Feeding ecology and trophic relationships of pelagic sharks and billfishes coexisting in the central eastern Pacific Ocean

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ABSTRACT: Large pelagic fishes are top predators inhabiting the water column of the tropical and subtropical Pacific Ocean. They are highly migratory organisms, and for Ecuadorian fisheries, they also represent important economic resources. We sampled 4 shark species, *Prionace glauca*, *Isurus oxyrinchus*, *Carcharhinus falciformis* and *Alopias pelagicus*, and 3 billfish species, *Xiphias gladius*, *Istiophorus platypterus* and *Makaira nigricans*, in order to gain understanding of their feeding ecology and trophic interactions through the use and combination of stomach content and stable isotope analyses. Results showed that $\delta^{13}C$ values were similar among the 7 pelagic predators (values ranged from −16.45‰ for *M. nigricans* to −16.73‰ for *C. falciformis*), suggesting that all of them exploit a similar marine area off the coast of Ecuador. $\delta^{15}N$ stable isotope values differed among species (the lowest value was 13.83‰ for *A. pelagicus* and the highest value was 18.57‰ for *P. glauca*), suggesting segregation in the use of the water column and/or diet. The sharks *I. oxyrinchus* and *P. glauca* had high values of $\delta^{15}N$ and preyed mainly on cephalopods in comparison with the other species, which preyed mainly on fish. In addition to the common use of cephalopods as prey for large pelagic fish, our results indicate that these shark species segregate their diet.

KEY WORDS: Large pelagic predators · Ecuadorian Pacific Ocean · Trophic ecology · Stable isotopes · Sympatric species

INTRODUCTION

Determining the feeding ecology of marine predators is pivotal to understanding their ecological roles and trophic relationships in the ecosystems they inhabit and to designing effective ecosystem-based management programs. This information can be used to predict the impacts of fishing or climate change on other components of the ecosystem (e.g. Dambacher et al. 2010). Large pelagic fishes and sharks are often classified as top- and meso-predators within marine ecosystems, although there are important differences in the trophic habits between species and populations (e.g. Abitía-Cárdenas et al. 2010, Galván-Magaña et al. 2013, Rosas-Luis et al. 2016a). These marine predators play an important ecological role in pelagic ecosystems, exerting top-down control on their prey (Baum & Worm 2009, Dambacher et al. 2010). However, the trophic role that individual species play within marine communities in many ecosystems is still unclear. Thus, consequences of the removal or reduction of their main trophic resources are difficult to predict (Montoya et al. 2006). More studies on species-specific trophic

ecology are essential, as they can provide information on trophic relationships and requirements and the ecological roles of these predators and can help inform the design of conservation strategies (e.g. Rosas-Luis et al. 2016a, Navarro et al. 2017).

The biology and ecology of large pelagic fishes in the northern Pacific have been thoroughly investigated (e.g. Sedberry & Loefer 2001, Kubodera et al. 2007, Bonfil 2008, Dambacher et al. 2010, Carlisle et al. 2012, Preti et al. 2012, Vögler et al. 2012, Li et al. 2016). In addition, there are reports that describe the trophic ecology of sharks for the southern Pacific waters of South America off the coast of Chile (López et al. 2009, López et al. 2010). In contrast, the biology and ecology of large pelagic sharks and billfishes in the central Ecuadorian Pacific is poorly studied (Galván-Magaña et al. 2013, Martínez-Ortiz et al. 2015, Rosas-Luis et al. 2016a). The shark species *Alopias pelagicus*, *Prionace glauca*, *Isurus oxyrinchus* and *Carcharhinus falciformis*, and the billfishes *Makaira nigricans*, *Istiophorus platypterus* and X*iphias gladius* are economically important for Ecuador's artisanal fishing fleet (Martínez-Ortiz et al. 2015). Thus, accurate studies focusing on the relationships and interactions of these marine predators in the pelagic environment of Ecuador are needed. Moreover, the IUCN classifies *P. glauca* as Near Threatened, *I. oxyrinchus*, *A. pelagicus* and *M. nigricans* as Vulnerable and *I. platypterus* as Least Concern. These pelagic predators are found in open waters of tropical, subtropical and temperate oceans (Walsh & Brodziak 2015). Their distribution and migratory behavior in the Pacific Ocean are related to productivity gradients, prey availability and adequate areas for spawning (Ibáñez et al. 2004, Galván-Magaña et al. 2013, Walsh & Brodziak 2015). Studies on feeding habits in the eastern Pacific reported that *I. oxyrinchus*, *P. glauca* and *X. gladius* prey on squids (*Dosidicus gigas* and *Ancistrocheirus lessueuri*), while *I. platypterus* and *M. nigricans* prey on scombrid fishes such as *Auxis* spp. (Ibáñez et al. 2004, Abitía-Cárdenas et al. 2010, Rosas-Luis et al. 2016a,b, Loor-Andrade et al. 2017a,b).

The study of the trophic ecology of marine predators has traditionally relied on stomach content analysis. Although these data allow high levels of taxonomic resolution, large pelagic fishes and sharks often have empty stomachs, and prey detection is highly conditioned by digestion rates (Hyslop 1980). Stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) have been used as a complementary tool to study different aspects of the feeding ecology of a wide range of marine organisms (Layman et al.

2007). This approach is based on the fact that $\delta^{15}N$ and $\delta^{13}C$ values are transformed from dietary sources to consumers in a predictable manner and integrate the diet of the consumer over different time periods depending on the tissue selected (Post 2002, Layman et al. 2007, Madigan et al. 2012). For example, muscle integrates information about feeding over several months (Madigan et al. 2012, Vander Zanden et al. 2015). $\delta^{15}N$ and $\delta^{13}C$ values are indicators of the consumers' trophic positions and dietary sources of carbon, respectively (Layman et al. 2007). $\delta^{13}C$ values are commonly used to infer the habitat (e.g. pelagic vs. benthic; France & Peters 1997, Post, 2002) and movements of organisms between different latitudinal locations (Ménard et al. 2007, Carlisle et al. 2015). $\delta^{15}N$ values are mainly used to determine the trophic level (DeNiro & Epstein 1981) and, in combination with $\delta^{13}C$ values, the diet of the predator (Parnell & Jackson 2013). Due to this dual information on prey and habitat provided by isotopic values, these ecological markers can also be applied to define trophic niches (Layman et al. 2007, Jackson et al. 2011). Although the outcomes of stomach content analysis and isotopic models should be interpreted with caution, their combination is valuable to a better understanding of the feeding ecology of marine predators (e.g. Richert et al. 2015, Navarro et al. 2017).

In this study, we combined stable isotope and stomach content analyses to examine the trophic relationships of 4 shark and 3 billfish species that coexist in the pelagic environment of oceanic waters off Ecuador. These species are economically important fishing resources and top predators in the ecosystem, and our study provides new insights into their ecological role within the pelagic community, providing novel data on how these pelagic predators are related to prey and how they exploit available prey resources. The main objective was to determine the diet and trophic relationships among the 7 predators.

MATERIALS AND METHODS

Study area and fieldwork procedures

Species were caught by the artisanal longline fisheries of Santa Rosa, Salinas, and Playita Mía, Manta, in Ecuador, working between 37 and 130 km off the Ecuadorian coast in the pelagic environment of oceanic waters (Fig. 1). Unfortunately, we did not have access to the exact geographical position where
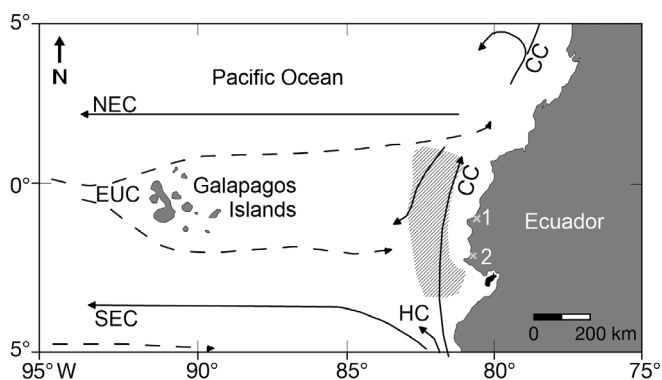
Fig. 1. Ecuadorian Pacific area. Striped polygon: main fishing area of artisanal fisheries in Ecuador; numbers indicate the fishing ports of (1) Playita Mía, Manta and (2) Santa Rosa, Salinas. Lines represent direction of marine currents: NEC: North Equatorial Current; EUC: Equatorial Undercurrent; SEC: South Equatorial Current; HC: Humboldt Current; CC: Coastal Current Map modified from Rincón-Martínez et al. (2010)

species where caught; nevertheless, the main fishing area for these species was plotted (see the sampling area in Fig. 1). This marine area is characterized by a constant flux of warm water from the Equatorial Current System and the eastern Pacific warm pool, and cold water from the Humboldt Current System (Bendix & Bendix 2006). The cold water flux moves in a south–north direction off Ecuador to the Panama basin and promotes high primary production (Bendix & Bendix 2006, Rincón-Martínez et al. 2010). The convergence of the 2 current systems promotes the formation of an eddy off the coast of Ecuador (Bendix & Bendix 2006) that influences the trophic web in the ecosystem.

Specimens of *Prionace glauca*, *Isurus oxyrinchus*, *Carcharhinus falciformis*, *Alopias pelagicus*, *Xiphias gladius*, *Istiophorus platypterus* and *Makaira nigricans* were provided by artisanal longline fisheries after they arrived at the fishing ports. The standard body length of each individual was recorded to the nearest 10 mm (precaudal length in sharks and fork length in billfishes). A small portion of the dorsal muscle of the caudal peduncle of billfishes and the dorsal muscle of the head of sharks caught from July to September 2014 was extracted and stored at −20°C until lipid extraction and isotopic analyses were conducted. Stomachs of all species were collected from February 2014 to January 2015, and prey items were identified for *A. pelagicus* and *C. falciformis*. For the other species, prey items were identified and reports were previously published (Rosas-Luis et al. 2016b, Loor-Andrade et al. 2017a).

Stable isotope analysis

Prior to isotopic determination, we extracted the lipid contents of the samples with chloroform and methanol following Bligh & Dyer (1959). All muscle samples were then freeze-dried and powdered, and 0.3 to 0.4 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Estación Biológica de Doñana (www.ebd.csic.es/lie/index.html). Samples were combusted at 1020°C using a continuous flow isotope-ratio mass spectrometer (Thermo Electron) by means of a Flash HT Plus elemental analyzer interfaced with a Delta V Advantage mass spectrometer. Stable isotope ratios were expressed in the standard δ-notation (‰) relative to Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$). Based on laboratory standards, the measurement error was ±0.1 and ±0.2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. The standards used were EBD-23 (cow horn, internal standard), LIE-BB (whale baleen, internal standard) and LIE-PA (razorbill feathers, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency.

Isotopic mixing model and trophic niche

To estimate the potential contributions of the different prey to the diet of each species, we applied stable isotope analysis in R (SIAR) Bayesian isotopic mixing models (Parnell et al. 2010). The SIAR model estimates the potential contribution of each prey item in the diet of the consumer based on $\delta^{13}C$ and $\delta^{15}N$ values of the consumer and its potential prey. This model runs in the free software R (R Development Core Team 2010) and allows the inclusion of variability in the stable isotope ratios of the predator and the potential prey (Parnell et al. 2010). To run the SIAR model, values of 2 prey groups (fish and cephalopods; Table 1) identified in the stomach contents, and whose stable isotope values had been obtained were included. The use of prey from stomach contents for isotope analysis is a methodology used in different studies with marine predators (e.g. Albo-Puigserver et al. 2015). This is an alternative when data on potential prey from the field is not available. The main limitation is that only fresh items can be used to reduce the potential biases associated with digestion. For this reason, we only selected prey items that were undigested. Moreover, the advantage of using prey from stomach contents is that we can guarantee that it is actual prey eaten by the predator. The iso-

Table 1. Mean ± SD of δ^{13}C and δ^{15}N values of prey identified in the stomach contents of billfishes and sharks

	n	δ^{13}C(‰)	δ^{15}N(‰)	C:N
FISH	61	−17.2 ± 0.5	12.4 ± 2.3	
Opisthonema libertate	2	−16.2 ± 0.1	13.3 ± 0.2	3.0
Pristigenys serrula	1	−17.5	11.0	3.1
Auxis sp.	8	−17.5 ± 0.9	12.1 ± 1.7	3.1
Katsuwonus pelamis	30	−17.1 ± 0.5	12.3 ± 3.0	3.1
Scomber japonicus	5	−16.8 ± 0.1	11.6 ± 0.5	3.2
Thunnus albacares	14	−17.2 ± 0.2	13.2 ± 1.1	3.1
Lagocephalus lagocephalus	1	−17.2	12.3	3.1
CEPHALOPODS	31	−16.8 ± 0.8	12.6 ± 2.1	
Ancistrocheirus lesueurii	3	−17.4 ± 0.1	12.3 ± 1.1	3.1
Dosidicus gigas	20	−16.0 ± 0.5	13.4 ± 1.9	3.0
Thysanoteuthis rhombus	1	−16.4	11.5	2.9

topic enrichment factor of a consumer in relation to the isotopic values of the prey consumed (1.9 ± 0.4 for δ^{15}N and 1.8 ± 0.3 for δ^{13}C according to Madigan et al. 2012) were used for billfish species. For sharks, we used discrimination factors of 3.7 ± 0.4 for δ^{15}N and 1.7 ± 0.5 for δ^{13}C according to Kim et al. (2012) and Carlisle et al. (2012).

As a measure of trophic width, we calculated the standard corrected ellipse area (SEA_c) for each species (Jackson et al. 2011) by using trophic enrichment factor (TEF)-corrected values (see 'Statistical analysis' below). This metric represents a measure of the total amount of isotopic niche exploited by a particular predator and is thus a proxy for the extent of the trophic niche exploited by the studied species (high values of SEA_c indicate high trophic width). Bayesian inference techniques allow for robust statistical comparisons between data sets with different sample sizes. Isotopic standard ellipse areas were calculated using the SIBER package (Jackson et al. 2011) included in the SIAR library.

Stomach content analysis

Prey items found in the stomach contents of *A. pelagicus* and *C. falciformis* were identified at a minimum taxonomic level using several references. Complete fish and cephalopods were identified from Fischer et al. (1995a,b) and Jereb & Roper (2010). Bones were identified with the help of Clothier (1950) and by comparison to the fish and skeleton collection of the project 'Trophic Ecology of Large Pelagic Species of Ecuador' (Universidad Laica Eloy Alfaro de Manabi). Cephalopod beaks were identified using Wolff (1984) and Clarke (1986). Frequency of occur-

rence (%FO) and numeric and gravimetric methods were used to quantify the diet. %FO was calculated according to the percentage of predators that fed on a certain prey. Number (%N) was the number of individuals of a certain prey relative to the total number of individual prey. Weight (%W) was the weight of a certain prey relative to the total weight of all prey (Cailliet 1976). The index of relative importance, IRI = (%N + %W) × %FO was calculated (Pinkas et al. 1971) and represented as a percentage, %IRI = IRI / SumIRI × 100. The raw data set of prey of *X. gladius*, *M. nigricans* and *I. platypterus*, used to calculate values of the prey-specific IRI (%PSIRI) by Loor-Andrade et al. (2017b), those of *P. glauca* by Loor-Andrade et al. (2017a) and those of the %IRI of *I. oxyrinchus* by Rosas-Luis et al. (2016b), were used to calculate the %IRI values of these species. A quantitative predator–prey network was generated for prey with a %IRI greater than 1%. The network was generated using the Food Web Designer software (Sint & Traugott 2016).

Statistical analysis

To correctly interpret and compare isotopic values among species, we adjusted the δ^{15}N and δ^{13}C values of the individuals by subtracting the specific TEF for sharks (Kim et al. 2012, Carlisle et al. 2012) and billfishes (Madigan et al. 2012). ANOVA and Tukey post hoc tests were used to compare the TEF-adjusted δ^{13}C and TEF-adjusted δ^{15}N values among species. ANOSIM with Bray-Curtis coefficients were applied using %IRI transformed to $\log(x + 1)$ of each prey item in the diet of predators (PRIMER v6.2; www.primer-e.com) (Clarke & Gorley 2001). ANOSIM is a non-parametric and multivariate ANOVA. It was used to test for significant differences between prey items and predators and groups of predator species. Data were permutated 999 times for a distribution to determine the p-value of ANOSIM's R statistic (R = 0 is identical, R = −1 or 1 is most divergent). SIMPER analysis was applied to identify prey species that could discriminate between groups of predators (Clarke & Warwick 2001). ANOSIM and SIMPER were conducted in Primer v7.0.11 (PRIMER-E).

RESULTS

A total of 130 samples of individual muscle tissue were analyzed to obtain stable isotope values

Table 2. Mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values and trophic enrichment factor (TEF)-adjusted $\delta^{15}N$ and $\delta^{13}C$ values of the sharks and billfishes sampled in the pelagic environment of Ecuador. The summary of the ANOVA tests examining variation in TEF-adjusted stable isotopes between species are also indicated. M: male; F: female; I: indeterminate. Length was measured as mean precaudal length for sharks and mean fork length in billfishes. Tukey test results are shown as subscript capital letters; for each species, the means of species with the same letter were not significantly different

Species	n	Length (cm)	Sex			TEF-adjusted		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
			M	F	I	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)		
SHARKS									
Isurus oxyrinchus	19	145.5 ± 19.81	7	1	11	−18.3 ± 0.3	14.8 ± 1.3$_A$	−16.6 ± 0.3	18.5 ± 1.3
Prionace glauca	20	165.7 ± 25.7	8	4	8	−18.3 ± 0.2	14.9 ± 1.8	−16.6 ± 0.2	18.6 ± 1.8
Alopias pelagicus	19	142 ± 25.1	8	4	7	−18.4 ± 0.3	10.1 ± 1.4$_B$	−16.7 ± 0.3	13.8 ± 1.4
Carcharhinus falciformis	13	120.8 ± 16.9	2	4	7	−18.4 ± 0.2	10.9 ± 1.4$_B$	−16.7 ± 0.2	14.6 ± 1.4
BILLFISHES									
Xiphias gladius	20	162 ± 38	0	8	12	−18.3 ± 0.3	12.4 ± 1.1$_C$	−16.5 ± 0.3	14.3 ± 1.1
Istiophorus platypterus	19	188.2 ± 29.9	2	9	8	−18.4 ± 0.3	13.2 ± 0.5$_C$	−16.6 ± 0.3	15.1 ± 0.5
Makaira nigricans	20	238.2 ± 43.3	0	14	6	−18.2 ± 0.2	12.7 ± 1.1$_C$	−16.4 ± 0.2	14.6 ± 1.1
ANOVA test						$F_{6,129} = 1.05$	$F_{6,129} = 34.65$		
p-values						0.41	<0.001		

(Table 2), and 641 stomachs were analyzed to determine food content (Table 3). The mean fork length for billfishes and mean precaudal length for sharks are shown in Table 2. *Makaira nigricans* was the largest fish captured, with a mean length of 238.2 cm.

Isotopic results

TEF-adjusted $\delta^{15}N$ values of muscle differed among species (Table 2), showing higher values in *Isurus oxyrinchus* and *Prionace glauca* followed by *Xiphias gladius*, *Istiophorus platypterus* and *M. nigricans*, with the lowest values in *Alopias pelagicus* and *Carcharhinus falciformis* (Table 2, Fig. 2). In contrast, TEF-adjusted $\delta^{13}C$ values did not differ among species (Table 2, Fig. 2). The niche breadth, measured by the SEA$_c$, was higher in *A. pelagicus* (SEA$_c$ = 1.53), *P. glauca* (1.36) and *I. oxyrinchus* (1.13), followed by *C. falciformis* (0.88), *M. nigricans* (=0.71), *X. gladius* (0.69) and *I. platypterus* (0.44). The niche overlap based on TEF-adjusted $\delta^{15}N$ and $\delta^{13}C$, and the SEA$_c$ between species, showed that *I. oxyrinchus* versus *P. glauca*, *C. falciformis* versus *A. pelagicus*, and *X. gladius* versus *M. nigricans* had the highest overlap (%overlap: 76, 67 and 50% respectively). The lowest overlap was found in *C. falciformis* versus *X. gladius* and *M. nigricans* (23 and 8%), *P. glauca* versus *I. platypterus*, *M. nigricans* and *X. gladius* (16, 10 and 1%), *M. nigricans* versus *I. platypterus* and *I. oxyrinchus* (15 and 6%), *X. gladius* versus *I. platypterus* (7%) and *A. pelagicus* versus *X. gladius* (3%) (Fig. 2).

SIAR and stomach content results

The results of the SIAR, %IRI and SIMPER analyses revealed that for *C. falciformis*, *I. platypterus* and *M. nigricans*, fish were the most important prey group. SIAR and %IRI values showed higher percentages of fish in the diet for the shark *C. falciformis* (SIAR = 0.68, %IRI = 99.66) and for the billfishes *I. platypterus* (SIAR = 0.52, %IRI = 96.2) and *M. nigricans* (SIAR = 0.56, %IRI = 97.05) (Figs. 3 & 4). Based on stomach contents, 3 fish species were identified as the main prey items, with *Auxis* sp. being the main prey item for *I. platypterus* and *M. nigricans*, and *Katsuwonus pelamis* and *Thunnus albacares* the main prey fishes for *C. falciformis* (ANOSIM: global R = 0.88, p < 0.05) (Table 3, Fig. 4). In addition, the SIMPER analysis revealed a similarity of 74.72% in the diet of *M. nigricans* and *I. platypterus*, with *Auxis* sp. contributing 58.88% to the diet and being the most important prey for both species. Stomach contents for *P. glauca*, *A. pelagicus*, *I. oxyrinchus* and *X. gladius* revealed that cephalopods were their main prey (%IRI = 94.31, 65.79, 56.11 and 98.52 respectively) (Table 3). For these species, the importance of cephalopods in the stomach contents was higher than that estimated from the SIAR models (Fig. 3). The diet of *A. pelagicus*, *I. oxyrinchus* and *X. gladius* was similar (57.42% similarity), with *Dosidicus gigas* found as the main prey (93.09% similarity contribution). The diet of *P. glauca* was different from the other species (76% dissimilarity) with *Ancistrocheirus lesueurii* found as the main prey.

Table 3. Percentage of the index of relative importance (IRI) for prey items identified in the stomach contents of large pelagic fishes during 2014 and 2015. 0.0 indicates values <0.1

Prey	*Isurus oxyrinchus* (n = 115)	*Prionace glauca* (n = 181)	*Alopias pelagicus* (n = 34)	*Carcharhinus falciformis* (n = 11)	*Xiphias gladius* (n = 117)	*Istiophorus platypterus* (n = 99)	*Makaira nigricans* (n = 84)
FISH	**43.7**	**3.4**	**34.2**	**99.7**	**1.5**	**96.2**	**97.0**
Aluterus monoceros	0.0						0.0
Auxis sp.	2.8	0.2	1.2	6.3	0.0	88.8	89.1
Auxis thazard						0.0	
Belonidae	0.1					0.3	
Coryphaena hippurus	0.8	0.0					0.0
Decapterus sp.	0.1	0.0					
Etrumeus teres					0.0	0.1	
Katsuwonus pelamis	11.5	0.8		71.7	0.0		2.2
Lagocephalus lagocephalus	2.9	0.0		4.1	0.1	4.7	0.9
Merluccius gayi	0.0		16.1		0.1	0.4	0.0
Mugil cephalus	0.0			1.3	0.0		1.7
Opisthonema libertate						0.0	
Pristigenys serrula	0.0						0.0
Ragalecus glesne	0.1		0.2				
Remora remora	0.0						
Thunnus albacares	0.1			14.2			
Scomber japonicus						0.1	
Scombridae sp.	0.3					0.0	0.0
Unidentified fish	4.3	0.4	0.1	1.2	0.08	2.4	3.4
CEPHALOPODS	**56.1**	**94.3**	**65.8**	**0.3**	**98.5**	**3.8**	**2.9**
Ancistrocheirus lesueurii	5.0	67	0.0		0.0		0.0
Argonauta sp.		0.6			0.0	0.0	
Dosidicus gigas	68.6	4.2	81.6	1.2	93.5	2.9	2.1
Gonatus sp.	0.0	8.9					
Histioteuthis sp.	0.2	0.0				0.0	
Histioteuthis dofleini	0.1	9.9	0.0		0.0		0.0
Histioteuthis heteropsis		0.0					
Hyaloteuthis pelagica	0.0				0.0		
Loligo sp.						0.1	
Mastigoteuthis dentata	0.0	0.1			0.0		
Ommastrephidae sp. 2	0.6	0.0			1.0	0.1	0.4
Ommastrephidae sp. 2	0.0						
Opistoteuthis sp.		0.2					
Octopodoteuthis sp.		0.0					
Octopodoteuthis sicula	0.1	1.1					
Onychoteuthis sp. 1	0.2	0.8					
Onychoteuthis sp. 2		0.4					0.0
Polydoteuthis boschmaii		0.1					
Sthenoteuthis oualaniensis	0.2				0.1	0.0	
Thysanoteuthis rhombus	0.3				4.3	0.0	0.0
Vampyroteuthis infernalis		0.1					
Unidentifified cephalopod	1.3	2.8	0.1		0.7	0.0	0.1
OTHER PREY	**0.2**	**2.2**			**0.0**		**0.0**
Delfinidae	0.1	0.0					
Unidentified mammal	0.1	1.7					
Unidentified prey	0.0	0.5			0.0		0.0

DISCUSSION

In the present study, we provide a global view of the feeding ecology and trophic relationships of 7 marine predators coexisting in the pelagic environment of the Ecuadorian Pacific. We revealed inter-specific differences in the main feeding strategies and similarities in the exploited habitat between species. Moreover, although the use of isotope analysis to investigate the trophic ecology of marine organisms is increasing, as our findings suggested it is necessary to combine both isotopic and stomach
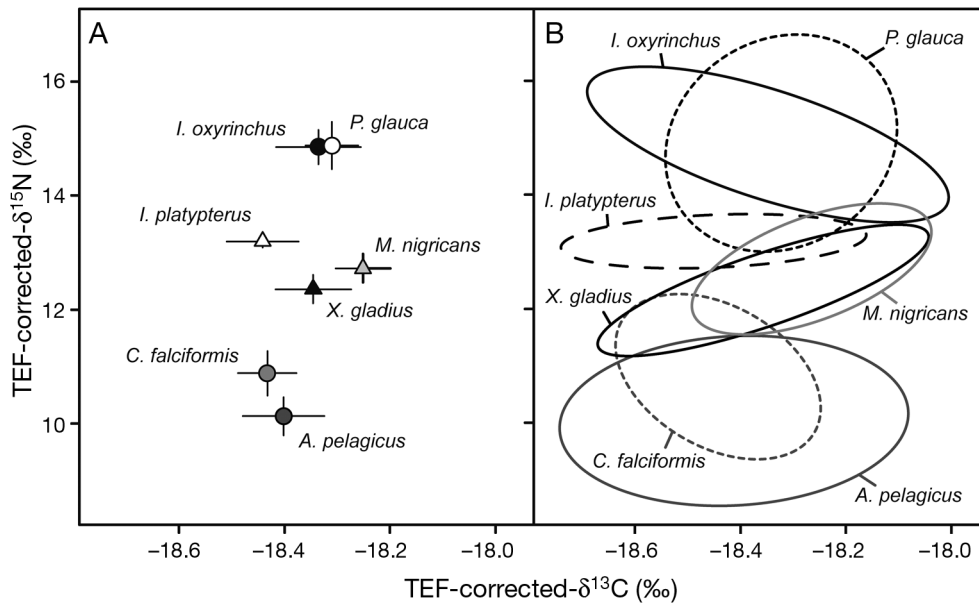
Fig. 2. (A) Mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values and (B) standard ellipse areas of the sharks and billfishes sampled in the pelagic environment of Ecuador adjusted for the trophic enrichment factor (TEF)
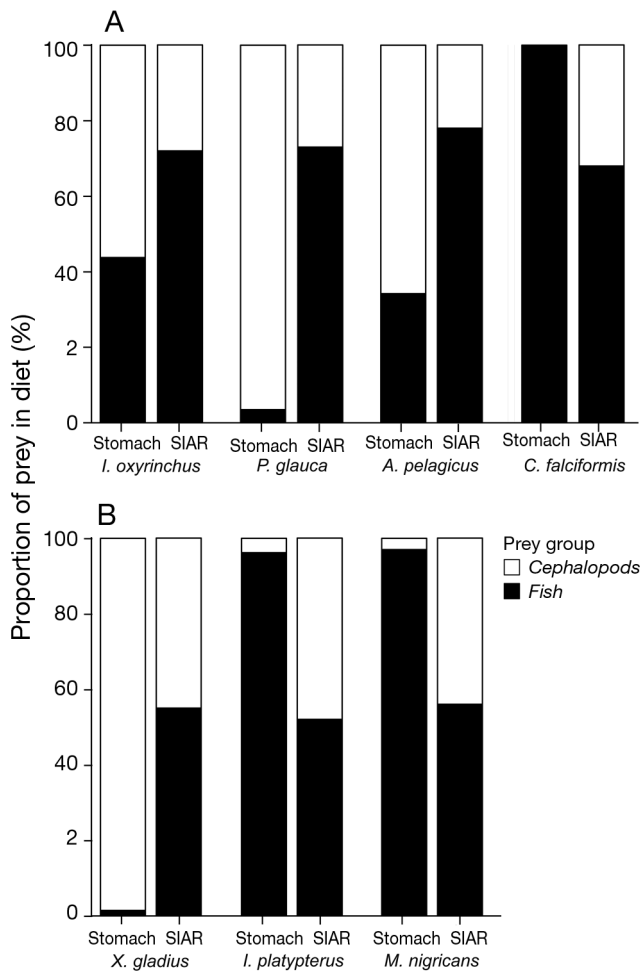

Fig. 3. Mean proportional contribution of fish and cephalopods to the diets of (A) sharks and (B) billfishes sampled in the pelagic environment of Ecuador

content approaches to minimize biases and interpretative mistakes.

The similarity in the $\delta^{13}C$ values between the 7 studied pelagic predators indicated that all of them exploit a similar marine area off the coast of Ecuador. In marine systems, differences in $\delta^{13}C$ values are generally related to the use of productive (close to the coast and upwelling areas) versus unproductive areas (oceanic waters) (France & Peters 1997, Post 2002, Ménard et al. 2007, Carlisle et al. 2015). In our case, these pelagic predators likely share the same waters due to the high productivity associated with the oceanographic conditions in the study area. These conditions favor and homogenize primary production, which directly promotes the concentration and abundance of these species in the fishing area where the artisanal Ecuadorian fishing fleet operates (Bendix & Bendix 2006, Rincón-Martínez et al. 2010). These findings support the hypothesis that the Ecuadorian Pacific is a productive area with a high availability of prey resources able to support a rich marine predator community, including the studied species and marine mammals (e.g. O'Hern et al. 2009, Block et al. 2011, Galván-Magaña et al. 2013). Based on the principle of competitive exclusion, similar species in the same area are expected to coexist in a state of ecological divergence or exclusion (Pianka 2000). However, in some cases, marine predators coexist in the same habitat because they segregate the exploitation of their main prey (Albo-Puigserver et al. 2015, Navarro et al. 2017). Along these lines, based on isotopic and stomach content results, the differences in the feeding strategies
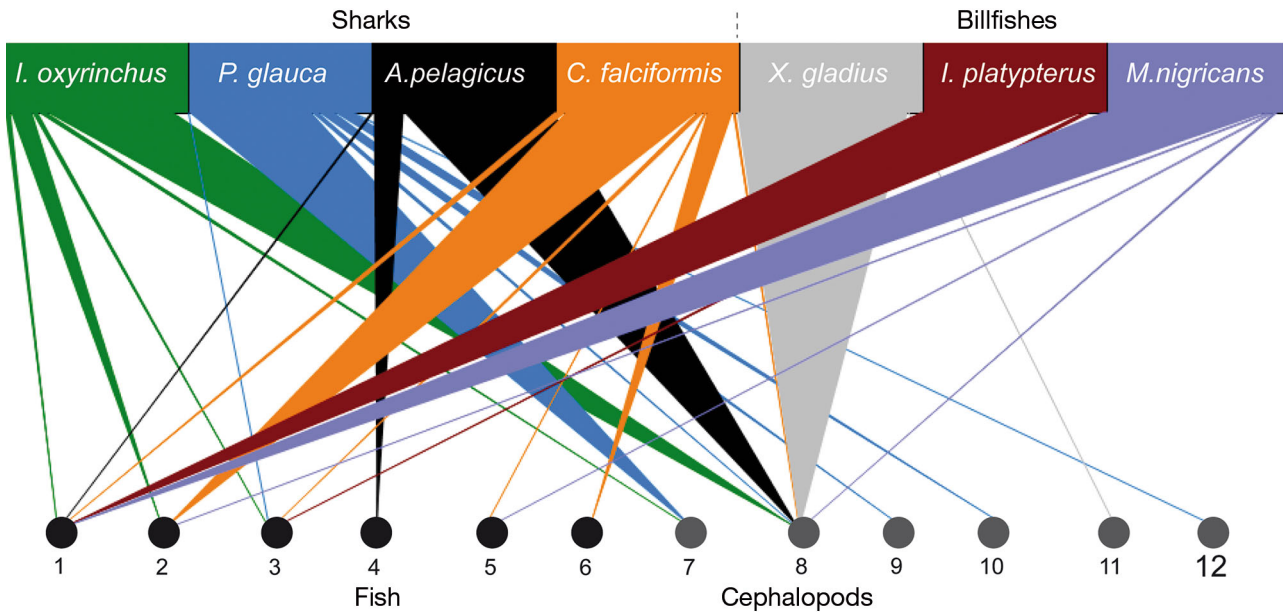
Fig. 4. Quantitative predator–prey network generated from the diet results based on the stomach content analysis of 4 sharks and 3 billfishes sampled in the pelagic environment of Ecuador. Only prey with a %IRI greater than 1% were represented. 1: *Auxis* sp.; 2: *Katsuwonus pelamis*; 3: *Lagocephalus lagocephalus*; 4: *Merluccius gayi*; 5: *Mugil cephalus*; 6: *Thunnus albacares*; 7: *Ancistrocheirus lesueurii*; 8: *Dosidicus gigas*; 9: *Gonatus* sp.; 10: *Histioteuthis dofleini*; 11: *Ommastrephidae* sp; 12: *Octopodoteuthis sícula*. The network was generated by using the Food Web Designer software (Sint & Traugott 2016)

found among species could be a mechanism allowing their coexistence in the same pelagic habitat.

Differences in $\delta^{15}N$ values suggest a segregation of species in the water column and in their use of different feeding resources. For example, prey resource partitioning related to the consumption of prey distributed at different depths has been found for *Isurus oxyrinchus* and *Xiphias gladius*. They fed mainly on the cephalopod *Dosidicus gigas*, a species mostly present at a depth of 1000 m (Nigmatullin et al. 2001). Although we did not estimate prey size, billfishes are known to consume smaller prey than sharks (Ménard et al. 2007, Galván-Magaña et al. 2013), which was reflected in the higher $\delta^{15}N$ values of the shark *I. oxyrinchus* in comparison to the billfish *X. gladius*. On the other hand, the lowest values were found for the sharks *Carcharhinus falciformis* and *Alopias pelagicus*; both species rarely dive to depths greater than 500 m (Bonfil 2008). *C. falciformis* fed mainly on fish while *A. pelagicus* fed on *D. gigas* and demersal fish, such as *Merluccius gayi*. In addition, the voracity and active predation of sharks were demonstrated with the trophic niche measured by the SEA_c. In general, sharks had higher SEA_c values than billfishes associated with the presence of a higher diversity of cephalopods in the diet. Despite their high values of SEA_c overlap, our results from stomach contents and those reported by Galván-

Magaña et al. (2013) and Rosas-Luis et al. (2016a) do not support competition for feeding resources in sharks. SEA_c represents the limit of the $\delta^{15}N$ and $\delta^{13}C$ values of individual predators in the model (Layman et al. 2007, Jackson et al. 2011), but does not identify the prey exploited by consumers. For that reason, the combination of stomach contents for the identification of prey items is necessary, and in this work it allowed us to demonstrate the segregation of *I. oxyrinchus*, which fed on *D. gigas*, and *Prionace glauca*, which fed on *Ancistrocheirus lesueurii*. Without the information from the stomach contents and prey identification, an incorrect assumption of overlap in the diet of these 2 predators would have been made.

Strong trophic niche segregation occurs between billfishes and sharks, with shark species occupying the highest and lowest trophic positions and billfishes occupying intermediate positions. Active teutophagous predators such as *P. glauca*, *I. oxyrinchus* and *X. gladius* showed high $\delta^{15}N$ values, probably related to the influence on stable isotope values of the higher concentrations of ammonia in their main prey, namely squids. Ammonia is the main nitrogen end-product in cephalopods (Boucher-Rodoni & Mangold 1989), and its concentration increases in active squids (Hoeger et al. 1987) that perform vertical migrations to deep waters. In fact, higher values of $\delta^{15}N$ in *I. oxyrinchus*, *P. glauca* and *X. gladius* are

also enforced by the deep and vertical migrations that these species perform for feeding (Sedberry & Loefer 2001, Ibáñez et al. 2004, Vögler et al. 2012), with *D. gigas* and other squid species with deep distributions being their main prey sources (Ibáñez et al. 2004, Kubodera et al. 2007, Preti et al. 2012, Rosas-Luis et al. 2016a,b, Loor-Andrade et al. 2017a,b). The billfishes *Makaira nigricans* and *Istiophorus platypterus* fed on similar prey, with the fish *Auxis* sp. as their main food source. However, this was not supported by the $\delta^{15}N$ values, which showed little overlap. Thus, this result may be explained by the different use of food sources, mainly through the consumption of small *Auxis* sp. by *M. nigricans*. This type of feeding segregation, where a predator feeds on the same but smaller prey compared to other predators, has been described previously in teutophagous whales, sharks and billfishes (Santos & Pierce 2005, Li et al. 2016).

The importance of the main prey groups (fish and cephalopods) of billfishes was similarly indicated by stomach content analysis and SIAR outputs, but this was not the case for sharks. SIAR outputs for sharks showed that fish were the main prey group while stomach content analysis showed that cephalopods were the most important. This is likely because the main hard structure used to identify cephalopods in stomach contents are beaks, and this may lead to overestimations of their importance in the diet due to their accumulation in the stomach over a long period (Galván-Magaña et a. 2013, Rosas-Luis et al. 2016a). Similar contrasting results related to the SIAR outputs were described for the squid *D. gigas* in the north Pacific (Miller et al. 2013). For this large squid, stomach content reports indicated the importance of myctophids and other fishes, but the SIAR outputs showed macrozooplankton as being one of the most important groups. The explanation for the high values of macrozooplankton for *D. gigas* was its voracity and high metabolic rates, promoting the consumption and rapid digestion of this prey, which are difficult to find and identify in stomach contents (Miller et al. 2013). On the other hand, it is important to mention that large pelagic fish are migratory species and could use feeding sources in different areas, which could have different baseline values of $\delta^{15}N$ and $\delta^{13}C$ (Richert et al. 2015). We think that if the pelagic ecosystem off Ecuador is a feeding area for these species, the $\delta^{15}N$ and $\delta^{13}C$ results could be good indicators of their trophic ecology. However, studies on foraging and migratory movements of these species are needed for a better interpretation of stable isotope results and a better understanding of their trophic ecology.

In conclusion, we present new data regarding the feeding ecology of 7 pelagic predators coexisting in the pelagic waters of Ecuador. Dietary analyses from 2 different methodological perspectives indicate that trophic partitioning explains the coexistence of the 7 species in the same area. This is the first time that isotopic values of these predators have been related to stomach content results for the pelagic area off Ecuador and represents an effort to study the ecosystem through the top predators and their trophic relationships. Trophic relationships inferred by the muscle isotopic values and stomach contents of the 7 predators reported in this work suggest that *D. gigas*, *A. lesueurii*, and *Auxis* sp. are key species in the trophic web of this ecosystem. The identification of *D. gigas* as a key prey item in the diet of these predators reveals the need for research on the dynamics and ecology of this squid, especially since a monospecific fishery of this species was established in 2014. Prey–predator regulation in the trophic web may be modified with the removal of these large pelagic fishes. Thus, it is necessary to design a global strategy for the management of these species and promote the sustainability of fisheries in Ecuador. The next step in the study of large predators in Ecuador must be oriented toward the integration of food web models including all ecosystem components.

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