

## Article

# Trophic Ecology during the Ontogenetic Development of the Pelagic Thresher Shark *Alopias pelagicus* in Baja California Sur, Mexico

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**Abstract:** The trophic ecology of the Pelagic Thresher shark (*Alopias pelagicus*) was evaluated based on chemical ecology using stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in the vertebrae and muscles. Individuals were caught between August 2013 and October 2019 on both the coasts of Baja California Sur, Mexico. In Bahía Tortugas, the mean vertebrae ( $n = 35$ ) values were  $12.72 \pm 1.06\text{‰}$  ( $\delta^{15}\text{N}$ ) and  $-14.79 \pm 0.61\text{‰}$  ( $\delta^{13}\text{C}$ ), while in muscles ( $n = 32$ ) these values were  $16.63 \pm 0.76\text{‰}$  ( $\delta^{15}\text{N}$ ) and  $-17.18 \pm 0.39\text{‰}$  ( $\delta^{13}\text{C}$ ). In Santa Rosalía, the mean vertebrae ( $n = 125$ ) isotopic values were  $14.4 \pm 1.59\text{‰}$  ( $\delta^{15}\text{N}$ ) and  $-14.18 \pm 0.51\text{‰}$  ( $\delta^{13}\text{C}$ ), while in muscles ( $n = 43$ ), these values were  $18.08 \pm 0.96\text{‰}$  ( $\delta^{15}\text{N}$ ) and  $-16.43 \pm 0.34\text{‰}$  ( $\delta^{13}\text{C}$ ). These results show higher  $\delta^{15}\text{N}$  values in Santa Rosalía as an effect of baseline isotopic differences between the two regions, whereas the  $\delta^{13}\text{C}$  values were lower in Bahía Tortugas, suggesting offshore ecological behavior ( $p < 0.05$ ). In Santa Rosalía, there were significant differences by sex for  $\delta^{15}\text{N}$  in muscle, whereas the  $\delta^{13}\text{C}$  showed ontogenetic shifts, indicating that neonates feed in coastal areas more commonly than juveniles or adults ( $p < 0.05$ ). Neither sex nor ontogenetic differences were observed in Bahía Tortugas ( $p > 0.05$ ), suggesting a high overlap between their isotopic niches. Therefore, *Alopias pelagicus* uses the same ecological niche throughout its life, and there is consistency between sexes. The mean trophic position for both tissues and regions was 4.5, which corresponds to a tertiary predator, without any differences between stages or sex. Due to their higher energetic needs, juveniles and females showed the greatest isotopic niche amplitude; thus, their ecological niche is the widest.

**Keywords:** chemical ecology; stable isotopes; vertebrae; muscle; trophic shifts



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## 1. Introduction

The Pelagic Thresher shark *Alopias pelagicus* (Nakamura inhabits tropical and temperate oceanic waters throughout the Indo-Pacific Ocean, including the eastern coast from Mexico to northern Peru, with no records in the Atlantic Ocean. It is found up to 300 m deep, and its length can reach up to 4.28 m [1]. Thresher sharks are characterized by their long tail, which measures half their body length, and that they use to corner and to disorient and stun the fish and pelagic invertebrates that are part of their diet. This species is considered to be oophagous, due to the egg capsules and fragments of shell found in the stomachs of embryos [2]. Liu et al. [3] established that the Pelagic Thresher shark has two embryos per litter, with a long gestation period of nine months, and they mature at the age of eight years, which suggests that this species is extremely vulnerable to overexploitation and in need of close monitoring.

Mexican fisheries land some of the largest shark catches in the world, dominated mainly by mustelids, but other species such as *A. pelagicus* also represent a significant

component [4]. Fisheries along the coasts of Baja California are diverse and opportunistic, while *A. pelagicus* and *Isurus oxyrinchus* (Rafinesque) are specifically targeted for their meat and fins [4].

There is scarce knowledge regarding their biology, with few studies carried out into their age, growth, and reproduction in the northwestern Pacific [3,5] and Ecuador [2]. The trophic ecology of *A. pelagicus* has mainly been examined in the waters off Ecuador [6–8], with Lara et al.'s [9] being the only dietary study in Mexican waters. Polo-Silva et al. [6] found the giant squid *Dosidicus gigas* (d'Orbigny) to be the main prey in its diet, followed by the lanternfish *Benthoosema panamense* (Tåning) and the squid *Sthenoethis oulaniensis* (Lesson). Similar results were obtained by Calle-Morán [7], who reported as the main prey the Red Flying Squid *Ommastrephes bartramii* (Lesueur), the Jumbo Squid *D. gigas*, the Purpleback Flying Squid *S. oulaniensis*, and the South Pacific Hake *Merluccius gayi* (Guichenot). Garcia-Olvera [10] reported anchovy *Engraulis mordax* (Girard) to be its main prey, followed by the family of lanternfishes Myctophidae, and *Paralabrax* spp. (Girard). The same topic has been recently studied in Indonesia for the first time [11], with the following identified as the three top prey species: the Frigate Tuna *Auxis thazard* (Lacepède), the Purpleback Flying Squid *S. oulaniensis*, and the Spiny Lanternfish *Dasyscopelus spinosus* (Steindachner).

The most common method used to analyze prey is to extract the stomach and identify stomach contents to the lowest taxonomical level possible, but it is also possible to obtain samples via non-lethal methods [12]. One technique that has been used previously is stomach flushing, also referred to as gastric lavage. This technique involves pumping water via a tube down the throat of the animal into the stomach, expelling the stomach contents via the mouth [12]. Another less widely used method is applying forceps to evert the stomachs of small sharks. However, since this involves either restraining the shark upside down, or anesthetizing the animal before reaching into its mouth, it is not appropriate for larger species [12].

Stable isotope analysis, particularly using nitrogen and carbon, allows us to evaluate the structure and dynamics of ecological communities, with this approach combining the benefits of both trophic-level and food web paradigms in food web ecology [13]. Moreover, this method is less invasive than others previously mentioned, since stable isotope samples can be taken by extracting blood or by performing biopsies [14]. Values of  $\delta^{15}\text{N}$  are used to estimate trophic levels and breadth; consumers are isotopically enriched by 3–4‰ relative to their prey [13,15]. In contrast, values of  $\delta^{13}\text{C}$  remain relatively unaffected by trophic level (0.5–1‰), providing information about trophic habitat use across the inshore-offshore gradient [15,16].

For slow-growing species such as sharks, the isotopic signals given off by the muscle tissue provide information about the prey assimilated by the predator 1–2 years prior to its consumption [14,16]. Other tissues such as vertebrae are metabolically inert and contain growth layers that can record ecological information over the lifespan of a single individual [16,17]. Ontogenetic shifts in the use of trophic habitat often reflect changes in survival strategies. While juveniles use their energy to grow, adults prioritize activities such as reproduction [18]. These changes in feeding habits are common in sharks and rays, as these species are able to shift their prey types and feeding areas as they grow [19]. Understanding the ecological life history of pelagic species is important to ensure their survival, especially in long-living species such as elasmobranchs [18].

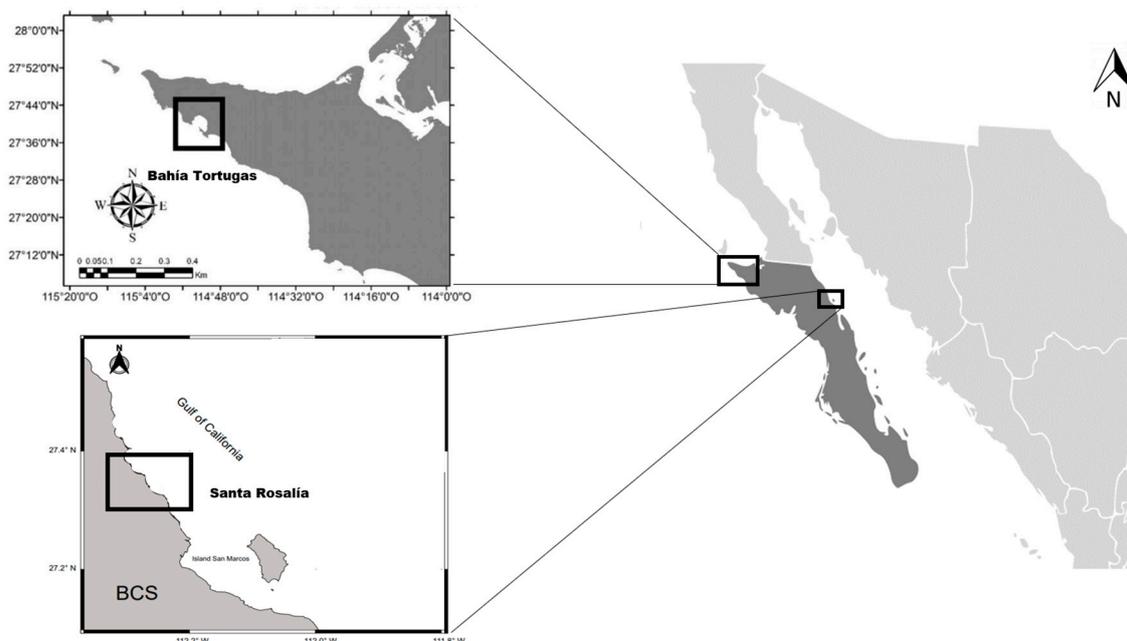
The combined assessment of trophic ontogeny in *A. pelagicus* from multiple regions, focusing on different tissues and life stages, allows us not only to analyze their feeding behaviors, but also to isotopically compare tissues with different metabolic rates and relate these to areas with unique oceanographic conditions.

Considering that stable isotopes of the Pelagic Thresher shark have only been only studied in Ecuadorian waters, the aim of this study is to examine the trophic ontogeny of this species in both the coastal areas of Baja California Sur, Mexico, by analyzing the nitrogen and carbon stable isotopes in vertebrae and muscles.

## 2. Materials and Methods

### 2.1. Study Area and Sample Collection

Samples were collected in two areas: the fishing town of Bahía Tortugas ( $27^{\circ}39'35''$  N;  $114^{\circ}52'35''$  W), located on the western coast of the Baja California Sur Peninsula, and Santa Rosalía ( $27^{\circ}20'20''$  N;  $112^{\circ}16'01''$  W), located on the eastern coast of Baja California Sur (Figure 1).



**Figure 1.** Location of two study areas, Bahía Tortugas and Santa Rosalía in Baja California Sur, Mexico.

Bahía Tortugas (BT) is known for the frequent upwelling phenomenon that occurs there, which provides nutrients to the surface that remain available for primary production. Therefore, this region exhibits a high phyto- and zooplankton biomass, which results in the high productivity of fishery resources [20].

Santa Rosalía (SR) is located in the Gulf of California, the only evaporation basin of the Pacific Ocean, due to its location between two hot land masses and the absence of freshwater inflow to the region. The variable depth of the Gulf of California, the characteristics of its habitat, and its unique location in a transition zone between temperate and tropical faunal regions endows the area with a unique biological richness [21].

In BT, samples of *A. pelagicus* were collected using artisanal long-line fishery equipment from August 2013 to August 2016. In SR, samples were collected by fishermen using gill nets from October 2017 to October 2019. The total and precaudal length (TL and PL) were measured and the sex was determined by the presence of claspers in males. Sexual maturity in males was established via the size and condition of the clasper (rotation, calcification, and semen presence) and the development of the testes. Males were divided into two reproductive stages. Juvenile/Immature: short and non-calcified clasper; testes soft, elongated, and not lobated. Adult/Mature: calcified claspers with fully lobated testes.

Approximately 20 g of muscle along with one or more vertebrae from the dorsal region near the head was sampled from each individual organism. The samples were stored in polyethylene bags properly identified and transported on ice to the CICIMAR-IPN. They were frozen in the laboratory until analysis.

### 2.2. Laboratory Analysis

The vertebrae were defrosted, cleaned, and dried. The radius of each vertebra was measured using a digital vernier and related to the precaudal length by linear regression. Based on this equation and on the size at maturity, as proposed by Romero-Cacedo et al. [2],

three ontogenetic stages in each vertebra were determined. For neonates, the first visible growth layer was sampled. Juvenile samples were those taken from the far end of the growth layer—up to 8.6 mm radius for males and 8.7 mm radius for females. For mature sharks, samples were taken from the outer edge of the vertebra when the radii exceeded 8.6 and 8.7 mm, respectively. Thus, for each vertebra of a mature shark, three samples were taken, while for each vertebra of an immature shark, two samples were taken. The samples were extracted using a microdrill with a 1 mm bit and were exposed for 24 h to a hydrochloric acid steam bath to remove inorganic carbon.

Approximately 5 g of each muscle sample was put into vials. As elasmobranchs retain urea and fat in their tissues, which can influence  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (depleting their heavy isotope values), these products had to be removed. Urea was extracted following Kim and Koch's [22] methods, using a mechanic bath (Branson M 8800) wherein each sample was washed three times with 10 mL deionized water for 15 min. The samples were then lyophilized at 0.123 mbar and  $-40\text{ }^\circ\text{C}$  for 48 h, and then ground and homogenized in an agate mortar. Lipids were not removed since Post et al. [23] suggested an arithmetic correction that could be used to remove lipids from the isotopic signature, as follows:

$$\delta^{13}\text{C}_{\text{corrected}} = \delta^{13}\text{C}_{\text{sample}} - 3.32 + 0.99 \times \text{C:N} \quad (1)$$

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were determined at the Instituto Andaluz de Ciencias de la Tierra in Granada, Spain, using a DELTA plus XL, Thermo-Finnigan isotope ratio mass spectrometer (IRMS, Bremen, Germany). The isotopic results are expressed as  $\delta$  values:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1] \quad (2)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  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  $\text{N}_2$  for nitrogen. The units are expressed as parts per thousand (per mil, ‰).

### 2.3. Data Analysis

The normality of data was assessed by the Kolmogorov–Smirnov–Lilliefors test and variance homogeneity by the Levene test. These were used to test the null hypothesis that a set of data originated from a normal and homoscedastic distribution. If they did, we used a parametrical test to assess for significant differences in mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between sexes, stages, and regions. In this case, the most appropriate test was a three-factor analysis of variance (ANOVA), considering that we had three independent categorical variables and one dependent continuous variable. This test reveals whether the variance arose by chance or was the influence by the factors. If the data were not normally distributed, we used a Kruskal–Wallis test, which is accurate when applied to non-parametric data with three categorical variables. Moreover, using this non-parametric test allowed us to perform statistical analysis without necessarily transforming the data.

Estimates of the trophic position (TP) were calculated using the R package tRophicPosition [24]. A different base organism was used for each region: particulate organic matter (POM) for SR ( $\delta^{15}\text{N}_{\text{POM}} = 11.1\text{‰}$ ,  $\text{TP}_{\text{POM}} = 1$ ) [25] and the pelagic red crab *Pleuroncodes planipes* (Stimpson) ( $\delta^{15}\text{N}_{\text{Pleuroncodes planipes}} = 12.10\text{‰}$ ,  $\text{TP}_{\text{Pleuroncodes planipes}} = 2$ ) [20] for BT. As the trophic discrimination factor (TDF), 1.95‰ was used for vertebral tissue and 2.44‰ for muscle tissue [14].

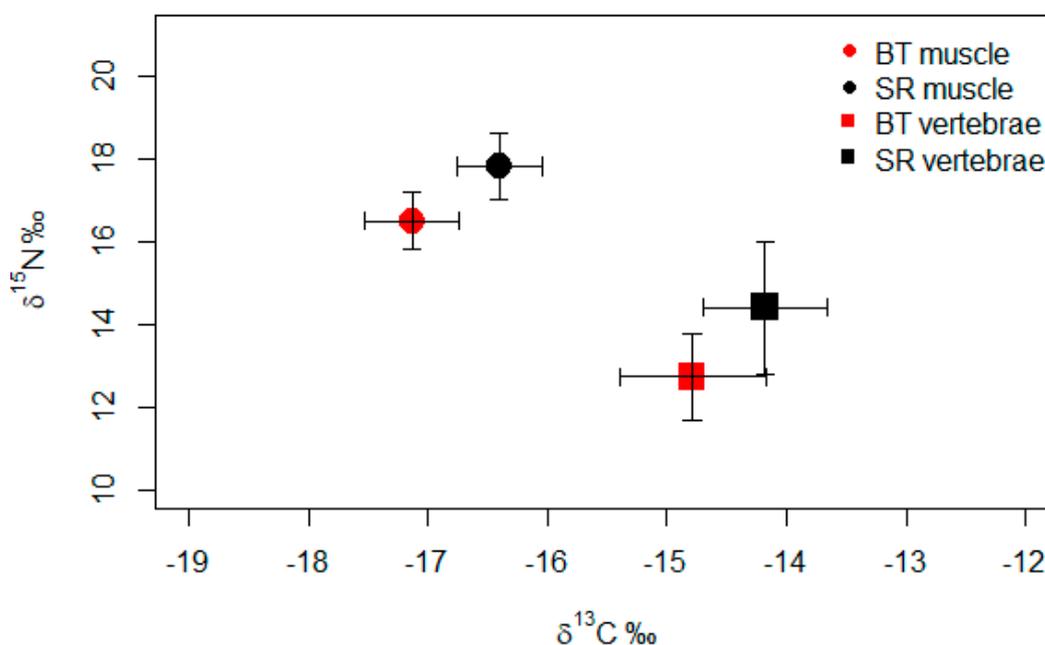
To determine the niche breadth and trophic overlap between stages, sexes, and regions, we used the package SIBER (Stable Isotope Bayesian Ellipses in R) from the program R [26]. This analysis uses measurements based on ellipses calculated by a covariance matrix that defines their area (Standard Ellipse Corrected Area, SEAc) to show the trophic niche breadth. Using this method, it is possible to obtain the overlap between ellipses, whereby values close to 1 represent high trophic overlap [26].

### 3. Results

For BT, a total of 35 vertebrae and 32 muscle samples were used. For SR, a total of 125 vertebrae and 32 muscle samples were used.

Non-parametrical tests were applied to the  $\delta^{13}\text{C}$  vertebrae data in BT ( $F = 8.405$ ,  $p = 0.007$ ), as well as to the  $\delta^{15}\text{N}$  ( $D = 0.094$ ,  $p = 0.007$ ) and  $\delta^{13}\text{C}$  ( $F = 6.178$ ,  $p = 0.003$ ) vertebrae data in SR, since these did not follow a normal or homoscedastic distribution.

The  $\delta^{15}\text{N}$  values in vertebrae and muscles were higher in SR than in BT ( $\chi^2 = 37.848$ ,  $p = 7.648 \times 10^{-10}$  and  $F = 49.54$ ,  $p = 8.84 \times 10^{-10}$ , respectively), and the  $\delta^{13}\text{C}$  also presented less negative vertebrae and muscle values in SR ( $\chi^2 = 26.854$ ,  $p = 2.194 \times 10^{-7}$  and  $F = 75.8$ ,  $p = 6.62 \times 10^{-13}$ , respectively) (Figure 2; Table 1). In BT and SR, the isotopic niches presented an overlap of 39% in the vertebrae and an overlap of 23% in the muscles. Moreover, the  $\delta^{15}\text{N}$  values were 4‰ higher in the muscles than in the vertebrae ( $\chi^2 = 125.07$ ,  $p < 2.2 \times 10^{-16}$ ) (Figure 2; Table 1).



**Figure 2.**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (mean  $\pm$  SD ‰) of *A. pelagicus* in Bahía Tortugas (BT) and Santa Rosalía (SR) in vertebrae and muscles.

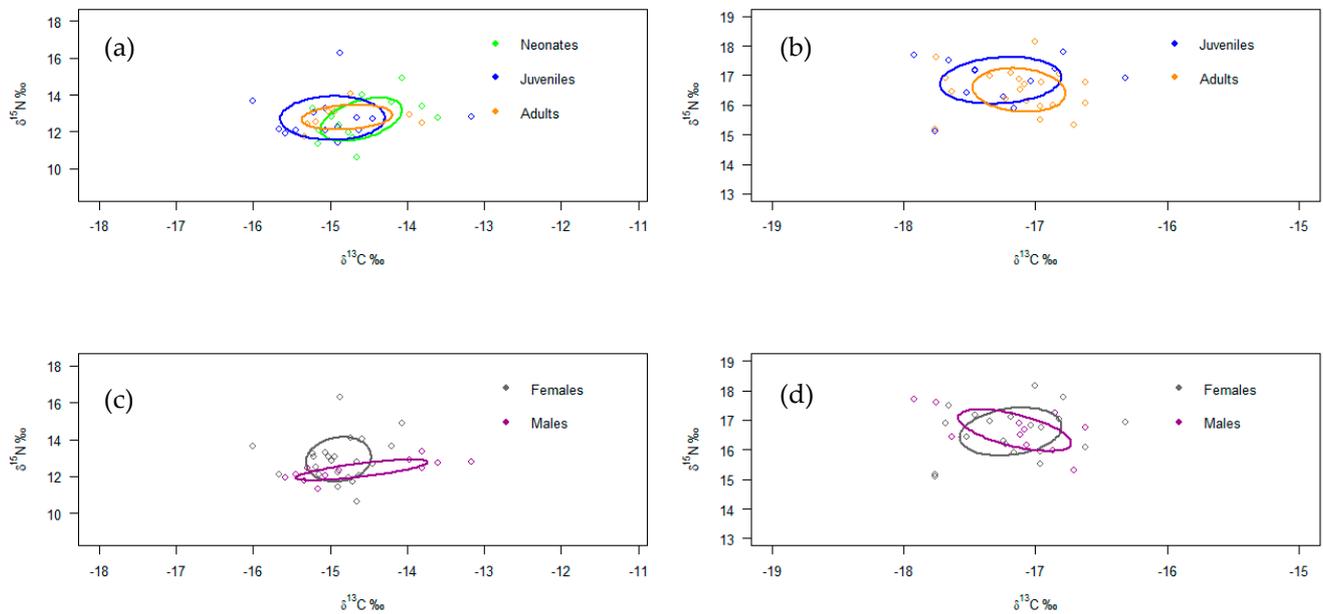
**Table 1.** Summary of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (mean  $\pm$  SD ‰) by maturity stage and sex in vertebrae and muscles of *A. pelagicus* in Bahía Tortugas (BT) and Santa Rosalía (SR).

	Vertebrae BT (n = 35)		Muscle BT (n = 32)		Vertebrae SR (n = 125)		Muscle SR (n = 43)	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Neonates	12.67 $\pm$ 1.17	-14.6 $\pm$ 0.53	16.83 $\pm$ 0.79	-17.26 $\pm$ 0.46	14.91 $\pm$ 1.59	-13.84 $\pm$ 0.41	17.78 $\pm$ 0.76	-16.48 $\pm$ 0.29
Juveniles	12.74 $\pm$ 1.18	-14.97 $\pm$ 0.68	16.51 $\pm$ 0.74	-17.12 $\pm$ 0.35	14.47 $\pm$ 0.87	-14.6 $\pm$ 0.42	17.83 $\pm$ 0.85	-16.32 $\pm$ 0.4
Adults	12.79 $\pm$ 0.67	-14.78 $\pm$ 0.59	16.61 $\pm$ 0.81	-17.19 $\pm$ 0.39	14.31 $\pm$ 1.64	-14.21 $\pm$ 0.52	17.95 $\pm$ 0.77	-16.42 $\pm$ 0.37
Females	12.92 $\pm$ 1.21	-14.59 $\pm$ 0.85	16.66 $\pm$ 0.70	-17.16 $\pm$ 0.43	14.67 $\pm$ 1.45	-14.08 $\pm$ 0.49	17.44 $\pm$ 0.74	-16.29 $\pm$ 0.33
Males	12.34 $\pm$ 0.56	-14.79 $\pm$ 0.61	16.63 $\pm$ 0.76	-17.18 $\pm$ 0.39	14.4 $\pm$ 1.59	-14.18 $\pm$ 0.51	18.08 $\pm$ 0.96	-16.43 $\pm$ 0.34
Mean	12.72 $\pm$ 1.06	-14.79 $\pm$ 0.61	16.63 $\pm$ 0.76	-17.18 $\pm$ 0.39	14.4 $\pm$ 1.59	-14.18 $\pm$ 0.51	18.08 $\pm$ 0.96	-16.43 $\pm$ 0.34

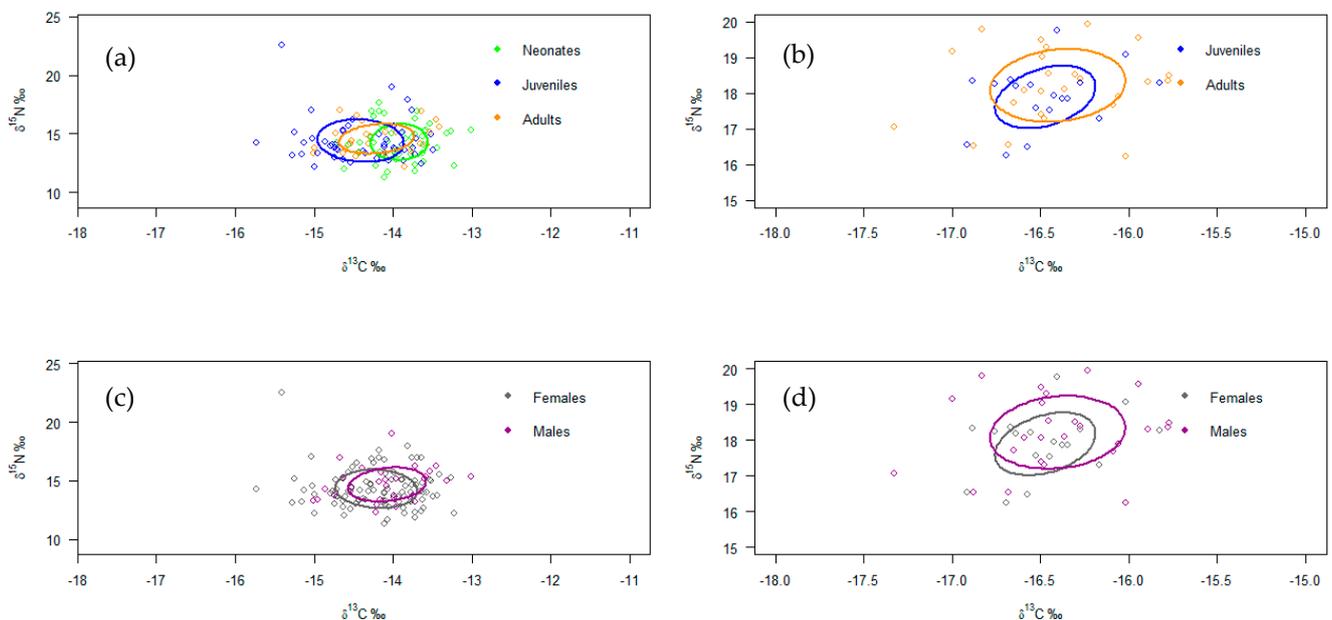
Statistical tests confirmed the similarity of trophic habits of *A. pelagicus* in BT between maturity stages (neonates, juveniles, and adults) and sexes (females and males) for both tissues (Table 2). In SR, the  $\delta^{15}\text{N}$  remained constant between stages for both tissues, while the values for female were higher than those for males in the muscles. The  $\delta^{13}\text{C}$  showed significant differences between stages in vertebral tissue (Table 2). Even when statistical differences were only significant in SR, the  $\delta^{13}\text{C}$  values were more negative in both regions

in juveniles and more positive in neonates (Table 1). However, females presented lower  $\delta^{13}\text{C}$  values than males in both areas and tissues (Table 1).

The SIBER analysis showed an overlap of between 41% and 68% in all groups (except sexes) for vertebrae in BT (27%) (Figures 3 and 4; Table 3). Juveniles and females presented wider ellipse areas for both tissues in BT and for vertebrae in SR (Table 3).



**Figure 3.** Isotopic niche (ellipses) by stage, sex, and tissue of *A. pelagicus* in Bahía Tortugas (BT). (a) Vertebral tissue by stage; (b) muscle tissue by stage; (c) vertebral tissue by sex; (d) muscle tissue by sex.



**Figure 4.** Isotopic niche (ellipses) by stage, sex, and tissue from *A. pelagicus* in Santa Rosalía (SR). (a) Vertebral tissue by stage; (b) muscle tissue by stage; (c) vertebral tissue by sex; (d) muscle tissue by sex.

**Table 2.**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ANOVA and Kruskal–Wallis analysis of vertebrae and muscles of *A. pelagicus* based on maturity stage and sex in Bahía Tortugas (BT) and Santa Rosalía (SR).

	Vertebra BT				Muscle BT				Vertebra SR				Muscle SR			
	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
	F	p	X <sup>2</sup>	p	F	p	F	p	X <sup>2</sup>	p	X <sup>2</sup>	p	F	p	F	p
Stage	0.22	0.64	2.97	0.23	1.59	0.22	1.19	0.28	1.08	0.58	19.42	$6.08 \times 10^{-5}$	2.93	0.09	0.38	0.54
Sex	2.37	0.13	0.09	0.75	0.02	0.88	0.007	0.93	2.21	0.14	1.11	0.30	5.68	0.02	0.042	0.84

**Table 3.** Stable Isotope Bayesian Ellipses in R (SIBER) analysis: Standard Ellipse Corrected Area, SEAc (%<sup>2</sup>) and overlap (%) from vertebrae and muscles of *A. pelagicus* based on maturity stages and sex in Bahía Tortugas (BT) and Santa Rosalía (SR).

	Vertebra BT		Muscle BT		Vertebra SR		Muscle SR	
	SEAc	Overlap	SEAc	Overlap	SEAc	Overlap	SEAc	Overlap
Neonates	1.9				1.79			
Juveniles	2.74	48			3.17	49		
Adults	1.4		41		1.94		54	
Males	1.1				2.28			
Females	1.67	27		44	2.68	68		46
	Overlap of neonates and adults: 41%				Overlap of neonates and adults: 55%			

The estimated TP values obtained in BT were 4.6 (vertebrae) and 4.4 (muscle). These values for SR were 4.3 (vertebrae) and 4.5 (muscle). No statistical differences were shown between regions, tissues, sexes, or maturity groups ( $p < 0.05$ ).

#### 4. Discussion

##### 4.1. Comparison between Regions

The Gulf of California presents relatively high baseline  $\delta^{15}\text{N}$  values due to the denitrification processes that occur in the minimum oxygen zone [27], where  $^{14}\text{N}$  is mostly consumed, leaving a  $^{15}\text{N}$ -enriched nitrate pool [28]. Thus,  $\delta^{15}\text{N}$  values of particulate organic matter in the Gulf of California are higher (11.1‰) than those on the western coast of Baja California Sur (8.5‰) [27]. The same pattern was shown for *A. pelagicus* in this study, and so the isotopic contrast between both coasts of Baja California Sur is thought to be related to this difference in the trophic baseline, and not to the fact that the sharks showed different trophic positions on each coast.

Fewer negative  $\delta^{13}\text{C}$  values are found in productive inshore waters, such as in upwelling regions, while more negative values are found in less productive offshore waters [28]. The results suggest that in BT, *A. pelagicus* feed in offshore habitats, while in SR, this shark species presents a trophic inshore habitat. Another explanation for these differences in  $\delta^{13}\text{C}$  values is the narrow continental shelf found in BT, which has a strong offshore influence. Hence, even when sharks fed in both regions at similar distances from the coast, those caught on the oceanic shelf (BT) showed more negative  $\delta^{13}\text{C}$  values. Moreover, environmental conditions such as temperature may also affect the availability of prey in each region. While in summer, the water in SR reaches 30 °C [29], in BT, it stays at 19 °C [30] Thus, the prey in BT come from deeper and colder waters, as compared to the shallower and warmer waters in SR.

Isotopic niches represent the ecological niche based on a determined area inside of the  $\delta$  space, where the coordinates are  $\delta^{13}\text{C}$  (environmental components) and  $\delta^{15}\text{N}$  (trophic components) [31]. The minimal overlap of isotopic niches from both study areas also indicates the different ecological niches of the Pelagic Thresher shark in each region, as discussed above.

#### 4.2. Comparison between Tissues

For both areas, the  $\delta^{15}\text{N}$  values were higher in muscles than in vertebrae. Likewise,  $\delta^{13}\text{C}$  values were more positive in vertebrae than in muscles. This isotopic variability among tissues has been previously explained by the fact that each tissue has a different metabolic turnover rate and a different TDF. For example, MacNeil et al. [32] demonstrated that vertebrae presented the slowest  $\delta^{15}\text{N}$  turnover rate due to slow layer growth. These authors also found that  $\delta^{13}\text{C}$  decreases with increasing metabolic tissue rates. It has also been observed that muscle presents a higher degree of enrichment (2.44‰) than vertebrae (1.95‰), which could have an influence on the nitrogen isotope ratio [14]. Due to this turnover rate, the vertebrae emit isotopic signals over the lifetime of the organism, while the muscles only yield isotopic information from the last month of the animal's life [17,32].

#### 4.3. Isotopic Analysis by Maturity Stages

No ontogenetic differences in  $\delta^{15}\text{N}$  values were found in *A. pelagicus*, which indicates that this species feeds on the same prey groups throughout its life. In BT, the  $\delta^{13}\text{C}$  values also stayed stable throughout the organism's life. Similar results were obtained in Ecuador by Calle-Morán [7], with no ontogenetic differences for any isotope ratio. Lara et al. [9] analyzed the stomach contents of *A. pelagicus* in BT and determined that the sardine *Sardinops sagax* (Jenyns) was the principal prey for mature and immatures sharks, suggesting the same feeding pattern despite maturity stage, thus supporting our results. On the other hand, Estupiñán-Montaño [8] showed that the  $\delta^{13}\text{C}$  values in neonates presented differences from those in juveniles and adults off the Galápagos Islands. Similar results were found in the present study for SR, where the vertebrae presented differences among all maturity stages, from which we can infer a possible ontogenetic inshore–offshore movement for *A. pelagicus*.

Lowe et al. [19] pointed out that sharks change their diet and ecological needs as they grow, thus exploiting different areas, since they are segregated by size. In this study, the findings for BT do not reflect this behavior, while in SR they do, corroborating that *A. pelagicus* shows flexibility in its trophic behavior depending on its habitat and the inherent requirements related to its development.

In both areas, juveniles present the lowest  $\delta^{13}\text{C}$  values and the widest isotopic niches, while neonates show the highest  $\delta^{13}\text{C}$  values and most narrow isotopic niches. It can be inferred that on both coasts of Baja California Sur, juvenile individuals of *A. pelagicus* present a wider and more offshore feeding habitat, while neonates present a narrow and more inshore feeding habitat. Calle-Morán [7] attributed this phenomenon to the limitation of neonates reaching offshore waters, causing their habitat to be restricted to inshore waters. Moreover, as juveniles are not completely developed, they might focus most of their energy on growing, and as such, they need more nutrients and probably a wider ecological niche.

#### 4.4. Isotopic Analysis by Sex

No differences in the  $\delta^{15}\text{N}$  values of *A. pelagicus* by sex were found in BT for any tissue, which is consistent with Lara et al.'s [9] findings in the same area, where males and females presented similar stomach contents. On the other hand, the  $\delta^{15}\text{N}$  values in SR assessed by sex, were similar in the vertebrae; however, in muscles, they showed significant differences. Once again, a contrast between the tissues is highlighted. These data suggest that male and female *A. pelagicus* in SR were feeding on prey that belonged to the same trophic level during its lifetime. Therefore, the overlap in their isotopic niches is high (68%). Nevertheless, assessments of the muscle indicate that, during the last months before their capture, males and females consumed different prey.

The  $\delta^{13}\text{C}$  values were similar between sexes in both study areas and tissues; however, females presented the lowest values. Moreover, they showed a larger ellipse area than males. Therefore, even if *A. pelagicus* did not segregate by sex on the coasts of Baja California Sur, females presented inclination towards a wider and more oceanic trophic habitat than

males. This may indicate that females have greater energy and nutritional needs than males, related to the requirements of pregnancy [16].

It is important to consider that ontogenetic and sex differences were only seen in SR, while in BT, all sharks presented the same trophic behavior, regardless of their sex and size. These results indicate that the ecological habitat in SR is more heterogeneous than in BT, as *A. pelagicus* presented a wider isotopic niche ( $2.6\text{‰}^2$ ) during its lifetime in the Gulf of California than on the western coast of Baja California Sur ( $2.1\text{‰}^2$ ).

#### 4.5. Trophic Position

Polo-Silva et al. [16] reported that the most appropriate TDF is that published by Kim et al. [33], who carried out a study on the feeding habits of the Leopard Shark *Triakis semifasciata* over 1250 days. Nevertheless, in the present study, we used the TDF proposed by Hussey et al. [14]. Even though the experiment was shorter (around 365 days), these authors developed the factor for vertebrae as well as for muscle.

In both study areas, the TP for the Pelagic Thresher shark was around 4.5, classifying it as a tertiary predator, with no changes by sex or stage. These results agree with those of Lara et al. [9] and Fernández-Aguirre (in process), who reported the sardine *Sardinops sagax* as the main prey for *A. pelagicus* in BT, and anchovy *Engraulis mordax* in SR. Since both these prey feed on plankton, they have a similar TP and consequently, *A. pelagicus* presented a similar TP in both areas.

No variability in the TP by sex or stage was found in this study, which was also reported by Polo-Silva et al. [6], who derived a TP of 3.9 for this species. On the other hand, Calle-Morán [7] obtained the same results (4.5) as we did in our study. Therefore, once more, it can be inferred that *A. pelagicus* shows flexible trophic behavior depending on the availability of prey in the surrounding environment.

## 5. Conclusions

The present study is the first to analyze the chemical ecology of *A. pelagicus* using stable isotopes in Mexican waters. It provides information on the trophic ontogeny of this species on the western and eastern coasts of Baja California Sur. On the western coast, both isotopes remained stable by sex and stage; thus, this area presented stable feeding habitats for *A. pelagicus*. On the eastern coast, the  $\delta^{13}\text{C}$  values showed no differences between sexes, and did so only between stages, as neonates probably fed close to the coast. On both coasts, neonates presented a narrow and onshore feeding habit, while juveniles presented a wide and offshore one. *A. pelagicus* was classified as a tertiary predator in our study area.

The use of stable isotopes to measure ontogenetic changes is a very useful tool, especially in environments that are highly variable, such as SR. The use of vertebrae as an indicator of ontogenetic changes is more utile; however, this type of tool is complementary rather than exclusive. For example, the isotopic signatures in muscles show variations that could be explained by the isotopic signatures in the vertebrae. This study makes a novel contribution to the ecological knowledge base (including habitat use) regarding the Thresher Shark in waters off the Mexican Pacific and Gulf of California coasts, providing potential tools for the better management of the species in both areas.

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