial variations of this species in the Ecuadorian waters. Further studies, therefore, should be addressed to assess seasonal and spatial variation in the feeding ecology of this large pelagic fish.

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