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Short communication

Feeding habits of juvenile yellowfin tuna (*Thunnus albacares*) in Ecuadorian waters assessed from stomach content and stable isotope analysis

José Luis Varela[a,b,c,*], Ketty María Intriago[a], Julio César Flores[a], Cristhian Ronald Lucas-Pilozo[a]

[a] Departamento Central de Investigación, Universidad Laica Eloy Alfaro de Manabí, Av. de Circunvalación, Manta, Ecuador
[b] Departamento de Biología, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI·MAR), Av. República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain
[c] 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 (≤ 50 cm in fork length, FL) fed mainly on laternfishes, 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 (≥ 60 cm, FL) preyed almost exclusively on bullet and frigate tunas (%AI = 90.85). Mean ± SD muscle isotope values were 12.14 ± 1.95‰ for δ^{15}N and −17.42 ± 0.27 for δ^{13}C‰; mean (± SD) liver values were 11.30‰ for δ^{15}N and −17.42 ± 0.27‰ for δ^{13}C. Overall, liver and muscle samples showed significant size-related differences in δ^{15}N and δ^{13}C. Stable isotope Bayesian ellipses, SEAc, did not show trophic overlap among size classes. A significant positive linear correlation was observed between δ^{15}N and δ^{13}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 (δ^{13}C) give information about dietary sources (Fry, 2006; Fry and Sherr, 1984), whereas nitrogen stable isotope ratios (δ^{15}N) are used as indicators of the consumer's trophic level (Post et al., 2007). Both δ^{13}C and δ^{15}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 (Guelinckx 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

* Corresponding author at: Departamento Central de Investigación, Universidad Laica Eloy Alfaro de Manabí, Av. de Circunvalación, Manta, Ecuador.
E-mail address: joseluis.varela@uca.es (J.L. Varela).

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descriptors of trophic niches and the values of $\delta^{13}C$ and $\delta^{15}N$ 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): FL = 0.8 + 0.96 × CFL.

Whole stomachs were collected from all the sampled fish and stored at −20 °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 °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 $\delta^{15}N$, carbon (%) and nitrogen (%). Prior to $\delta^{13}C$ 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}C and ^{15}N ($\delta^{13}C$ and $\delta^{15}N$, repectively) 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 δ notation according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry, 1987). Standard materials are Vienna Pee Dee belemnite for carbon and atmospheric 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 × 100/total weight of all prey items), (2) frequency of occurrence (% Oi = number of stomachs containing prey item i × 100/total number of non-empty stomachs), and (3) alimentary index expressed as percentage (%AIi = [(%Oi × % Wi)/(Σ%Wi × % Oi)] × 100) (Kawakami and Vazzoler, 1980). Mean percent weight was calculated as $\%MW_i = (1/P)\sum_{j=1}^{P}(S_{ij}/\sum_{i=1}^{Q} S_{ij}) \times 100$, 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 (≤ 50 cm in FL), class II (50–60 cm in FL) and class III (≥ 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: %Pi = (Σprey i weight/Σweight of all prey in the stomach containing prey i x 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: $B_i = [1/(n-1)][(1/\sum P_{ij}^2) - 1]$, whereBi 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{15}N$ and LF, the strength of the correlation being determined by the by 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). SEA_c overlap values ≥ 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