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Trophic shift in the diet of the pelagic thresher shark based on stomach contents and stable isotope analyses

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Abstract

The trophic ecology of the pelagic thresher shark (*Alopias pelagicus*) was analysed using stomach contents and carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis. The sharks were caught in Ecuadorian waters between June and December 2003. Approximately 24 prey species were found in the stomachs of 111 sharks. The most common species were *Dosidicus gigas*, *Benthosema panamense* and *Sthenoteuthis oualaniensis*. Mean (\pm SD) muscle isotope values of the pelagic thresher shark were $-16.0 \pm 0.3\text{‰}$ for $\delta^{13}\text{C}$ and $13.7 \pm 1.1\text{‰}$ for $\delta^{15}\text{N}$; mean (\pm SD) vertebrae values were -16.7 ± 2.0 for $\delta^{13}\text{C}$ and 9.4 ± 1.8 for $\delta^{15}\text{N}$. There were no significant sex-related differences in muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; however, female vertebrae had significantly higher mean (\pm SD) $\delta^{13}\text{C}$ values ($-16.4 \pm 2.0\text{‰}$) than males ($-18.8 \pm 0.3\text{‰}$). Inter-individual variation in $\delta^{15}\text{N}$ values was observed in both tissues and was likely related to foraging in different ecosystems with distinct isotope values. Both stomach content and isotope data suggest that the pelagic thresher shark is a specialist predator in Ecuadorian waters. In general, little is known about the foraging ecology and movement patterns of this globally distributed but elusive top marine predator, and how these fundamental ecological traits vary among populations. By using two independent but complementary approaches, our study addresses these questions for the pelagic thresher sharks in Ecuadorian waters. Such information is critically important for the design of effective management strategies for these highly exploited but poorly understood predators.

Key words: *Alopias pelagicus*, diet, Ecuador, sharks, stable isotopes

Introduction

The pelagic thresher shark *Alopias pelagicus* Nakamura, 1935 inhabits oceanic and coastal areas throughout the tropical Indo-Pacific and Eastern Pacific. This shark is found at depths from the surface to 150 m (Compagno 2001). Important abiotic factors, such as temperature and oceanic currents, influence its distribution (Dingerkus 1987). This species is especially vulnerable to over-exploitation because its habitat occurs within the range of several gillnet and longline fisheries (Watts & Wu 2005; Lack & Sant 2008) and is subject to high levels of by-catch mortality from tuna fisheries in the Gulf of California, Red Sea, and probably

Southeast Asia (Clarke et al. 2006). In Ecuador, marine resources are widely exploited with commercial landings ranging from 5000 to 11,000 t per year that include large top predators, including billfish, dolphinfish and sharks. The pelagic thresher shark represents $\sim 36\%$ of the total Ecuadorian catch of sharks; more than 150,000 pelagic thresher sharks were caught between 2003 and 2006 (Martínez-Ortiz et al. 2007). This trend is not limited to Ecuador, as overfishing has reduced the population size of this species worldwide and its slow growth rate limits the potential for population recovery. Pelagic thresher sharks are currently listed as vulnerable by the International Union for the Conservation of Nature (IUCN; Dulvy et al. 2008).

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Little is known about the life history and diet of the pelagic thresher shark. The few biological studies include research on reproduction and growth rates (Otake & Mizue 1981; Liu et al. 1998; Romero-Caicedo 2007; Salomon-Aguilar et al. 2009). Its diet, based on the stomach contents of 20 pelagic thresher sharks caught during Japanese fishery surveys in the eastern tropical Pacific Ocean, found that small midwater fishes of the families Paralipididae, Phosichthyidae and Gempylidae were the dominant dietary components (Moteki et al. 2001). These sharks were captured in a limited area near the Galapagos Islands, and the sample size ($n=20$) was small. Polo-Silva et al. (2009) and Calle (2010) describe the diet of pelagic thresher sharks ($n=94$ and $n=62$, respectively) caught during the rainy season (January to June) in Ecuadorian waters and found a high degree of diet overlap between sexes and maturity stages; the main prey were squids (*Dosidicus gigas*, Orbigny, 1835 and *Sthenoteuthis oualaimiensis* Lesson, 1830) and fish (*Benthoosema panamense* Täning, 1932).

These dietary studies are based on stomach content analysis (SCA), which represents a snapshot of ingested food in the days prior to death (Cortés 1999; Vaudo & Heithaus 2011; Kim et al. 2012a). SCA yields information on dietary diversity, but may not be representative of dietary preferences over longer timescales unless intensive sampling is possible. A complementary technique to traditional SCA is stable isotope analysis (SIA), which can yield dietary (and movement) information integrated over a variety of timescales, depending on the type of tissue that is analysed (DeNiro & Epstein 1978; Fry & Parker 1979; Martínez del Rio et al. 2009). Nitrogen isotope values ($\delta^{15}\text{N}$) are typically used to estimate trophic levels because consumers are isotopically enriched in comparison to their common prey by 3–5‰ (DeNiro & Epstein 1981; Bearhop et al. 2004; Post 2002). Thus, $\delta^{15}\text{N}$ of each animal becomes an index for trophic position relative to the $\delta^{15}\text{N}$ of the known trophic position of a primary producer or consumer (Vander-Zanden et al. 1997). In contrast, values of $\delta^{13}\text{C}$ remain relatively unaffected by trophic level and increase, on average, by only $\sim 1\text{‰}$ per trophic step from phytoplankton to apex predators. Thus, carbon isotopes are commonly used as an indicator of ‘baseline’ shifts in isotope values that occur at the base of the food web over space and time (DeNiro & Epstein 1978; Créach et al. 1997; Peterson & Whitfield 1997; Newsome et al. 2010). In marine ecosystems, $\delta^{13}\text{C}$ values of top predators have been used to assess high versus low latitude and inshore versus offshore habitat exploitation (Hobson et al. 1994; Kelly 2000; Newsome et al. 2010).

For large, slow-growing ectotherms like sharks, the isotopic signature in muscle tissue most likely provides information about the food assimilated by an individual over the past 1–2 years prior to death (MacNeil et al. 2006; Hussey et al. 2010, 2012; Logan & Lutcavage 2010; Kim et al. 2012a; Malpica-Cruz et al. 2012). Accretionary tissues that are metabolically inert and contain growth layers, such as vertebrae or teeth, can record a chronology of ecological information over the lifetime of a single individual (Estrada et al. 2006; Kerr et al. 2006; Newsome et al. 2010; Hussey et al. 2012). In many shark species, including the white shark (*Carcharodon carcharias* Linnaeus, 1758), the blue shark (*Prionace glauca* Linnaeus, 1758) and the silky shark (*Carcharhinus falciformis* Müller & Henle, 1839), a single growth layer in vertebrae represents one year of growth and has been used to accurately age individuals (Estrada et al. 2006; Blanco-Parra et al. 2008; Sánchez-de Ita et al. 2011). Overall, SIA of different tissues (e.g. muscle and vertebrae) collected from the same individual can provide an enhanced perspective of the ecology of marine top predators over a range of timescales.

Combining isotopic analysis with information on diet gleaned from stomach contents can help establish the trophic position and diet composition of top marine consumers (Hansson et al. 1997; Fantle et al. 1999; Jardine et al. 2003). The purpose of this study was to examine the trophic structure among different age and sex classes of the pelagic thresher sharks in the Ecuadorian Pacific through use of SCA and SIA. We expected that pelagic thresher sharks in this region would not show sex or maturity stage-related differences in their isotopic composition because previous studies based on stomach content analysis suggest a high degree of individual dietary overlap (Polo-Silva et al. 2009; Calle 2010). We also estimated the trophic position of this predator using both methods (SCA and SIA) and compared our results with previous dietary information obtained for this and some closely related species. Finally, we compared muscle and vertebral isotope values because these two tissues represent different timescales (Martínez del Rio et al. 2009; Hussey et al. 2012) and may be useful for examining shifts in individual diet composition or habitat fidelity over time.

Materials and methods

The pelagic thresher sharks were landed at the port of Manta, Ecuador ($0^{\circ}56'59.25''\text{S}$, $80^{\circ}42'33.14''\text{W}$) between June and December 2003. The actual location of catch of each individual is unknown, but within 160 km of Manta. Approximately 5–10 sharks were sampled every week depending on the

catch. Total length (cm) was measured and the sex and maturity (mature/immature) of each shark was recorded. Length, calcification, and rotation of claspers, and the presence/absence of semen, were used to determine maturity for males. Mature males have a completely calcified clasper with 360° rotation, an open rhipiodon and semen. Immature males lacked these features. Mature females showed copulation marks, with the entrance of the cloaca at least 3 cm in diameter, and ovaries weighed more than 100 g. Pregnant and mature females were differentiated by the presence of eggs or embryos in both uteri (Liu et al. 1998; Romero-Caicedo 2007).

Stomach content analysis

In the field, stomach contents were stored in plastic bags on ice and then frozen until laboratory analysis. Identification of prey depended on the stage of digestion. For fish that were almost completely digested, we used axial skeletons and otolith keys (Clothier 1950; García-Godos 2001). Fishes at an initial stage of digestion were identified using Fischer et al. (1995) and Chirichigno & Vélez (1998). Crustaceans and other remains were identified using Garth & Stephenson (1966). Cephalopod mandibles (beaks) were identified according to Clarke (1986) and Wolff (1984) and were also used to extrapolate the weight and mantle length of each squid species.

To determine if the number of stomachs collected were enough to represent the diet of the pelagic thresher shark, we used the procedure suggested by Ferry et al. (1997) and applied by Páez-Rosas et al. (2010), Baque-Menoscal et al. (2012) and Moreno-Sánchez et al. (2012). The method involves calculating the accumulated mean and standard deviation of prey diversity curves using the Shannon–Wiener H Index (Krebs 1999). Diversity curves were generated using a script written in MATLAB, with 500 random permutations (Adams 2004). The permuted values for each accumulated sample size were then used to calculate the coefficient of variation (CV). The sample size where CV decreased below 0.05 was considered as an indicator of the diversity curve, reached an asymptote, and provided an assessment of the appropriate sample size required for characterizing the diet of the species. Additionally, we used the method proposed by Bizzarro et al. (2007) to assess objectively whether the curve had reached an asymptote. Specifically, we used linear regression to fit a line to the last 4 points from the diversity accumulation curve and determined if its slope was significantly different from a line with a slope of zero. If the slope of this line did not differ significantly from zero, then the diversity accumulation curve was deemed to have reached an asymptote.

Three indices were used to quantify the relative importance of prey species: (1) percentage by number, (2) percentage by weight and (3) percentage by frequency of occurrence (Hyslop 1980). These data were used to determine the Index of Relative Importance (IRI; Pinkas et al. 1971). To standardize the IRI to percentage (%), the IRI was calculated for each prey type and divided by the total IRI for all prey items (Cortés 1997). To determine whether the sharks demonstrate different degrees of resource use, we used Levin's standardized index (B_i ; Krebs 1999), which measures the species' trophic breadth to distinguish between specialists and general consumers.

$$B_i = 1/n - 1 \left\{ \left(1 / \sum P_{ij}^2 \right) - 1 \right\}, \quad (1)$$

where P_{ij}^2 is the ratio of prey i in the diet of predator j and n is the total number of prey species in the diet. To observe the effect of sex and maturity stage on dietary composition, we used a one-way non-parametric permutation multivariate analysis of variance (PERMANOVA), using Bray–Curtis distances (Anderson 2001). This method allows multivariate data to be analysed on the basis of any distance or dissimilarity measure of choice, with P values obtained using permutations. A principal coordinates analysis (PCoA) was used to visualize diet data. PCoA produces an ordination equivalent to a principal components analysis (PCA) when a Bray–Curtis similarity matrix is used as the basis of the analysis (Braccini 2008; Mendonça 2009; Fanelli & Cartes 2010).

As we found no differences in stomach contents between maturity stages in either sex, based on PERMANOVA (Supplementary material Table SI), stomach content data were grouped by sex and maturity for all analyses.

Stable isotope analysis

Approximately 5 g of white muscle was removed from the dorsal anterior section of each shark. The samples were oven dried at 60°C for 24 h. Lipids were then extracted from each muscle sample with ~25 ml 1:1 chloroform:methanol following the microwave-assisted extraction protocol (MARS 5, CEM). Samples were then placed in a drying oven at room temperature for 12 h to allow all solvents to evaporate (Renoe 1994).

We used a low-speed cutting drill to extract samples of material from vertebrae for SIA. Samples from vertebrae of immature sharks represent an average of all growth bands present in the vertebrae. For mature sharks, we sampled material from the outer edge of the vertebra (~1 cm) that represents

the most recent period of growth and approximately represent the last three years of life prior to death (Liu et al. 1999; Supplementary material Figure S1). Approximately 3–4 mg of vertebral powder was demineralized using ~1.5 ml EDTA to remove the inorganic carbon (Kerr et al. 2006; Kim & Koch 2012). Pre-treated samples of muscle and vertebrae were ground and homogenized in an agate mortar; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined at the Carnegie Institution of Washington, using an elemental analyser (NC 2500, Carlo-Erba) interfaced with a Finnigan Delta Plus XL isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Isotopic results were expressed as δ values, $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratios of the sample and standard, respectively. The standards were Vienna-Pee Dee Belemnite limestone for carbon and atmospheric N_2 for nitrogen. The units are expressed as parts per thousand (per mil, ‰). The within-run standard deviation of an acetanilide standard was $\leq 0.2\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The weight-percentage C:N ratios of each tissue sample were in the expected range (2.9–3.3 for muscle and 2.8–3.4 for vertebrae)

for pure protein, indicating that lipid extraction and demineralization were sufficient (Post 2007; Matich et al. 2010; Kim et al. 2012b; Hussey et al. 2012).

Data analyses

Normality was assessed by the Kolmogorov–Smirnov–Lilliefors test and variance homogeneity by the Bartlett test. If the data conformed to a normal distribution for both isotopes, we used a Student's t -test to assess for significant differences in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between sex and maturity. If the data were not normally distributed, we used a non-parametric Mann–Whitney U test.

Estimation of trophic level (TL) was based on two models, depending on the type of data. When using stomach contents, we used the model proposed by Cortés (1999).

$$TL = 1 + \left(\sum_{j=1}^n P_j \times TL_j \right) \quad (2)$$

where P_j is the proportion of each prey category j in the diet of the predator, TL_j is the trophic level of each prey category j and n is the total number of prey species in the diet. TL estimates were calculated using

Table I. Prey species of pelagic thresher sharks in Manta, Ecuador, expressed in absolute and percent values by number (N), weight (W), frequency of occurrence (FO) and index of relative importance (IRI). Morphotypes 2 and 3 belong to the family Ophichthidae; morphotype 4 belongs to the family Serranidae. Trophic level (TL) for each prey was taken from Pauly et al. (1998), Cortés (1999) and Dambacher et al. (2010).

Prey species	Number	%N	Weight(g)	%W	FO	%FO	%IRI	Habitat	TL
Teleosts									
<i>Ophichthus</i> sp.	1	0.07	172	0.19	1	1.18	<0.1	Benthic	2.4
Morphotype 2	3	0.22	460.9	0.52	2	2.35	<0.1	Benthic	2.4
Morphotype 3	2	0.15	27.7	0.03	2	2.35	<0.1	Benthic	2.4
<i>Sardinops sagax</i>	13	0.95	69.4	0.08	4	4.71	0.1	Epipelagic	2.7
<i>Synodus</i> sp.	1	0.07	17.6	0.02	1	1.18	<0.1	Benthic	3.4
<i>Bentosema panamense</i>	1095	79.7	1500.3	1.69	30	35.29	30	Mesopelagic	3.0
<i>Symbolophorus evermanni</i>	1	0.07	7.4	0.01	1	1.18	<0.1	Mesopelagic	3.2
<i>Merluccius gayi</i>	20	1.46	429	0.48	6	7.06	0.1	Benthic	4.2
<i>Paralabrax callaensis</i>	1	0.07	316.4	0.36	1	1.18	<0.1	Coastal	4.0
Morphotype 4	1	0.07	83	0.09	1	1.18	<0.1	Coastal	4.0
<i>Larimus argenteus</i>	3	0.22	79.8	0.09	2	2.35	<0.1	Coastal	3.1
<i>Auxis thazard</i>	26	1.89	1173.1	1.32	13	15.29	0.5	Epipelagic	4.3
<i>Gempylus serpens</i>	1	0.07	12	0.01	1	1.18	<0.1	Mesopelagic	4.2
Lutjanidae	1	0.07	60	0.07	1	1.18	<0.1	Coastal	4.0
<i>Brama japonica</i>	1	0.07	35.9	0.04	1	1.18	<0.1	Epipelagic	4.1
<i>Lagocephalus lagocephalus</i>	1	0.07	100	0.11	1	1.18	<0.1	Epipelagic	2.6
Cephalopods									
<i>Dosidicus gigas</i>	149	10.84	71,742.3	80.61	58	68.24	66	Mesopelagic	4.0
<i>Sthenoteuthis oualaniensis</i>	22	1.6	12,697.0	14.27	15	17.65	2.9	Mesopelagic	3.2
<i>Pholidoteuthis boschmaii</i>	2	0.15	0	0	1	1.18	<0.1	Mesopelagic	3.2
<i>Abraliopsis affinis</i>	1	0.07	12	0.01	1	1.18	<0.1	Mesopelagic	3.4
<i>Ancistrocheirus lesueurii</i>	3	0.22	0.5	<0.01	3	3.53	<0.1	Mesopelagic	3.4
<i>Mastigoteuthis dentata</i>	6	0.44	0.3	<0.01	3	3.53	<0.1	Batipelagic	3.2
<i>Loliopsis diomedae</i>	13	0.95	3.1	<0.01	1	1.18	<0.1	Coastal	3.2
Octopoda	6	0.44	–	–	1	1.18	–	Benthic	3.8
Crustaceans									
<i>Pennaeus stylirostris</i>	1	0	2	<0.01	1	1.18	<0.1	Benthic	2.6

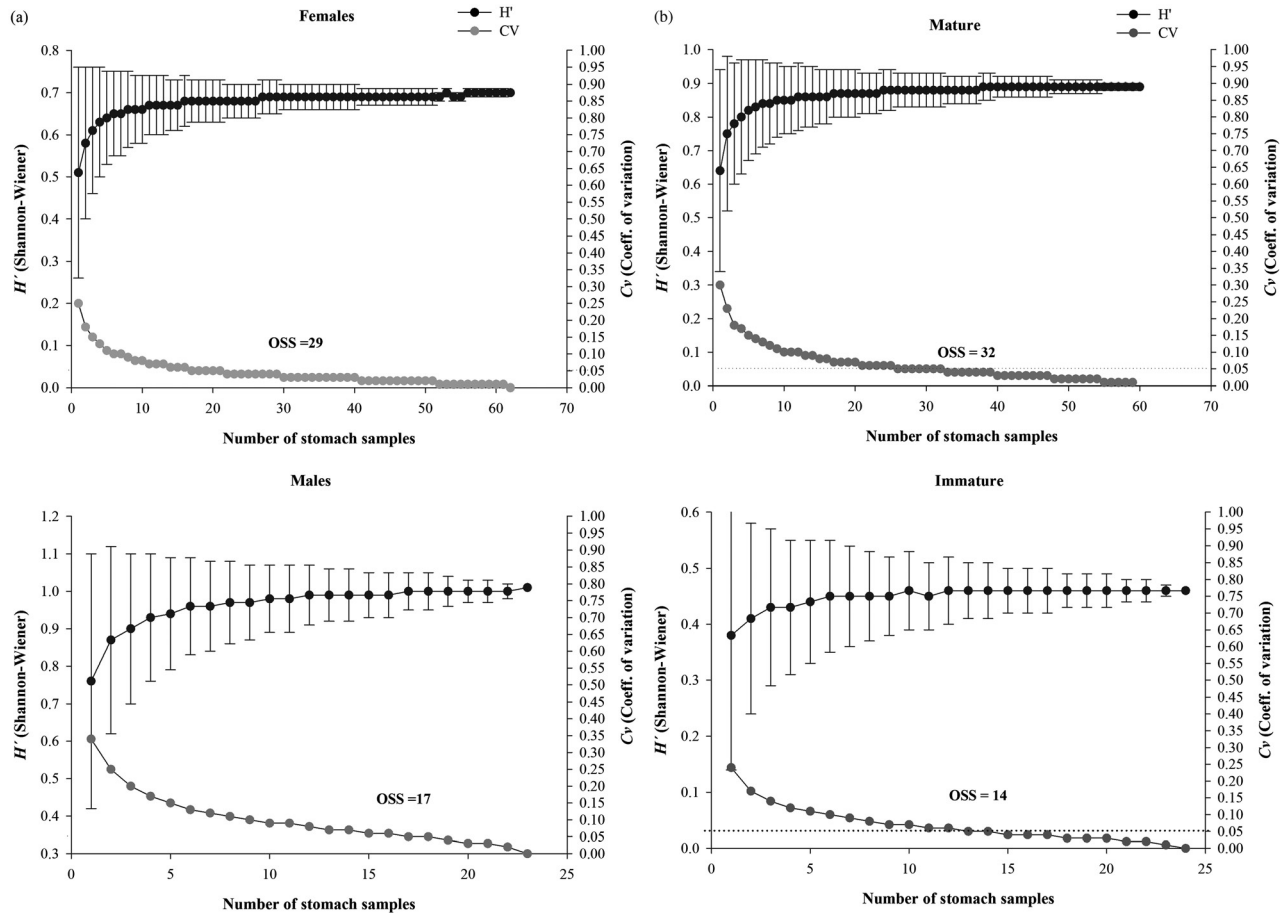


Figure 1. Mean (\pm SD) cumulative prey diversity curves and coefficients of variation (Cv) for the pelagic thresher shark *Alopias pelagicus* based on diet samples from (a) females and males and (b) mature and immature individuals; cumulative prey diversity is based on the Shannon–Wiener index (H') (OSS = optimum sample size).

all prey found in each stomach (Table I). TL values for all prey were derived from studies of Pauly et al. (1998), Cortés (1999) and Dambacher et al. (2010).

Trophic level estimates derived from isotopic data were calculated from $\delta^{15}N$ values and the equation proposed by Post (2002).

$$TL = \lambda + (\delta^{15}N_{muscle-predator} - \delta^{15}N_{base})/\Delta_n \quad (3)$$

where λ is the trophic position of the organism used to define the base. As the reference species should share the same habitat as the predator (Post 2002), we used the $\delta^{15}N_{base}$ of the lamp fish *B. panamense* (9.9‰; Calle 2010), a selective zooplankton-feeder from the Ecuadorian Pacific, which has a TL of 3.0 (Dambacher et al. 2010); $\delta^{15}N_{muscle-predator}$ is the direct signature of $\delta^{15}N$ for the pelagic thresher shark and Δ_n is the trophic discrimination factor per trophic level. To obtain an estimate of TL based on stable isotopes, we performed a sensitivity analysis by using different trophic discrimination factors (TDF) determined by Hussey et al. (2010; $\Delta_n = 2.3\text{‰}$), Kim et al. (2012a; $\Delta_n = 3.7\text{‰}$) and Post (2002; $\Delta_n = 3.4\text{‰}$) in elasmobranchs and teleosts.

We also compared isotopically derived TL estimates with those obtained from the SCA and other published dietary studies to gauge whether our results were consistent with previous research.

Results

Stomach content analysis

From July to December 2003, the stomach contents of 111 pelagic thresher sharks (27 males, 84 females) were collected. Females ranged from 160 to 340 cm total length, with a mean of 280.0 ± 0.3 cm and males ranged from 200 to 330 cm, with a mean of 278.0 ± 0.3 cm. Analysis of the cumulative diversity curves showed no variation at the endpoints of each curve in females and mature individuals (Student's t -test, $t = 61.7$, $p = 0.26$ and $t = 82.0$, $p = 0.1$, respectively) and reached an asymptote at 29 and 17 stomach samples, respectively (Figure 1a,b). However, cumulative diversity curves for males and immature sharks reached an asymptote at 17 and 14 stomachs, respectively (Figure 1a,b). Regression analysis of the last four points of the curve showed a

Table II. Index of relative importance (IRI) of the main prey of the pelagic thresher shark *Alopias pelagicus* by month in Manta, Ecuador. Values in parentheses represent the sample size for each month.

Prey species	%IRI					
	Months					
	Jul (5)	Aug (12)	Sep (25)	Oct (20)	Nov (14)	Dec (9)
<i>Behthosema panamense</i>	4.1	75.6	24.3	14.6	29.2	39.0
<i>Merluccius gayi</i>	6.1	–	–	–	–	–
<i>Loliolopsis diomedea</i>	8.9	–	–	–	–	–
<i>Sardinops sagax</i>	7.0	–	–	–	–	–
<i>Dosidicus gigas</i>	63.6	13.1	73.6	78.0	63.9	57.5
<i>Sthenoteuthis oualaniensis</i>	–	10.3	2.0	6.0	2.4	–
<i>Auxis thazard</i>	–	–	–	–	–	2.6

slope that was moderately significant from zero (Student’s *t*-test: for males *t*-test, $t=2.67$ and $p=0.054$, for immature sharks $t=2.0$ and $p=0.06$, respectively), but trophic level estimates in these groups may not be conclusive because of relatively low samples sizes for males ($n=23$) and immature sharks ($n=24$).

Stomach content analysis showed that approximately 77% ($n=85$) of stomachs contained prey, of 24 species, including one crustacean, 7 cephalopods, and 16 teleost fishes (Table I). According to the %IRI, the three most common components were *D. gigas* (66%), *B. panamense* (30%) and *S. oualaniensis* (2.9%) (Table I). The monthly dietary data showed that sharks mainly fed on these three species across all seasons, but the sample sizes were small in some months (Table II). Other fish, such as the Pacific sardine *Sardinops sagax* Jenyns, 1842, hake *Merluccius gayi* Guichenot, 1848 and squid *Loliolopsis diomedea* Hoyle, 1904, were also important prey in some months (Table II).

Approximately 73% ($n=62$) of female stomachs contained 20 prey species, of which *D. gigas*, *B. panamense* and *S. oualaniensis* were dominant, and frigate tuna (*Auxis thazard* Lacepède, 1800) and hake (*M. gayi*) were secondary prey. Approximately 27% ($n=23$) of male stomachs contained 11 prey species. As for females, *D. gigas*, *B. panamense* and *S. oualaniensis* were the dominant prey, and *A. thazard* were secondary (Table III; Supplementary material Table SII).

Table III. PERMANOVA analysis based on Euclidean distance matrix of prey data from stomach contents of pelagic thresher sharks *Alopias pelagicus* based on sex and maturity stages.

Factor	FD	F	P
Sex			
Males × Females	1	0.60	0.45
Maturity stage			
Mature × Immature	1	2.44	0.11

Immature sharks (total length: 160–253 cm) fed on 5 prey species found in 28% of stomachs ($n=24$); the sample size of immature sharks was small compared to other sex and age categories. Stomachs of mature sharks (total length: 254–343 cm) contained 19 prey species found in 72% of stomachs ($n=61$) (Supplementary material Table SIII). Among mature sharks, the main prey species were *D. gigas*, *B. panamense* and *S. oualaniensis*. In immature sharks, the proportion of

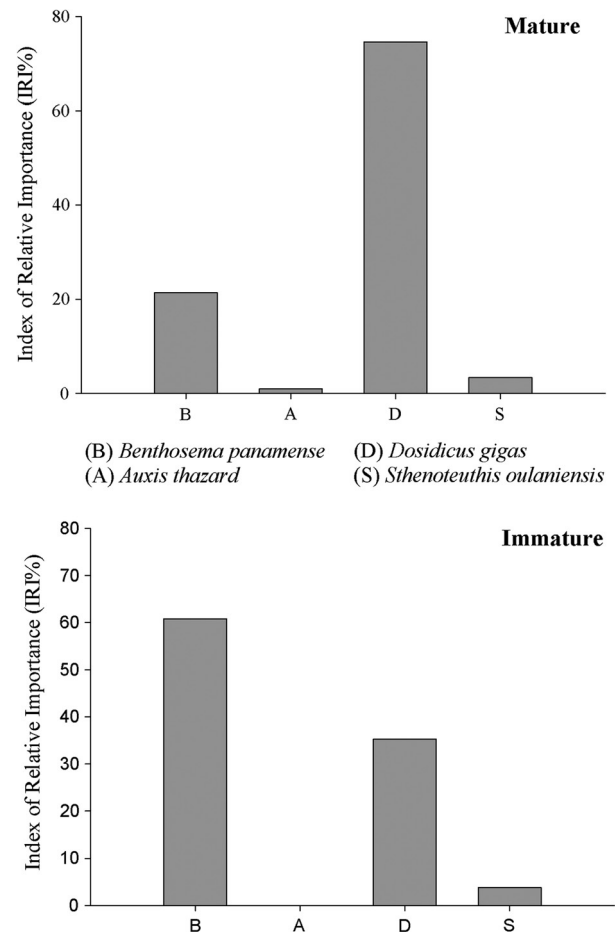


Figure 2. Relative importance index (IRI) of the main prey of immature and mature pelagic thresher shark *Alopias pelagicus* in Manta, Ecuador.

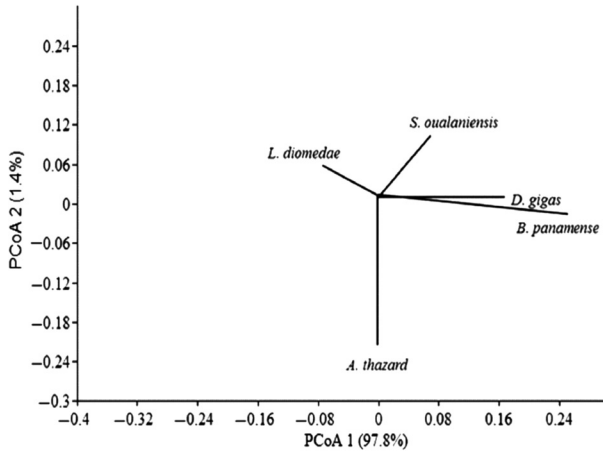


Figure 3. Principal Coordinates Analysis (PCoA) of the stomach contents of the pelagic thresher shark *Alopias pelagicus* based on the Bray–Curtis similarity, which indicates primary prey consumed by each sex and maturity stage.

B. panamense was higher than the proportion of *D. gigas* (Figure 2).

Overlap and trophic breadth

The PERMANOVA confirmed the similarity of diet between sex and maturity stages (Table III), which was driven by the large contributions of *B. panamense* and *D. gigas*, explaining 97.8% of total variation on the first axis (Figure 3). The analysis of trophic breadth (Levin’s Index) for the pelagic thresher shark showed values less than 0.6, which suggests that this shark is a specialist predator (Table IV).

Stable isotope analysis

Isotopic comparison between sexes and maturity. A total of 40 muscle samples and 29 vertebrae were separated by sex (muscle: 30 females and 10 males; vertebrae: 23 females and 6 males) and maturity stage (muscle: 29 mature and 11 immature; vertebrae: 19 mature

and 10 immature). Because all data sets showed normal distributions, we applied Student’s *t*-test to assess significant differences in mean isotope values. Significant differences between sexes were found for vertebrae $\delta^{13}\text{C}$ values ($t=2.71, p=0.01$), with males having lower values than females (Figure 4a; Table V). There were no significant differences between immature and mature sharks for muscle or vertebrae $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Figure 4b; Table V). We found a high degree of inter-individual variation in $\delta^{15}\text{N}$ values in muscle ($\sim 5\text{‰}$) and vertebrae ($\sim 4\text{‰}$) in both mature and immature individuals. We also found a high degree of inter-individual $\delta^{13}\text{C}$ variation in vertebrae ($\sim 6\text{‰}$), but not in muscle in either maturity stage (Figure 4b).

Isotopic comparison between tissues. The PERMANOVA was applied to test for significant differences in mean isotope values. $\delta^{13}\text{C}$ values did not differ significantly between tissues ($F=1, F=4.54, P=0.10$), but $\delta^{15}\text{N}$ were significantly different ($F=1, F=118.8, P=0.0001$); muscle $\delta^{15}\text{N}$ values were 4.2% higher on average than vertebrae.

Trophic level

The estimated trophic level obtained for each sex and maturity group, using data from stomach content analysis and $\delta^{15}\text{N}$ values, were similar. As expected, mean estimated TL derived from isotopic data varied, depending on which TDF was used in the analysis (Table IV). Using the TDF estimated by Hussey et al. (2010), TL values were higher (4.6–5.0) than estimates derived from the TDF proposed by Post (2002) and Kim et al. 2012a (4.0–4.3) and values estimated from stomach content analysis (4.1–4.3; Table IV). Overall, TL estimates suggest that the pelagic thresher shark is a secondary–tertiary carnivore (Mearns et al. 1981).

Table IV. Trophic breadth and level based on the stomach content and stable isotope analysis by sex and maturity stage in pelagic thresher shark in Manta, Ecuador.

Shark groups	Trophic breadth Levin Index	Stomach content Cortés (1999) Index	Trophic level		
			Isotopes		
			Hussey et al. (2010) $\Delta_n=2.3$	Post (2002) $\Delta_n=3.4$	Kim et al. (2012) $\Delta_n=3.7$
Female	0.04	4.1	5.0	4.0	4.0
Male	0.04	4.3	4.9	4.3	4.2
Mature	0.05	4.2	4.6	4.1	4.0
Immature	0.03	4.2	4.8	4.2	4.1
Mean	0.02	4.2	4.7	4.2	4.0

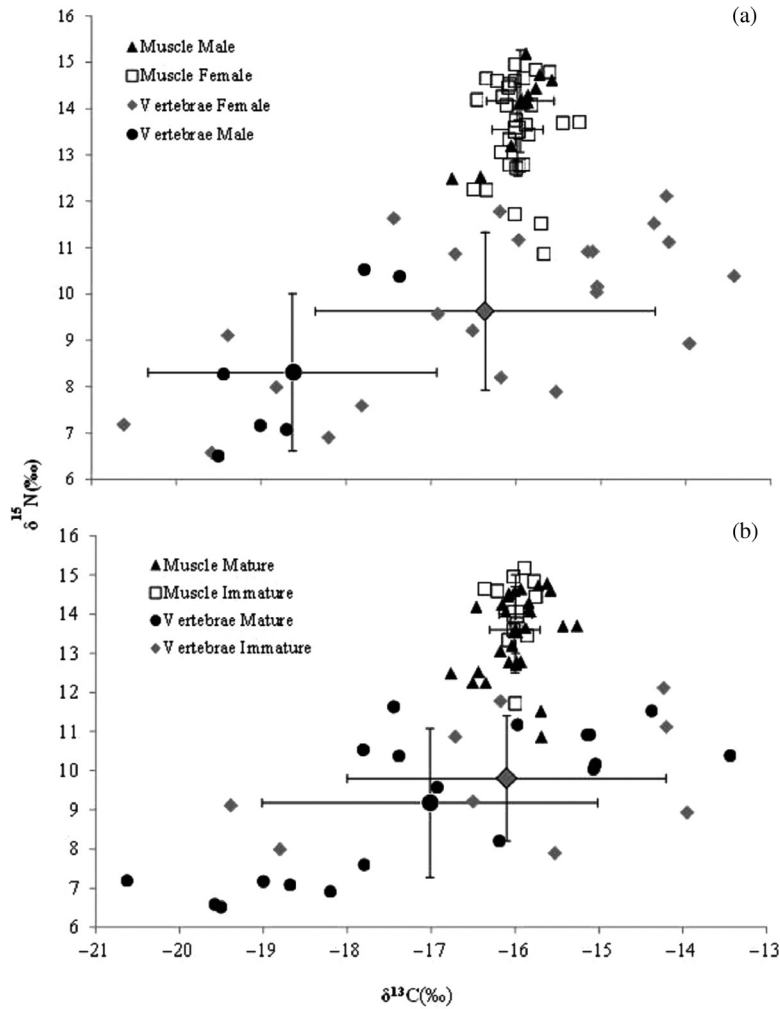


Figure 4. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (small symbols) and mean \pm SD (large symbols and error bars) for muscle and vertebrae of the pelagic thresher shark *Alopias pelagicus* captured in Manta, Ecuador, grouped by (a) sex and (b) maturity stage.

Table V. Muscle and vertebrae $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD in ‰) of pelagic thresher shark *Alopias pelagicus* collected in Manta, Ecuador and summary of ANOVA tests between sexes and maturity stages for each tissue.

		$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
		Muscle					
Shark groups	<i>n</i>	Mean	T_{40}	<i>P</i>	Mean	T_{40}	<i>P</i>
Female	30	-16.0 ± 0.3	-0.29	0.76	13.5 ± 1.0	-1.54	0.13
Male	10	-15.9 ± 0.3			14.2 ± 1.1		
Mature	29	-16.0 ± 0.3	T_{40} 0.26	0.79	13.6 ± 1.1	T_{40} -1.19	0.23
Immature	11	-16.0 ± 0.2			14.0 ± 1.0		
		Vertebrae					
Female	23	-16.4 ± 2.0	T_{29} 2.71	0.01*	9.6 ± 1.7	T_{29} 1.67	0.10
Male	6	-18.6 ± 0.9			8.3 ± 1.7		
Mature	19	-17.0 ± 2.0	T_{29} -1.05	0.30	9.2 ± 1.9	U 65	0.31
Immature	10	-16.1 ± 1.9			9.8 ± 1.6		

P values with * are significant ($P < 0.05$).

Discussion

Diet composition

Analysis of foraging behaviour and trophic level in sharks based solely on stomach contents only considers food recently consumed. By combining SCA with stable isotope analysis, our approach provides information on trophic position (Jennings et al. 2002; Blanco-Parra et al. 2012) and the relative importance of different prey (Hobson et al. 1997; Abrantes & Barnett 2011; Bethea et al. 2011) assimilated over multi-year timescales. Together, these two techniques provide a better description of the relationship between elusive predators and their prey in oceanic ecosystems.

Analyses of the cumulative prey diversity curves indicate that our sample size represents an accurate assessment of the diet of pelagic thresher sharks in this region, which is dominated by three species: the Humboldt squid (*D. gigas*), the meso-pelagic lamp fish (*B. panamense*) and the purple-back squid (*S. oualaniensis*). These prey are abundant and widely distributed in the eastern tropical Pacific Ocean near the west coast of South America (Taïpe et al. 2001; Argüelles et al. 2008), likely because there are large zooplankton stocks in the region (Ayón & Girón 1997; Brodeur & Yamamura 2005). Previous studies show that juvenile Humboldt squid are important prey for other pelagic shark species in Ecuadorian waters and other regions of the eastern Pacific Ocean (Aguilar-Castro 2003; Castañeda & Sandoval-Londoño 2007; Estupiñan-Montaño et al. 2009). At night, juvenile Humboldt squid are found near the surface of coastal waters, while adult squid remain between 10 and 35 m. Young and mature squid return to deeper waters during the day (Markaida & Sosa-Nishizaki 2003). The lantern fish occur from the surface to a depth of > 500 m, and are found over continental shelves and oceanic waters throughout the world (Fischer et al. 1995). Lantern fish remain at depth (~200 m) during the day and migrate to surface waters at night (Fischer et al. 1995). They are also important prey for Humboldt squid in the Gulf of California and Peruvian waters (Nesis 1970; Bennet 1978; Markaida & Sosa-Nishizaki 2003).

Throughout our study (July–December 2003), the proportion of these three main prey in shark stomachs remained nearly constant (Table II). Polo-Silva et al. (2009) conducted a similar study in this region in January–June and found that these same prey species were common in the stomachs of pelagic thresher sharks. These results suggest that there is no seasonality and little inter-annual variability in the diet of this top predator in this area. Our

data suggest that there is substantial overlap between the diurnal movements and habitat preferences of the prey and the pelagic thresher shark, which inhabit epipelagic waters at depths from 0 to 150 m (Compagno 2001).

The principal prey of the pelagic thresher shark did not differ between males (23 stomachs) and females (62 stomachs), a pattern also observed by Polo-Silva et al. (2009). Females analysed by Polo-Silva et al. (2009) had a greater diversity of prey, which may indicate that females have greater energy requirements than males to support their larger size and the energy costs associated with reproduction (Wetherbee & Cortés 2004). This pattern has also been observed in the sandbar shark (*Carcharhinus plumbeus* Nardo, 1827), silky shark (*C. falciformis*) and narrownose smooth-hound shark (*Mustelus schmitti* Springer, 1939) (Van der Molen & Caille 2001; McElroy et al. 2006; Cabrera-Chávez-Costa et al. 2010). Bedford (1992) found that the diet of the common thresher shark (*A. vulpinus* Bonnaterre, 1788) seasonally segregates by sex off the Pacific coast of the United States.

Mature sharks consumed a greater diversity of prey (19 species) than immature sharks (5 species). Immature sharks may preferentially consume *B. panamense* because sharks of this age class lack hunting experience and *B. panamense* form large schools that are relatively easy to capture and are smaller and slower than *D. gigas*. Mature sharks preferred *D. gigas* as their primary prey. Studies of other shark species confirm shifts in diet and diversity of prey are associated with body size (Lowe et al. 1996; McElroy et al. 2006; Cabrera-Chávez-Costa et al. 2010) and the ability to catch larger prey increases with size and development of sensory organs in sharks (Cortés & Gruber 1990; Simpfendorfer et al. 2001; Alonso et al. 2002; Wetherbee & Cortés 2004; Estupiñan-Montaño et al. 2009).

We found that pregnant female pelagic thresher sharks in this region consumed prey of similar diversity and abundance as non-pregnant females. This result contrasts with silky sharks (*C. falciformis*) in the eastern Pacific Ocean, where pregnant females do not feed and must store energy reserves for reproduction before pregnancy (Cabrera-Chávez-Costa et al. 2010).

We did not find significant $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ differences in muscle between sexes (Table V), which is corroborated by stomach contents analysis, where no differences in trophic level occurred between the sexes. Several studies (Michener & Schell 1994; Auriolles et al. 2006; Newsome et al. 2010; Graham et al. 2010) show that variations in the isotopic composition of

carbon among top predators are partly driven by baseline differences in the isotopic composition of primary producers in nearshore (coastal) versus offshore (oceanic) food webs. Isotope differences in vertebrae suggest that males forage more often in offshore oceanic habitats, as males have significantly lower $\delta^{13}\text{C}$ values (-18.6‰) than females (-16.4‰) (Figure 4a). Higher $\delta^{13}\text{C}$ values in females likely indicate that they remain in coastal habitats upon maturity (> 7 years; Liu et al. 1999).

Overlap and trophic breadth

Multivariate analysis confirmed that prey composition were similar for all groups. Polo-Silva et al. (2009) reported similar results and showed a high degree of overlap in prey preference between sexes and maturity stages. Our SCA shows that the trophic niche was narrow for all groups (Table IV), which would classify this predator as a specialist during the dry season in equatorial waters.

$\delta^{15}\text{N}$ values of both muscle and vertebrae, showed a large degree of variation (Figure 4a,b). Given that stomach contents are consistent from season to season, the observed variation in $\delta^{15}\text{N}$ values would suggest that the pelagic thresher sharks caught off Ecuadorian shores may be sourced from a variety of eastern tropical Pacific oceanic ecosystems with distinct baseline nitrogen isotope values (see following section). It should be noted that lipid extraction could also alter $\delta^{15}\text{N}$ values, because the solvents used in this process (chloroform and methanol) slightly change $\delta^{15}\text{N}$ in teleosts (Sotiropoulos et al. 2004) and sharks (Hussey et al. 2010; Logan & Lutcavage 2010; Kim et al. 2012a) by altering the amino acid composition of muscle. Unfortunately, the effect on lipids and urea were not evaluated, because we did not compare paired lipid-extracted and bulk tissues. The potential influence of lipid-extraction on $\delta^{15}\text{N}$ values, however, should be minimal because we observed a mean (\pm SD) C:N ratio of 3.1 ± 0.1 in muscle samples that were lipid-extracted, which is within the range for pure muscle protein (Post et al. 2007; Hussey et al. 2012; Kim et al. 2012a).

Isotopic comparison between tissues

Comparing isotopic values between muscle and vertebrae, we found that muscle $\delta^{15}\text{N}$ values (13.6‰) were significantly higher than vertebrae (9.4‰) by over 4‰ . We offer two possible hypotheses to explain this difference. First, variation in the amino acid composition among tissues can influence bulk tissue $\delta^{15}\text{N}$ values, because individual amino acid $\delta^{15}\text{N}$ values can vary by as much as 30‰ in a

single tissue (Popp et al. 2007; Lorrain et al. 2009). Unfortunately, tissue amino acid composition has not been determined in pelagic thresher sharks; however, data exists for other elasmobranch species (Mizuta et al. 2001; Kittiphattanabawon et al. 2010) and tissue amino acid composition tends to be highly conserved among species. To test this hypothesis, we compared the proportion of amino acid in vertebrae and muscle in four other elasmobranch species (Mizuta et al. 2001; Kittiphattanabawon et al. 2010) with $\delta^{15}\text{N}$ values of individual amino acids in yellowfin tuna caught in the eastern tropical Pacific (Popp et al. 2007). Trophic level estimates for yellowfin tuna (4.2) were similar to estimates we obtained for pelagic thresher sharks, based on stomach contents and stable isotope analysis (TL range 4.0–4.4). Calculations of bulk tissue $\delta^{15}\text{N}$ values for muscle and vertebrae using this approach showed that bulk muscle tissue would have a slightly lower ($\sim 1\text{‰}$) $\delta^{15}\text{N}$ value than vertebrae. Thus, differences in amino acid composition between tissues do not explain why muscle $\delta^{15}\text{N}$ values were more than 4‰ higher than vertebrae $\delta^{15}\text{N}$ values.

Second, the 4‰ difference between muscle and vertebrae $\delta^{15}\text{N}$ values could be driven by basin-scale movements across spatial gradients in baseline $\delta^{15}\text{N}$ values. Popp et al. (2007) and Graham et al. (2010) found large variations in $\delta^{15}\text{N}$ values among tuna species in the Equatorial Pacific Ocean and compound-specific amino acid $\delta^{15}\text{N}$ analysis of muscle from these species verified that this variation was driven by latitudinal gradients in baseline $\delta^{15}\text{N}$ values. Thresher sharks may also travel great distances in search of food and may spend large periods of time in other regions of the Equatorial Pacific Ocean. The large degree of variation ($\sim 5\text{--}6\text{‰}$) in muscle or vertebrae $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values among mature sharks caught off the Ecuadorian coastlines (Figure 4a,b) suggests that adults could come from other areas of the Equatorial Pacific Ocean that have different baseline isotope values.

At present, it is impossible to determine if this second hypothesis is responsible for the observed isotopic variation among tissues. It is possible that both dietary and baseline effects could be important in explaining the observed $\delta^{15}\text{N}$ difference between tissues and that sharks caught near Manta, Ecuador represent a combination of resident and migratory individuals. Further work examining isotope values of principal prey species and tagging studies may be able to disentangle these potential explanations.

Trophic level

We used both stomach content and stable isotope analysis of muscle to determine trophic levels (TL). Estimates of TL based on SCA varied from 4.1 to 4.3; estimates derived from SIA varied from 4.0 to 5.0 (Table IV). To calculate pelagic thresher shark TL from bulk $\delta^{15}\text{N}$ values, we used different $\delta^{15}\text{N}$ TDFs from previous studies (2.3‰, 3.4‰ and 3.7‰). As expected, estimates of trophic level were higher (4.6–5.0) using the $\delta^{15}\text{N}$ TDF (2.3‰) reported by Hussey et al. (2010) than other TDF values (Post 2002; Kim et al. 2012a; Table IV). Several studies have shown that $\delta^{15}\text{N}$ TDFs can vary as a result of growth rate, diet protein quantity/quality, and the principal form of nitrogenous waste (e.g. ammonia versus urea) produced by organisms (Vander Zanden & Rasmussen 2001; Caut et al. 2009; Martínez del Río et al. 2009; Hussey et al. 2010; Kim et al. 2012a). Although TDFs have not yet been determined experimentally for pelagic thresher sharks, the isotopically derived TL estimate using the TDFs reported for leopard sharks (*Triakis semifasciata*, Girard 1855) by Kim et al. (2012a) was very similar to the TL estimate derived from stomach content analysis (Table IV). It is important to note that the TDFs proposed by Hussey et al. (2010) and Kim et al. (2012a) were determined in different shark species. We believe the $\delta^{15}\text{N}$ TDFs reported in Kim et al. (2012a) are more accurate because their controlled feeding study was long enough (~1250 days) for muscle tissue to fully incorporate the experimental diet. In contrast, the sharks studied by Hussey et al. (2010) probably did not reach steady state with the experimental diet because of the shorter duration of the experiment (~365 days).

Overall, our results suggest that pelagic thresher sharks in this region have a mixed diet, including squid and fish of intermediate to high (3.2–3.3) trophic levels (Cortés 1999), which indicates that this shark is a secondary–tertiary carnivore in this part of the Equatorial Pacific Ocean. Cortés (1999) reported a 4.0 TL in blue shark (*P. glauca*) and bigeye thresher shark (*A. superciliosus* Lowe, 1841) caught in California waters. While Cortés (1999) analysed a smaller number of stomachs ($n=20$), the results were similar to those presented here, suggesting that closely related species have a similar trophic level in different geographical areas of the eastern Pacific Ocean. Lastly, our results show good agreement between trophic level estimates derived from stomach contents and stable isotope analysis and suggest these independent but complementary approaches are

reliable methods to use for assessing diet of top marine predators.

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