Stable isotope analysis reveals partitioning in prey use by *Kajikia audax* (Istiophoridae), *Thunnus albacares*, *Katsuwonus pelamis*, and *Auxis* spp. (Scombridae) in the Eastern Tropical Pacific of Ecuador

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Kajikia audax, *Thunnus albacares*, *Katsuwonus pelamis*, and *Auxis* spp. occupy high and middle-level trophic positions in the food web. They represent important sources for fisheries in Ecuador. Despite their ecological and economic importance, studies on pelagic species in Ecuador are scarce. This study uses stable isotope analysis to assess the trophic ecology of these species, and to determine the contribution of prey to the predator tissue. Isotope data was used to test the hypothesis that medium-sized pelagic fish species have higher $\delta^{15}N$ values than those of the prey they consumed, and that there is no overlap between their $\delta^{13}C$ and $\delta^{15}N$ values. Results showed higher $\delta^{15}N$ values for *K. audax*, followed by *T. albacares*, *Auxis* spp. and *K. pelamis*, which indicates that the highest position in this food web is occupied by *K. audax*. The stable isotope Bayesian ellipses demonstrated that on a long time-scale, these species do not compete for food sources. Moreover, $\delta^{15}N$ values were different between species and they decreased with a decrease in predator size.

Keywords: Ecuadorian waters, Feeding ecology, Food web, Marine ecology, Pelagic fishes.

Kajikia audax, *Thunnus albacares*, *Katsuwonus pelamis* e *Auxis* spp. ocupam posições tróficas intermedias e/ou elevadas nas cadeias alimentares. Estas espécies representam um importante recurso pesqueiro no Ecuador. Apesar da sua importância económica e ecológica, estudos nestas espécies pelágicas no Ecuador são raras. Este estudo usa isótopos estáveis para avaliar o seu nível trófico de modo a determinar a contribuição das suas presas para os tecidos destes predadores. Dados dos isótopos foram usados para testar a hipótese de que estas espécies de peixes pelágicos possuem valores mais elevados de $\delta^{15}N$ do que daqueles das presas consumidas, e que não existe uma sobreposição entre os valores de $\delta^{13}C$ e $\delta^{15}N$. Resultados mostram que valores mais elevados de $\delta^{15}N$ para *K. audax*, seguidos por *T. albacares*, *Auxis* spp. e *K. pelamis*, indicam que a posição mais elevada na cadeia alimentar é ocupada por *K. audax*. Elipses Bayesianas de isótopos estáveis demonstram que, a uma escala de longo-termo, estas espécies de peixes não competem pelos recursos. Adicionalmente, os valores de $\delta^{15}N$ são diferentes entre espécies de peixe estudadas e estes valores decrescem com a diminuição do tamanho do predador.

Palavras-chave: Águas Equatorianas, Ecologia alimentar, Cadeia alimentar, Ecologia marinha, Peixes pelágicos.

INTRODUCTION

The striped marlin *Kajikia audax* (Philippi, 1887), the skipjack tuna *Katsuwonus pelamis* (Linnaeus, 1758) and the yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788) are pelagic fishes widely distributed in the oceans (Smith, Brown, 2002) and are the most important fishing sources for local and international fishing fleets in Ecuador (Schaefer *et al.*, 2009; Martinez-Ortiz *et al.*, 2015; Tanabe *et al.*, 2017). The capture of these species has an economic value of approximately 73 million US dollars per year for Ecuador (Martínez-Ortiz *et al.*, 2015). These economic gains have promoted the study and development of fisheries, mainly for *T. albacares* (Martinez-Ortiz *et al.*, 2015). For *K. audax*, *K. pelamis* and the small tunas *Auxis* spp., there is a lack of biological and ecological knowledge for Ecuador. Hence, it is necessary to assess the trophic web to detect shifts or impacts in the ecosystem resulting from the extraction of these species and to establish relationships or differences in their trophic strategies.

The pelagic fishes *K. audax*, *T. albacares*, *K. pelamis* and *Auxis* spp. are important components in the ecosystem and facilitate energy transfer between low and top trophic levels because they are preyed on by sharks, fishes, seabirds, and marine mammals (Wang *et al.*, 2003; Arizmendi-Rodríguez *et al.*, 2006; Galván-Magaña *et al.*, 2013; Rosas-Luis *et al.*, 2016; Diop *et al.*, 2018). They are also active predators of fishes, cephalopods, and crustaceans (Alverson, 1963; Loor-Andrade *et al.*, 2017; Rosas-Luis *et al.*, 2017; Varela *et al.*, 2017). In addition, these species are efficient transfers of biomass to other areas and water depths since they are fast-moving species that perform horizontal and vertical movements (Holland *et al.*, 1990).

The study of the trophic ecology of sympatric species in marine environments is achieved by using traditional stomach content analysis, and more recently the analysis of stable isotopes of carbon (denoted as $\delta^{13}C$) and nitrogen (denoted as $\delta^{15}N$) (Peterson,

Fry, 1987). Stable isotope analysis allows the characterization of migratory movements (Wunder, 2012; Segers, Broders, 2015) and is useful for obtaining information about sympatric species (Vanderklift *et al.*, 2006; Cabanillas-Terán *et al.*, 2016). δ^{15}N is an indicator of a consumer's trophic position, as the value in consumer tissues becomes higher compared to their prey (McCutchan *et al.*, 2003; Vanderklift, Ponsard, 2003). δ^{13}C values can indicate primary sources in a trophic network (McCutchan *et al.*, 2003). In marine environments, δ^{13}C values indicate the inshore/pelagic *versus* offshore/benthic contribution to food intake, indicating areas with low and high primary production respectively (Hobson *et al.*, 1994; Cherel, Hobson, 2007; Navarro *et al.*, 2013). The stable isotopes of δ^{15}N and δ^{13}C have been used to study the feeding behavior of large pelagic fishes in the central and north Pacific Ocean (Graham *et al.*, 2007; Acosta-Pachón *et al.*, 2015; Li *et al.*, 2016; Young *et al.*, 2018). In Ecuadorian waters, isotope values showed that sympatric species, such as the billfish *Istiophorus platypterus* (Shaw, 1792), the blue marlin *Makaira nigricans* Lacepède, 1802, and the swordfish *Xiphias gladius* Linnaeus, 1758, do not compet for food sources (Rosas-Luis *et al.*, 2017). The isotope analysis and stomach contents demonstrated that *X. gladius* consumed prey from deeper waters, while *I. platypterus* and *M. nigricans* fed mainly in upper waters (Rosas-Luis *et al.*, 2017). For *Thunnus albacares*, Varela *et al.* (2017) found that stable isotope ellipses had no overlap among size classes and suggested that the prey size increases as the tuna grow. These studies allowed the understanding of the food web; however, it is necessary to include *K. audax*, *K. pelamis*, and *Auxis* spp. in the analysis, to better explain the trophic structure of the ecosystem.

Pelagic fishes are important and abundant components in the marine ecosystem of Ecuador (Martínez-Ortiz *et al.*, 2015), but there is a lack of knowledge related to the trophic role of the sympatric species *K. audax*, *K. pelamis*, and *Auxis* spp. Therefore, this study represents an effort to identify the trophic relationships that these species have in the marine ecosystem off the coast of Ecuador, with a main objective to compare the δ^{15}N and δ^{13}C values of each species found in their muscle tissue. Moreover, we aim to explore the hypothesis that medium-sized pelagic fish species have higher δ^{15}N values than those of the prey they consumed, and that the prey consumed are different for each predator. Our results represent the first attempt to study tissue samples of the fishes *K. audax*, *K. pelamis*, and *Auxis* spp. collected in the fishing ports of Ecuador and analyzed by isotopic analysis.

MATERIAL AND METHODS

Study area. The marine environment off the coast of Ecuador is characterized by warm waters coming from the Equatorial Current System, with the influence of cold waters from the Humboldt Current System (Bendix, Bendix, 2006). High primary production areas are promoted by the convergence of the two current systems off the coast of Ecuador (Bendix, Bendix, 2006; Rincón-Martínez *et al.*, 2010). Fisheries in Ecuador are characterized by two main groups the longline fishery, targeting large and medium pelagic fishes, and a fishery that uses gillnets to capture cephalopods and other fishes (Martínez-Ortiz *et al.*, 2015). The longline fishery works in areas between 37 and 130 km off the Ecuadorian coast in the pelagic environment of oceanic waters and the

gillnets from the shore to 130 km off the Ecuadorian coast (Rosas-Luis *et al.*, 2017).

Samples. *Kajikia audax, T. albacares, K. pelamis* and the group *Auxis* spp. were collected from catches brought to the fishing ports of Playita Mía, Manta, Ecuador and Santa Rosa, Salinas, Ecuador during June 2014 and May 2015 (Fig. 1). The total body length (TL) was recorded to the nearest 10 mm. *Auxis* spp. grouped the frigate tuna, *Auxis thazard* (Lacepède, 1800), and the bullet tuna, *Auxis rochei* (Risso, 1810) since separation by morphological characteristics was not possible. Additionally, the Patagonian squid *Doryteuthis gahi* (d'Orbigny, 1835) and the dart squid *Lolliguncula diomedae* (Hoyle, 1904) were collected in the same fishing ports. A small portion of the dorsal muscle of the caudal peduncle of fishes and the mantle of squids was extracted and stored at –20°C in the laboratory of trophic ecology at the Universidad Laica Eloy Alfaro de Manabí until lipid extraction and isotopic procedures. Furthermore, prey items, collected from the stomach contents of predators reported by Rosas-Luis *et al.* (2017), were taken to characterize their values. Samples included complete individuals of the Peruvian anchovy *Engraulis ringens* Jenyns, 1842, the Peruvian hake *Merluccius gayi* (Guichenot, 1848), the Reinhardt's cranch squid *Liochranchia reinhardti* (Steenstrup, 1856), and the pelagic octopod *Japetella* sp. (Tab. 1).
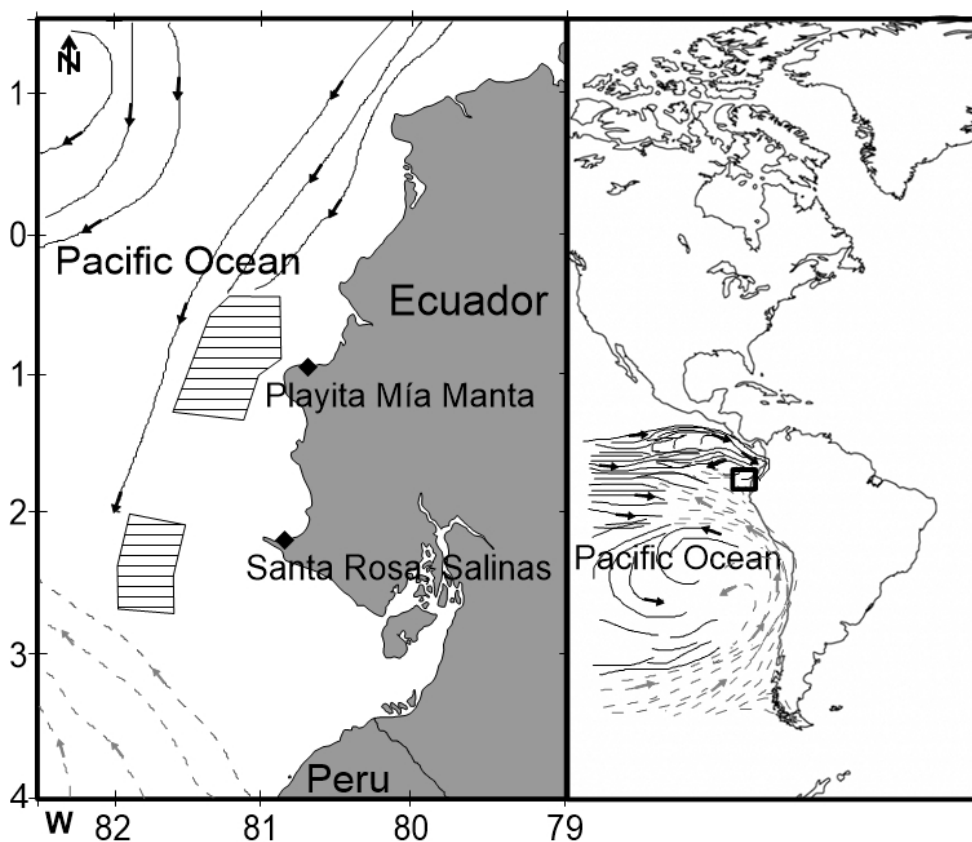

FIGURE 1 | Ecuadorian waters in the Pacific Ocean. Polygons indicate areas where artisanal longline fisheries operate. Black lines represent the flux of warm waters from the Equatorial Current System, and gray dotted lines represent the cold waters from the Humboldt Current System.

TABLE 1 | Mean and standard deviation (SD) of the length, $\delta^{13}C$ and $\delta^{15}N$ values of pelagic fishes and cephalopods sampled in Ecuadorian waters. The length obtained for squids was the dorsal mantle length, and for fishes the total length. * = isotope values were taken from Rosas-Luis *et al.* (2017); these samples were taken at the same time as those in the current work.

Species	n	Length (cm) ±SD	$\delta^{13}C$ (°/$_{oo}$)		$\delta^{15}N$ (°/$_{oo}$)	
Fishes						
Kajikia audax	16	274.8 ±31.46	-16.6	±0.23	14.9	±0.67
Thunnus albacares	14	42.4 ±2.87	-17.2	±0.23	13.2	±1.06
Katsuwonus pelamis	30	39.4 ±3.20	-17.3	±0.46	11.1	±1.52
Auxis spp.	8	28.1 ±0.99	-17.9	±0.33	11.3	±1.20
Scomber japonicus	5*	14.1 ±2.12	-16.9	±0.06	11.7	±0.47
Engraulis ringens	1	15.2	-16.3		12.1	
Lagocephalus lagocephalus	1*	32	-17.2		12.3	
Merluccius gayi	1	45.1	-16.3		11.4	
Pristigenys serrula	1*	19.4	-17.5		11.0	
Opisthonema libertate	5*	18.0 ±1.50	-16.2	±0.16	13.3	±0.25
Cephalopods						
Dosidicus gigas	20*	43.4 ±3.34	-16.0	±0.54	13.4	±1.86
Loligunculla diomedae	2	10	-16.6	±0.71	12.8	±0.3
Ancistrocheirus lesueurii	3*	24.3 ±1.15	-17.4	±0.11	12.4	± 1.08
Liocranchia reindarthi	1	20	-16.9		12.0	
Tysanoteuthis rhombus	1*	45	-16.5		11.5	
Doryteuthis gahi	4	20.2 ±0.50	-15.0	±0.15	11.1	±0.28
Japetella sp.	1	12	-16.1		12.9	

Lipid extraction and isotopic analysis. To avoid biases in the $\delta^{13}C$ values, lipid extraction was applied to all tissue samples (Post *et al.*, 2007). Lipids were extracted from all muscle samples with chloroform and methanol following the protocol of Bligh, Dyer (1959). All samples were then freeze-dried and powdered, with 0.3 to 0.4 mg of each sample packed into tin capsules. Isotopic analyses were performed at the Estación Biológica de Doñana, Spain. Samples were combusted at 1,020°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.

Diet. The diet of *K. audax* was taken from Loor-Andrade *et al.* (2017) and for *T. albacares*, diet information was based on Varela *et al.* (2017). These previous reports used samples from the same area and the same sampling time of this work (Tab. 2). Unfortunately, there were no reports on the feeding habits of *Auxis* spp. and *K. pelamis* in the study area; thus, for increased clarity, fish diets from outside the study area were used for *K. pelamis*, based on Tanabe (2001) (Tropical Western Pacific Ocean), and for *Auxis* spp. based on Siraimeetan (1985) (Tuticorin coast, Gulf of Mannar).

TABLE 2 | Percentage of the index of relative importance (IRI) of pelagic fishes. Data summarized from *Kajikia audax* followed Loor-Andrade *et al.* (2017), *Thunnus albacore* followed Varela *et al.* (2017), *K. pelami* followed Tanabe (2001), and *Auxis* spp. followed Siraimeetan (1985).

Prey/Predator	%IRI			
	Thunnus albacares	*Kajikia audax*	*Auxis* spp.	*Katsuwonus pelamis*
Auxis spp.	90.85	44.30		
K. pelamis		1.90		
Fishes	5.04	37.70	39	
Cephalopods	0.16	5.40	19	0.30
Crustaceans			42	3.54
Fish larvae				96.16

Trophic width. As a measure of trophic width (Jackson *et al.*, 2011), we calculated the corrected standard ellipse area (SEAc) for *K. audax*, *T. albacares*, *K. pelamis*, and *Auxis* spp. This metric represents a measure of the total amount of isotopic niche exploited by a predator and is thus a proxy for the extent of the trophic niche exploited by the studied species (high values of SEAc indicate high trophic width) (Jackson *et al.*, 2011). The corrected standard ellipse area (SEAc) based on the Bayesian ellipse area was proposed as an unbiased metric with respect to the sample size, particularly for the Bayesian method, which incorporates a robust comparison considering uncertainty with smaller sample sizes, resulting in larger ellipse areas. The SEAc was calculated by a covariance matrix of the samples. The sample variance provides an unbiased estimate of the population variance for data x and y, that defines their shape and area (Jackson *et al.*, 2011). The SEAc was fitted using R 3.1.0 for Windows (R Development Core Team, 2017). Isotopic standard ellipse areas were calculated using the SIBER package (Jackson *et al.*, 2011) included in the SIAR library, with R 3.1.0 for Windows (R Development Core Team, 2017). The Niche Overlap Metric was calculated as the probability that an individual from the predator species will be found within the niche of the other predator species with an alpha=0.95 using the nicheROVER routine in R (Swanson *et al.*, 2015).

C and N contributions. *Kajikia audax*, *T. albacares*, *K. pelamis*, and the *Auxis* spp. were used as single species in the isotopic analysis because the number of tissue samples was greater than 7 for each species (Tab. 1). A sample number greater than 7 is

considered adequate for posteriori statistical analysis (Jackson *et al.*, 2011).

The Stable Isotope Analysis in R (SIAR) was used to calculate the proportion of $\delta^{13}C$ and $\delta^{15}N$ isotopes in the diets of the predators (Parnell *et al.*, 2010). The prey species of *K. audax* and *T. albacares* were mixed into composite groups to obtain a high number of isotope values despite their different body size (Tab. 1). Groups were established considering that they were consumed by predators (according to previous reports for the area, Tab. 2). The fish group for *K. audax* and *T. albacares* was composed of *K. pelamis*, *Auxis* spp., *O. libertate*, *Scomber japonicus* Houttuyn, 1782, *E. ringens*, *L. lagocephalus*, *M. gayi*, and *P. serrula*. The cephalopod group for *K. audax* and *T. albacares* was composed of *L. reinhardti*, *Japetella* sp., *Dosidicus gigas*, *T. rhombus*, and *A. lesueurii*. Unfortunately, we had no muscle tissue of prey consumed by *K. pelamis* and *Auxis* spp., thus the contribution of $\delta^{13}C$ and $\delta^{15}N$ for these species was not calculated. The Trophic Discrimination Factor (TDF) used was 1.9 ± 0.4 for $\delta^{15}N$ and 1.8 ± 0.3 for $\delta^{13}C$ related to Pacific bluefin tuna (Madigan *et al.*, 2012), which were the most appropriate for the species in this work.

Statistical analysis. Size, $\delta^{13}C$ and $\delta^{15}N$ differences between species were tested using one-way ANOVA tests, and significant differences ($p \le 0.05$) between pairs of species were identified with a post hoc Tukey test. All tests were performed in the IBM SPSS statistics software v.19 (IBM, 2010).

RESULTS

Isotope values. The mean $\delta^{13}C$ values of *K. audax*, *T. albacares*. *K. pelamis* and *Auxis* spp. ranged between -17.9‰ and -16.6‰ (Tab. 1). The mean $\delta^{13}C$ value of *K. audax* was -16.6‰, higher than those of *T. albacares*, *K. pelamis*, and *Auxis* spp. ($F_{3,59} = 25.69$, $p < 0.05$). *T. albacares* and *K. pelamis* had similar $\delta^{13}C$ values (Tabs. 1–3). The mean $\delta^{15}N$ values ranged between 11.1‰ and 14.9‰ (Tab. 1). Significant differences in the $\delta^{15}N$ values were found between species ($F_{3,59} = 35.73$, $p < 0.05$) (Tabs. 1–3) with *K. audax* showing the highest values. The post hoc Tukey test showed similar $\delta^{15}N$ values for *K. pelamis* and *Auxis* spp. (Tab. 3).

TABLE 3 | Results of the Tukey post hoc test for the comparison of $\delta^{13}C$ and $\delta^{15}N$ values among groups and species and overlap probability. Bold numbers are significant values.

Group	Group	p value		Overlap probability
		$\delta^{13}C$	$\delta^{15}N$	
Kajikia audax	*Thunnus albacares*	**0.00**	**0.00**	31.9
	Katsuwonus pelamis	**0.00**	**0.00**	35.6
	Auxis spp.	**0.00**	**0.00**	0.4
Thunnus albacares	*Katsuwonus pelamis*	0.87	**0.00**	71.8
	Auxis spp.	**0.00**	**0.00**	23.4
Katsuwonus pelamis	*Auxis* spp.	**0.00**	0.99	46.1

Trophic width. The broadest SEAc was observed for *K. pelamis* (1.6), followed by *Auxis* spp. (1.4) (Fig. 2). Narrow SEAcS were recorded for *K. audax* (0.51) and *T. albacares* (0.7). A high overlap probability was found between *T. albacares* and *K. pelamis* (71.8%) (Tab. 3; Fig. 2). Moderate overlap probability was found among *K. audax*, *T. albacares* and *K. pelamis*, *T. albacares* and *Auxis* spp., and *K. pelamis* and *Auxis* spp. (Tab. 3; Fig. 2). Low overlap probability was found between *K. audax* and *Auxis* spp. (0.4%) (Tab. 3; Fig. 2).

δ¹³C and δ¹⁵N contribution of prey groups in the diet. The results of the SIAR analysis showed that fishes were the most important $\delta^{13}C$ and $\delta^{15}N$ contributors (up to 87%) for *T. albacares*, while cephalopods were the most important contributors for *K. audax* (up to 53%) (Fig. 3). The summary of the diet reports based on stomach contents indicated that fishes represent 95.8% of the diet of *T. albacares* and 83.9% of the diet of *K. audax*, with cephalopods being the second most represented group, but significantly less important (4.2 and 16.1%, respectively; Fig. 3).
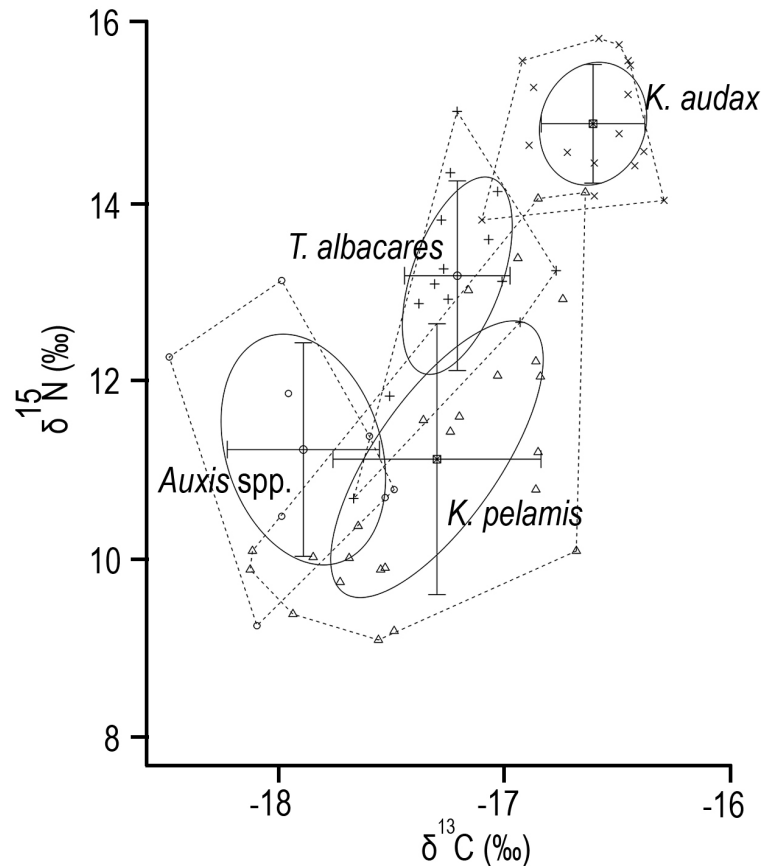

FIGURE 2 | Mean and standard deviation of δ¹⁵N and δ¹³C values and corrected standard ellipse areas (SEAc) based on δ¹³C and δ¹⁵N values of pelagic fish species off the coast of Ecuador. Symbols represent the individual organisms: triangles are for *Katsuwonu pelamis*, circles are for *Auxis* spp., crosses are for *Thunnus albacares* and X's are for *Kajikia audax*.
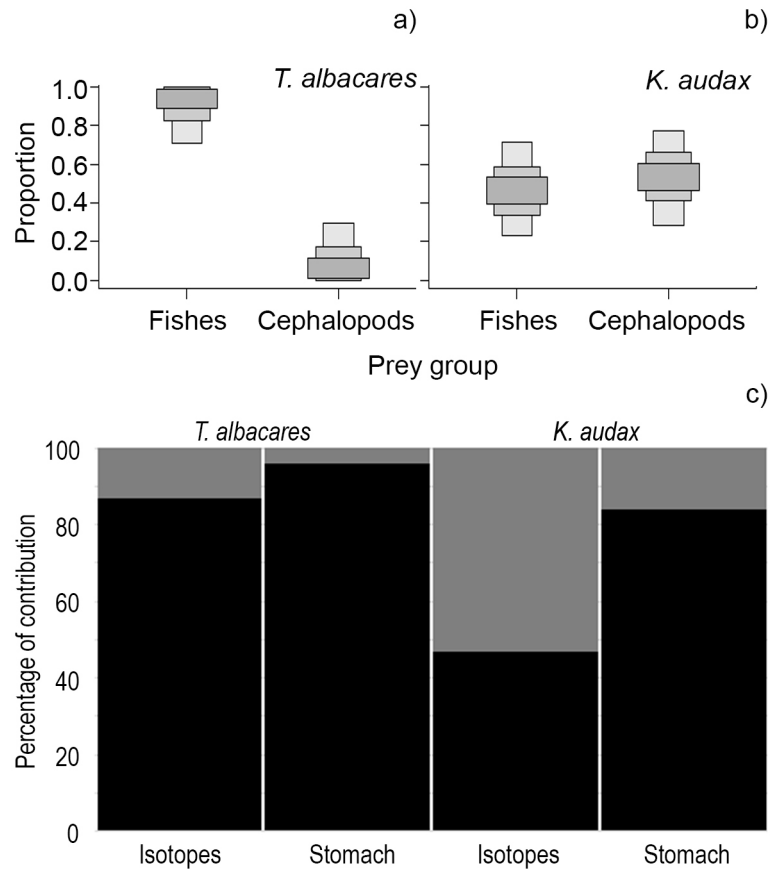
FIGURE 3 | Modeled proportion of prey groups in the diet of **A.** *Thunnus albacares* and **B.** *Kajikia audax* obtained using a stable isotope analysis in R. **C.** is the comparison between the mean proportional contribution of fish (black area) and cephalopods (gray area) to the diets of *T. albacares* and *K. audax*, left bars were based on $\delta^{15}N$ and $\delta^{13}C$ isotopes, and right bars were based on stomach content identification reported by Varela *et al.* (2017) for *T. albacares*, and Loor-Andrade *et al.* (2017) for *K. audax*.

DISCUSSION

In this study, isotope analysis allowed the identification of the trophic width and overlap between *Auxis* spp., *K. pelamis*, *T. albacares*, and *K. audax*. The ellipse metrics provided quantitative and integrated information about sources and niche breadth (Boecklen *et al.*, 2011), contributing to the ecological knowledge of pelagic and commercial species in the marine ecosystem of Ecuador. The $\delta^{13}C$ values suggest that *K. audax* has a different trophic strategy, probably consuming prey from a trophic chain based in high productivity areas, while *Auxis* spp. may be moving to low productivity areas and consuming different prey sources. *T. albacares* and *K. pelamis* had similar $\delta^{13}C$ values, thus indicating that they coexist in the same areas. Based on these results, the discussion is focused on explaining the trophic strategy and interactions of these sympatric species.

The highest $\delta^{13}C$ values were recorded for *K. audax*, followed by *T. albacares* and *K. pelamis*. On the one hand, the highest $\delta^{13}C$ values were related to high productivity

ocean areas (France, Peters, 1997; Ménard *et al.*, 2007; Carlisle *et al.*, 2014), coinciding with marine areas where fishing activity in Ecuador occurs (Martinez-Ortiz *et al.*, 2015). These species have been described as fishery sources with high abundances in waters where upwelling events favor the enrichment of primary production, such as the Humboldt Current and the Gulf of California (Stock *et al.*, 2017). For the Ecuadorian waters, these species are usually found in catches close to the coast (Martinez-Ortiz *et al.*, 2015). On the other hand, low values of δ13C in *Auxis* spp., compared to those of the other species, could be the result of feeding habits related to pelagic and open waters, affecting the signal in the muscle samples. *Auxis* spp. and other scombrid fishes, including *T. albacares* and *K. pelamis*, are fast-moving species in interior as well as more distant coastal waters (Holland *et al.*, 1990; Schaefer *et al.*, 2009), resulting in a different feeding strategy consuming small pelagic fishes, such as *S. japonicus* and myctophids, and pelagic crustaceans (Varela *et al.*, 2017), that could be available in and outside of the studied area. The trophic width as indicated by the SEAc showed that the fishes *Auxis* spp. and *K. pelamis* had broader isotopic ellipse areas, and that *K. audax* had the narrowest area. The consumption of similar prey by predators was confirmed by the isotope values and niche overlap probability between *Auxis* spp. and *K. pelamis* (46%). Nevertheless, it is necessary to identify the potential prey of these species. If they are voracious and active predators, the results will show a wide range of prey as observed in squids of similar size (Rosas-Luis *et al.*, 2014).

The highest δ15N values were recorded for *K. audax* and *T. albacares*, and the lowest were found in *K. pelamis* and *Auxis* spp. These values agree with the assumption that the increase in δ15N values results from prey ingestion, because the type and size of prey consumed affect the δ15N values in the predator tissue. The consumption of large prey increases the δ15N values (Post, 2002; Hussey *et al.*, 2014). The largest predator in this study was *K. audax,* which consumes large prey such as *K. pelamis* and *Auxis* spp. (Loor-Andrade *et al.*, 2017). The δ15N values allowed the comparison of *K. audax* with top predators and *T. albacares* with mid-level predators, which corresponds to the trophic position calculated for *T. albacares* in Ecuadorian waters (Varela *et al.*, 2017) and for *K. audax* in the north Pacific Ocean (Torres-Rojas *et al.*, 2013). The lowest level position was found in *K. pelamis* and *Auxis* spp.

As top predators, *K. audax* and *T. albacares* segregate from the other species, as suggested by the stomach contents and isotope results. They share food resources, with *K. audax* feeding mainly on *Auxis* spp. and other fishes and cephalopods, and *T. albacares* feeding mainly on *Auxis* spp. (Varela *et al.*, 2017). For *T. albacares* and *K. pelamis,* a higher overlap probability was recorded; thus, it can be suggested that these species also share food resources in Ecuadorian waters. However, a comparison of the δ15N values indicates that *T. albacares* had higher δ15N values than *K. pelamis.* Thus, they could consume prey that are located in the same area, but of different sizes (large prey for *T. albacares*), as was reported for these species in the Gulf of California (Alatorre-Ramírez *et al.*, 2017). The isotope and stomach content results are complementary because the isotope values support the evidence of prey contribution, taking into account the turnover rate of muscle tissue (several months) (Madigan *et al.*, 2012; Vander-Zanden *et al.*, 2015) and the estimated diet with stomach content identification, hours or days depending on the prey tissue (Olson, Boggs, 1986; Acosta-Pachón, Ortega-García, 2019). Thus, these results highlight the importance of *Auxis* spp. in the diets of *T. albacares* and *K. audax*,

and cephalopods in the diet of *K. audax*. In Ecuadorian waters, *K. audax* and *T. albacares* seem to be opportunistic predators that feed on available and abundant species. The fishes *Auxis* spp. could be abundant in the area because they have been reported as components in the diet of top predators (Loor-Andrade *et al.*, 2017; Rosas-Luis *et al.*, 2017; Varela *et al.*, 2017). More descriptions of the feeding habits of marine species are needed to corroborate the trophic relationships in the ecosystem, and other species such as *Auxis* spp. and *K. pelamis* should be included in the analysis.

In conclusion, our results suggest that the $\delta^{13}C$ values of *T. albacares* and *K. pelamis* overlap, indicating that they share similar foraging areas or a similar trophic strategy. Their $\delta^{15}N$ values allowed the categorization of the food web; the highest position in the food web was occupied for the large species *K. audax* and middle trophic positions for *T. albacares*, *K. pelamis*, and *Auxis* spp. confirming the hypothesis that medium-sized pelagic fish species accumulate $\delta^{15}N$ isotopes according to the size of prey consumed (large predators consumed larger prey than medium-sized predators). In addition, the different predator size allows the use of the same habitat by partitioning in the prey consumed by each predator. Considering these results and the fact that the fishes *K. audax*, *T. albacares* and *K. pelamis* are important for fisheries in Ecuador, it is necessary to identify the impact that fisheries have on natural populations. The SEAc of *Auxis* spp. could be related to our analysis of two species as a single group, which likely do not have similar feeding habits. Unfortunately, it was not possible to segregate the two species during the morphological identification, and no stomach content samples were taken. Thus, future research will require the use of genetic and morphological methods to separate the two species and to continue trophic ecology studies of all species caught in fisheries to better understand the food web of the marine ecosystem.

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