



# Dietary ontogeny of the blue shark, *Prionace glauca*, based on the analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in vertebrae

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## Abstract

Ontogenetic changes in habitat and food preferences are common in nature; they reflect changes in the needs of organisms during their lifetime. Studying the dietary ontogeny of pelagic species is a difficult task, because of migratory processes and the inaccessibility of their habitats. As a result, their life history remains poorly understood, or even unknown. Here, we studied the dietary ontogeny of 18 blue sharks, *Prionace glauca*, using isotopic analysis in vertebrae. A total of 132 samples of vertebral collagen were taken (64 from males and 68 from females). The wide range of  $\delta^{13}\text{C}$  values ( $-16.8$  to  $-13.1\text{‰}$ ) suggests that these sharks use both coastal and oceanic areas for feeding. Small juveniles and adults preferred coastal areas, while medium-sized and large juveniles preferred oceanic areas. The estimated  $\delta^{15}\text{N}$  values ( $9.5$ – $19.0\text{‰}$ ) suggest that *P. glauca* is a top predator that occupies various trophic levels and/or it feeds across areas with different baseline  $\delta^{15}\text{N}$  (trophic position  $3.9$ – $8.4$ ). Isotopic enrichment and differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  thus suggest ontogenetic changes in habitat use and prey consumption between maturity stages. The use of hard anatomical structures (vertebrae) is highly relevant because they integrate information on the dietary ontogeny of this shark species.

## Introduction

Ontogenetic changes in the foraging behavior of predators are constant and often reflect a shift in life history priorities: from a juvenile strategy that maximizes growth (e.g., Heupel

et al. 2007) to an adult strategy that involves changes related to reproductive strategies (Lowe et al. 1996; Estupiñán-Montaño et al. 2009, 2018). The study of ontogenetic changes provides insights into niche utilization, as well as into the impact of selective predation on the structure and functioning of ecosystems (Juanes et al. 2001; Newman et al. 2012).

Historically, the study of ontogenetic changes in the trophic ecology of sharks (elasmobranchs) has been difficult, due to the migratory nature of these animals and to the inaccessibility of their habitat. These difficulties leave large portions of their life history unknown (Hazen et al. 2012). This lack of information is of concern, considering the vulnerability of elasmobranchs to overexploitation by fishing (Baum et al. 2003; Dulvy et al. 2008) and their importance as apex predators (Cortés 2002; Baum and Worm 2009; Grubbs 2010). Efficient conservation and management strategies for elasmobranchs depend on accurate information about trophic ecology and the early life stages of these species throughout ontogeny (Cortés 2002; Grubbs 2010; Carlisle et al. 2015).

Artisanal and industrial fishing is an important economic activity in Ecuador (Martínez-Ortíz et al. 2015). The artisanal fishery includes a long-line fishery, targeting dolphinfish (*Coryphaena hippurus*), tuna fish (*Thunnus* spp.), billfish, and sharks. From the 30 shark species caught in Ecuador, the

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blue shark, *Prionace glauca*, is the second most commonly captured species (Martínez-Ortíz et al. 2007), with sizes between 130 and 300 cm in total length. In Ecuador, few regulations for fishing this shark take into account data on biology and ecology, which is not the case of other countries that perform more research on this species (e.g., México) (Blanco-Parra et al. 2008; Carrera-Fernández et al. 2010; Rosas-Luis et al. 2017).

Studies performing stomach content analysis on *P. glauca* have shown that this species feeds on small pelagic crustaceans (Miller and Brodeur 2007; Preti et al. 2012; Hernández-Aguilar et al. 2016), cephalopods (Galván-Magaña et al. 2013; Loor-Andrade et al. 2017; Rosas-Luis et al. 2017), fish (McCord and Campana 2003; Pardo-Gandarillas et al. 2007), birds (Rivera et al. 2015), marine mammals (Markaida and Sosa-Nishizaki 2010), and other sharks (Kubodera et al. 2007; Markaida and Sosa-Nishizaki 2010). Because of the feeding pattern revealed by these studies, *P. glauca* has been considered an opportunistic consumer (Vaske-Júnior and Rincón-Filho 1998; Rivera et al. 2015). In contrast, in Ecuador *P. glauca* has been classified as a teuthophagous predator (i.e., that feeds on cephalopods) with specialist habits (Galván-Magaña et al. 2013; Loor-Andrade et al. 2017; Rosas-Luis et al. 2017).

Various authors have used stable isotopes analysis to study the feeding pattern of *P. glauca* more accurately. Stable isotope analysis has been performed on different tissues, including muscle (Hernández-Aguilar et al. 2016; Maya-Meneses et al. 2016; Rosas-Luis et al. 2017), liver (MacNeil et al. 2005), teeth (Polo-Silva et al. 2012), and cartilage (MacNeil et al. 2005). In these studies, the reported  $\delta^{13}\text{C}$  values ( $-19.3$  to  $-14.9\text{‰}$ ), and the reported  $\delta^{15}\text{N}$  values ( $11.7$ – $18.6\text{‰}$ ), suggest that *P. glauca* prefers to hunt in oceanic areas with different isotopic values reflecting differences as they consume a wide variety of prey (Hernández-Aguilar et al. 2016; Maya-Meneses et al. 2016; Rosas-Luis et al. 2017). It is thus clear that there is conflicting information on the true nature of the feeding behavior of *P. glauca*.

Despite the abundance of information on the trophic ecology of *P. glauca*, there is no data on the ontogenetic changes in the diet of this species. This lack of this information is a matter of concern because ontogenetic changes in the diet greatly affect recruitment and survival of juveniles, which is of great importance in maintaining the health of long-lived marine fish, such as elasmobranchs (Cortés 2002; Grubbs 2010).

The trophic ecology and the dietary ontogeny of various shark species have been studied through stomach content analysis and through stable isotopes analysis. While stomach content analysis generates information on the food that was consumed recently (i.e., in the last few hours or days), stable isotope analysis focuses on the isotopic composition of a tissue (e.g., muscle, blood, or skin), which reflects the

prey assimilated over a longer time frame, as well as the environment of the prey (Kim et al. 2012). Furthermore, anatomical structures formed by accretion (e.g., fish otoliths and shark vertebrae) reflect changes in the diet of organisms throughout their life (Estrada et al. 2006; Koch 2007; Kim et al. 2012). Thus, stable isotope analysis of these anatomical structures helps in identifying ontogenetic changes in the diet and the habitat of sharks (and other organisms) throughout their life history (Koch 2007; Kim et al. 2012; Carlisle et al. 2015).

Therefore, the aim of this study was to analyze the ontogenetic changes in the diet and habitat preferences of *P. glauca* of the eastern Tropical Pacific and the Ecuadorian Pacific, through the analysis of stable isotopes of carbon and nitrogen in vertebral collagen. We sought to gain information on the dietary patterns of *P. glauca*, on its dietary ontogeny, and on *P. glauca*'s trophic level, so that we can better understand the role that this species plays in oceanic and coastal food webs throughout its life cycle.

## Materials and methods

### Sample collection

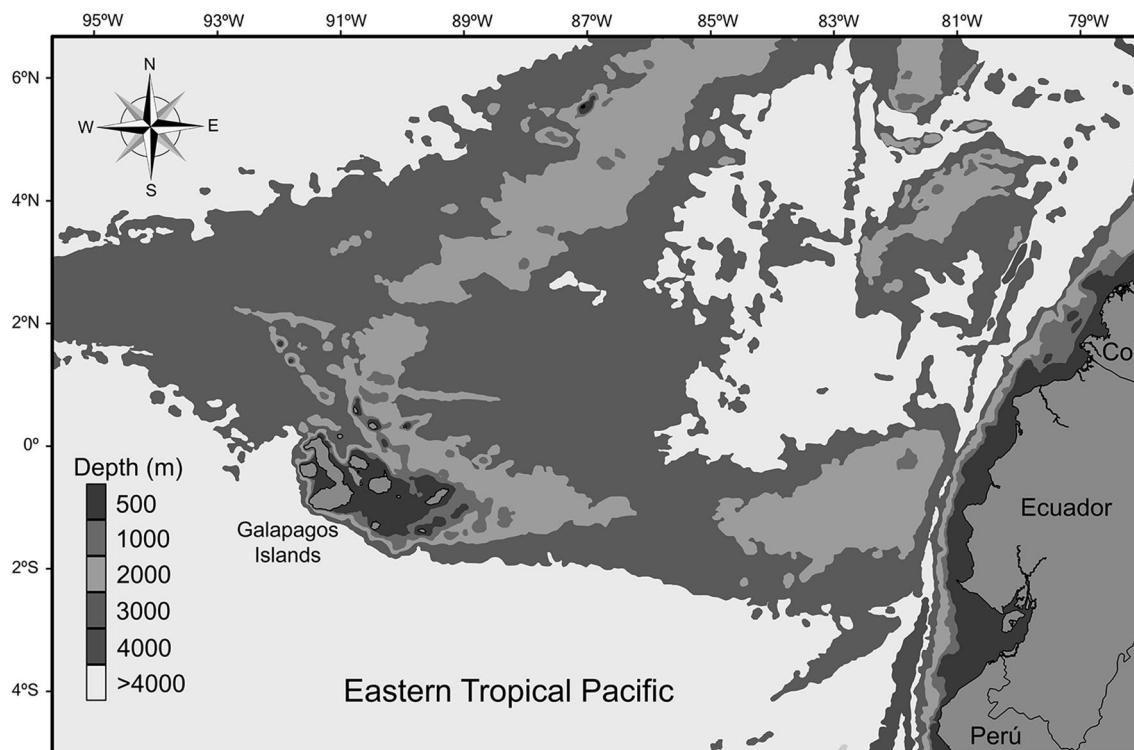
In 2011, a total of 18 blue sharks, *P. glauca*, were confiscated from illegal fishing operations in the Galápagos Marine Reserve, Ecuador (Fig. 1). For each specimen, total length (TL, in cm), precaudal length (in cm), and sex were recorded. Vertebrae were collected from the dorso-anterior part of the specimen (between the head and the first dorsal fin); vertebrae were then stored in pre-labeled plastic bags, and transported to the Galápagos Science Center of the Universidad San Francisco de Quito (Galápagos Campus), where they were cleaned.

For each vertebra, the neural arch and connective tissue were removed, leaving the vertebral body completely clean; vertebrae were then dried at room temperature. The vertebrae were subsequently transferred to the Laboratory of Fish Ecology of the Centro Interdisciplinario de Ciencias Marinas in La Paz, México of the Instituto Politécnico Nacional.

### Sample preparation and analysis

Each vertebra was sampled systematically using a microdrill with a 0.5-mm drill bit, to obtain vertebral collagen samples between 0.3 and 2.6 mg along the whole vertebra (from the centrum towards the outside edge).

To remove residual inorganic carbon, the samples were placed in a dryer and exposed to 37% HCl vapor for 12–14 h (Hedges and Stern 1984). Vertebral collagen samples were then stored in 3.2 × 4-mm tin capsules. The C:N ratio was estimated to determine whether the treatment applied to



**Fig. 1** Geographic location of Galápagos Islands, Ecuador

vertebral collagen was effective; a ratio  $\leq 3.5$  indicates that demineralization was effective (Hussey et al. 2012).

Stable isotope analysis was carried out at the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC-UGR), Spain. The carbon and nitrogen isotopic compositions of organic matter (vertebral collagen) were determined using an online Carlo Erba NA 1500 NC elemental analyzer (Milan, Italy) coupled online via ConFlo III interface to a Delta Plus XP mass spectrometer (EA-IRMS; ThermoQuest, Bremen, Germany).

The stable isotope composition is reported as  $\delta$  values per mil (‰) based on the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000, \quad (1)$$

where  $R$  is the isotope ratio ( $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ) of the sample or the standard (V-PDB and AIR for carbon and nitrogen, respectively). Commercial  $\text{CO}_2$  and  $\text{N}_2$  were used as the internal standard for the isotopic analyses. Internal standards of  $-30.63\text{‰}$  and  $-11.65\text{‰}$  (V-PDB) have been used for  $\delta^{13}\text{C}$  analysis and internal standards of  $-1.0\text{‰}$  and  $+16.0\text{‰}$  (AIR) for  $\delta^{15}\text{N}$ . A precision calculated, after correction of the mass spectrometer daily drift, from standards systematically interspersed in analytical batches was better than  $\pm 0.1\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The standard for reporting carbon measurement is V-PDB (Vienna-PDB) and for

nitrogen measurements is atmospheric nitrogen (AIR). Reference gases and in-house standards (with different C:N ratios and isotopic composition) were calibrated against International Reference Materials (USGS-24 and IAEA-C6 for carbon; IAEA-N1, IAEA-N2 and IAEA-N3 for nitrogen).

### Dietary ontogeny

Ontogenetic isotopic enrichment patterns were inferred from a sampling starting point located 2 mm from the vertebral centrum. Relative enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  were calculated using the algorithm proposed by Estrada et al. (2006):

$$\text{Enrichment } Y = \left( \frac{\delta^z Y_{x\text{mm}} - \delta^z Y_{2\text{mm}}}{\delta^z Y_{x\text{mm}}} \right), \quad (2)$$

where  $Y$  is the element of interest ( $^{13}\text{C}$  or  $^{15}\text{N}$ ),  $z$  is the atomic mass of the element, and  $x$  is the location of each vertebral collagen sample, relative to the location of the first sampling point (in mm).

The TL for each vertebral radius sampled was estimated using the following equation:  $\text{TL} = 66.931 + 11.184$  (vertebral radius, VR), using a coefficient of determination ( $R^2$ ) of 0.41 (Estupiñán-Montaño, unpublished data). In addition, age ( $t$ ) at each vertebral collagen sampling location (i.e., each millimeter) was estimated according to the von Bertalanffy growth function:

$$L_t = L_\infty \left[ 1 - e^{-K(t-t_0)} \right], \quad (3)$$

where  $L_t$  is the predicted length at  $t$  age,  $L_\infty$  is the mean asymptotic total length,  $K$  is the growth rate ( $\text{years}^{-1}$ ), and  $t_0$  is the theoretical age at which the shark had zero length. Age at every millimeter (vertebral collagen sampling location) was thus estimated as follows:

$$t = -\frac{\ln\left(1 - \frac{L_t}{L_\infty}\right)}{K} + t_0. \quad (4)$$

The parameters of the von Bertalanffy growth function were obtained from Blanco-Parra et al. (2008), based on sample collected close to the current study area. Blanco-Parra et al. estimated  $L_\infty$  299.85 and 237.5 cm LT for males and females, respectively;  $K_{\text{males}}$  was  $0.10 \text{ years}^{-1}$ ;  $K_{\text{females}}$  was  $0.15 \text{ years}^{-1}$ ;  $t_0$  was  $-2.44$  and  $-2.15$  years for males and females, respectively.

Maturity stages were determined based on the size of sharks at birth (45 cm TL; Nakano 1994) and the size at which 50% of the sharks reach maturity ( $TL_{50}$ ). In males,  $TL_{50}$  is 187 cm (Briones-Mendoza et al. 2016). In females,  $TL_{50}$  is 175 cm (Cruz-Ramírez et al. 2012). Based on the estimated maturity stages, the sharks were grouped into four categories: small juveniles (SJuv, 89–100 cm TL), medium-sized juveniles (MJuv, 100–133 cm TL), large juveniles (LJuv, 134–179 cm TL for males; and 134–168 cm TL for females) and adults (males  $\geq 190$  cm LT, females 179–190 cm TL).

### Trophic position

The trophic position (TP) of *P. glauca* was estimated by size, sex, and maturity stage, implementing a Bayesian approach within the R statistical environment (R Development Core Team 2018), using the *tRophicPosition* package (version 0.7.5; Quezada-Romegialli et al. 2018). Analyses of TP were performed using the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *P. glauca*; while the signatures of the zooplankton ( $\delta^{13}\text{C} = -20.1 \pm 0.7\text{‰}$  SD and  $\delta^{15}\text{N} = 5.3 \pm 0.8\text{‰}$  SD; Páez-Rosas et al. unpublished data), were used as isotopic baseline with  $\lambda = 2$ . A Bayesian model of one baseline and two trophic discrimination factors (TDF) was run with two parallel chains and 20,000 adaptive interactions, assuming a  $\lambda$  of the baseline taxa = 2. Accordingly, *P. glauca*'s TP value was estimated adopting a TDF of  $\Delta^{13}\text{C} = 4.2 \pm 0.7\text{‰}$  SD and  $\Delta^{15}\text{N} = 2.5 \pm 1.1\text{‰}$  SD (Kim et al. 2012) for carbon and nitrogen, respectively.

### Niche breadth and isotopic overlap

Niche breadth and isotopic overlap between individuals, sexes, and maturity stages were estimated using the Stable

Isotope Bayesian Ellipses method in R (Jackson et al. 2011), which is available in the Stable Isotope Analysis in R package (Jackson et al. 2011). This analysis uses ellipses, calculated by a covariance matrix that defines their shapes and areas (Jackson et al. 2011), to estimate trophic niche breadth (Standard Ellipse Corrected Area, SEAc). This method also allows isotopic overlap to be estimated, based on the overlap between ellipses.

### Statistical analyses

The normality and homoscedasticity of the isotopic data were tested with the Kolmogorov–Smirnov and Levene's test, respectively. Parametric (ANOVA, Student's  $t$  test) or non-parametric (Kruskal–Wallis test, Wilcoxon signed-rank test) analyses of variance were used to test for isotopic differences between categories (sex, maturity stages, and TP). A post hoc multiple comparison test (Tukey's test and Dunn's test) was then performed to identify specific differences between categories.

## Results

The 18 vertebrae of *P. glauca* (nine males and nine females) allowed 132 samples of vertebral collagen to be obtained (64 from males and 68 from females). The C:N ratio was in the range of 2.9–3.5, indicating demineralization was sufficient (Table 1).

### Food sources

The  $\delta^{13}\text{C}$  values of *P. glauca* varied between  $-16.8$  and  $-13.1\text{‰}$  (mean  $\pm$  SE =  $-14.9 \pm 0.10\text{‰}$ ; Kolmogorov–Smirnov test,  $D = 0.06$ ,  $P = 0.79$ ; Levene's test,  $F = 0.73$ ,  $P = 0.77$ ). Isotopic analysis by sex showed that  $\delta^{13}\text{C}$  was statistically lower in females (Student's  $t$  test,  $t_{129.55} = 2.707$ ,  $P = 0.008$ ). The  $\delta^{13}\text{C}$  values of males ranged from  $-16.8$  to  $-14.1\text{‰}$  ( $-15.1 \pm 0.10\text{‰}$ ) while females ranged from  $-16.8$  to  $-13.1\text{‰}$  ( $-14.8 \pm 0.10\text{‰}$ ).

The maturity stages (combining males and females) also showed significant statistical differences in  $\delta^{13}\text{C}$  (ANOVA,  $F_{3,28} = 11.83$ ,  $P \leq 0.01$ ). The differences were between MJuv and SJuv (post hoc Tukey's test,  $P < 0.01$ ; Table 2, Fig. 2a), MJuv and LJuv (Tukey's test,  $P < 0.01$ ; Table 2, Fig. 2a), and MJuv and adults (Tukey's test,  $P < 0.01$ ; Table 2, Fig. 2a).

A comparison of the  $\delta^{13}\text{C}$  values of males and females at each maturity stage (Table 2) showed that there were no significant differences between SJuv of both sexes ( $t_{9.62} = 0.42$ ,  $P = 0.69$ ), between LJuv of both sexes ( $t_{55.49} = -1.39$ ,  $P = 0.18$ ) and between adults of both sexes ( $t_{6.38} = -1.40$ ,  $P = 0.21$ ). However,  $\delta^{13}\text{C}$  values of MJuv

**Table 1**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as a function of vertebral radius (VR, in mm), estimated total length ( $\text{TL}_{\text{estimated}}$  in cm), estimated age (in years), maturity stage, C:N ratio and trophic position in *Prionace glauca* in Ecuadorian waters

Vertebral radius (mm)	N	TL <sub>estimated</sub> (Mean ± SE)	Age <sub>estimated</sub> (Mean ± SE)	Maturity stage	Ratio (C:N) (Mean ± SE)	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		Trophic position	
						Range	Mean ± SE	Range	Mean ± SE	95% CI	Mode
2	16	89.3 ± 0.01	1.1 ± 0.01	Small juveniles	3.29 ± 0.04	-15.7 to -13.1	-14.6 ± 0.16	10.4–18.3	15.7 ± 0.47	5.6–6.8	6.2
3	13	100.5 ± 0.01	1.6 ± 0.01	Median juveniles	3.25 ± 0.04	-16.8 to -14.4	-15.4 ± 0.20	15.7–18.0	16.9 ± 0.20	6.1–7.3	6.6
4	11	111.7 ± 0.01	2.1 ± 0.02	Median juveniles	3.27 ± 0.05	-15.9 to -13.5	-15.3 ± 0.22	9.4–19.0	15.9 ± 0.85	5.7–6.8	6.2
5	17	122.9 ± 0.01	2.8 ± 0.01	Median juveniles	3.20 ± 0.04	-15.9 to -14.4	-15.3 ± 0.10	14.9–18.6	16.7 ± 0.22	6.0–7.2	6.5
6	15	134.0 ± 0.01	3.4 ± 0.01	Large juveniles	3.19 ± 0.04	-15.9 to -14.1	-15.0 ± 0.15	12.6–18.4	15.9 ± 0.38	5.8–6.9	6.2
7	12	145.2 ± 0.01	4.2 ± 0.01	Large juveniles	3.22 ± 0.04	-15.5 to -14.2	-14.9 ± 0.13	12.2–18.2	15.9 ± 0.52	5.7–6.8	6.2
8	15	156.4 ± 0.01	5.0 ± 0.01	Large juveniles	3.13 ± 0.04	-15.9 to -14.1	-15.0 ± 0.13	12.2–17.9	15.2 ± 0.48	5.5–6.0	5.9
9	14	167.6 ± 0.01	5.8 ± 0.04	Large juveniles	3.22 ± 0.04	-16.0 to -14.1	-14.7 ± 0.12	11.5–17.0	14.2 ± 0.50	5.1–6.1	5.5
10	11	178.8 ± 0.01	7.0 ± 0.09	Adults	3.16 ± 0.05	-15.4 to -13.7	-14.5 ± 0.16	11.3–17.6	14.6 ± 0.68	5.2–6.2	5.7
11	8	190.0 ± 0.01	8.0 ± 0.18	Adults	3.16 ± 0.05	-15.7 to -14.1	-14.6 ± 0.19	10.5–17.6	12.7 ± 0.85	4.6–5.4	4.9

CI credibility interval

differed significantly between sexes ( $t_{34.82} = -2.56, P = 0.01$ ; Table 2, Fig. 2b).

### Trophic position

The  $\delta^{15}\text{N}$  values of *P. glauca* varied between 9.5 and 19.0‰ ( $15.5 \pm 0.20\text{‰}$ ; Kolmogorov–Smirnov test,  $D = 0.18, P < 0.01$ ; Levene’s test,  $F = 1.02, P = 0.44$ ). The  $\delta^{15}\text{N}$  values of males varied between 10.5 and 17.9‰, while the values of females varied between 9.5 and 19.0‰ (Wilcoxon signed-rank test,  $T = 2726, N = 132, P = 0.01$ , Table 2).

Maturity stages showed significant statistical differences in  $\delta^{15}\text{N}$  values (Kruskal–Wallis test,  $H_3 = 21.134, P < 0.01$ ) between MJuv and LJuv (post hoc Dunn’s test,  $P < 0.01$ ), and between MJuv and adults (Dunn’s test,  $P < 0.0003$ ; Table 2, Fig. 2a).

A comparison of the  $\delta^{15}\text{N}$  values of males and females at each maturity stage showed that there were significant differences between sexes in LJuv ( $T = 599, N = 60, P = 0.028$ ) and in adults ( $T = 48, N = 15, P = 0.003$ ), but not in SJuv ( $T = 28, N = 16, P = 0.72$ ) or MJuv ( $T = 210, N = 41, P = 1$ ; Table 3, Fig. 2b).

The estimated TP of *P. glauca* according to  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  varied from 3.9 to 8.4 (Mode [95% CI] = 6.1 [5.6–6.6],  $N = 396$ ). Likewise, TP estimated by sex suggests that both sexes have similar TP, varied between 5.1 and 7.2 for males, and 5.4 and 7.3 for females (Table 2). Besides, the estimated TP for maturity stage for sexes combined suggests that adults (3.8–5.2) have lower TP than SJuv. (5.5–7.6), MJuv. (5.7–7.8) and LJuv. (5.0–6.9) (Table 1). On the other hand, the estimated TP for maturity stage of males and females separately, suggest that adult males (3.7–5.2) mostly use resources the minor TP, while that SJuv. (5.4–7.5), MJuv. (5.7–7.8), LJuv. (4.7–7.0) of males, and females (SJuv. [5.1–7.5], MJuv. [5.4–7.8], LJuv. [5.2–7.5] and adults [5.2–7.2]) (Table 2).

### Dietary ontogeny

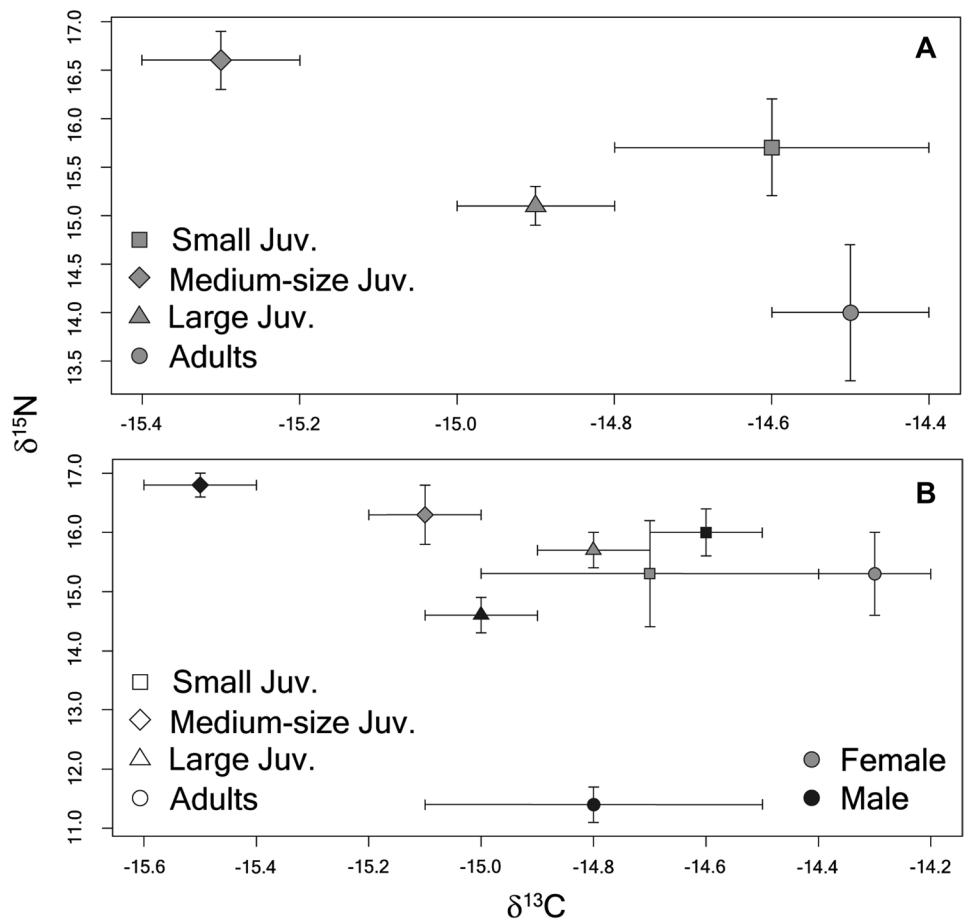
The reconstruction of isotopic enrichment patterns revealed that the vertebral collagen samples taken between 3 mm and 9 mm from the centrum of the vertebrae were enriched in  $^{13}\text{C}$ , relative to the sampling starting point located at 2 mm from the centrum (Table 1, Fig. 3). In contrast, samples taken at 10 mm and 11 mm from the centrum of the vertebrae were depleted in  $^{13}\text{C}$  (Table 1, Fig. 3). A clear enrichment in  $^{15}\text{N}$  was also observed between 3 mm and 7 mm from the centrum of the vertebrae, while samples at/ or over 8 mm from the centrum tended to be depleted in  $^{15}\text{N}$  (Table 1, Fig. 3). Using the isotopic values of SJuv (Table 1) as a starting point, the reconstruction of isotopic enrichment patterns by maturity stage indicates that MJuv were

**Table 2**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as a function of maturity stage, sex, estimated total length ( $\text{TL}_{\text{estimated}}$ , in cm) and age ( $\text{Age}_{\text{estimated}}$ , in years), and trophic position in *Prionace glauca* in Ecuadorian waters

Maturity stage	Sex	N	TL estimated (mean $\pm$ SE)	Age estimated (mean $\pm$ SE)	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		Trophic position	
					Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	95% CI	Mode
Small juveniles	Male	8	89.3 $\pm$ 0.00	1.1 $\pm$ 0.00	-15.0 to -14.1	-14.6 $\pm$ 0.14	14.6–17.8	16.0 $\pm$ 0.35	5.8–6.9	6.3
	Female	8	89.3 $\pm$ 0.00	1.0 $\pm$ 0.00	-15.7 to -13.1	-14.7 $\pm$ 0.31	10.4–18.3	15.3 $\pm$ 0.89	5.5–6.6	6.0
Median Juveniles	Male	21	112.7 $\pm$ 2.17	2.3 $\pm$ 0.12	-16.8 to -14.8	-15.5 $\pm$ 0.10	15.6–18.0	16.8 $\pm$ 0.15	6.0–7.2	6.6
	Female	20	112.8 $\pm$ 2.13	2.2 $\pm$ 0.11	-16.2 to -13.5	-15.1 $\pm$ 0.14	9.5–19.0	16.3 $\pm$ 0.49	5.8–7.0	6.4
Large juveniles	Male	26	152.9 $\pm$ 6.03	4.7 $\pm$ 0.41	-16.0 to -14.1	-15.0 $\pm$ 0.11	11.5–16.9	14.8 $\pm$ 0.37	5.2–6.2	5.7
	Female	30	148.9 $\pm$ 2.24	4.5 $\pm$ 0.17	-15.7 to -14.1	-14.8 $\pm$ 0.08	12.2–18.4	15.7 $\pm$ 0.34	5.7–6.7	6.1
Adults	Male	9	185.0 $\pm$ 10.5	7.2 $\pm$ 0.71	-15.7 to -14.1	-14.8 $\pm$ 0.20	10.5–14.8	12.1 $\pm$ 0.66	4.1–4.8	4.2
	Female	10	182.2 $\pm$ 1.71	7.6 $\pm$ 0.21	-15.0 to -13.7	-14.3 $\pm$ 0.14	11.8–17.6	15.3 $\pm$ 0.70	5.5–6.6	6.0
Overall	Male	64	136.3 $\pm$ 4.00	3.8 $\pm$ 0.25	-16.8 to -14.1	-15.1 $\pm$ 0.07	10.5–17.9	15.2 $\pm$ 0.25	5.5–6.6	6.0
	Female	68	136.2 $\pm$ 3.65	3.9 $\pm$ 0.26	-16.2 to -13.1	-14.8 $\pm$ 0.07	9.5–19.0	15.8 $\pm$ 0.25	5.7–6.8	6.2
Small juveniles	Combined	16	89.3 $\pm$ 0.01	1.1 $\pm$ 0.01	-15.0 to -14.1	-14.6 $\pm$ 0.14	14.6–17.8	16.0 $\pm$ 0.35	5.7–6.7	6.2
Median juveniles	Combined	41	112.8 $\pm$ 4.83	2.2 $\pm$ 0.33	-16.8 to -13.5	-15.3 $\pm$ 0.09	9.5–19.0	16.6 $\pm$ 0.32	6.0–7.2	6.5
Large juveniles	Combined	56	150.8 $\pm$ 4.11	4.6 $\pm$ 0.28	-16.0 to -14.1	-14.9 $\pm$ 0.08	11.5–18.4	15.3 $\pm$ 0.26	5.5–6.5	6.0
Adults	Combined	19	183.5 $\pm$ 7.15	7.4 $\pm$ 0.48	-15.7 to -13.7	-14.5 $\pm$ 0.14	10.5–17.6	13.8 $\pm$ 0.43	5.0–6.0	5.5

CI credibility interval

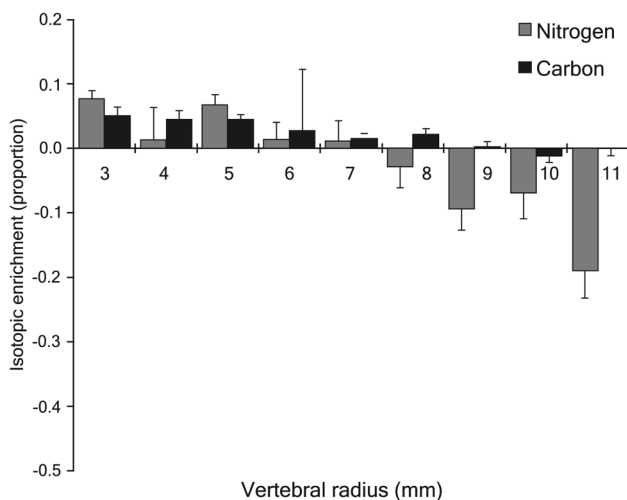
**Fig. 2** Isotopic values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  SE) by maturity stage of *Prionace glauca* at Galápagos Islands. **a** Both combined sexes; **b** separated sexes



**Table 3** Mean  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and trophic position based on estimated age in *Prionace glauca* in Ecuadorian waters

Vertebral radius (mm)	Age <sub>estimated</sub> (years)	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Trophic position Mode (95% C.I.)
			Mean $\pm$ SE	Mean $\pm$ SE	
2	1	16	$-14.6 \pm 0.2$	$15.7 \pm 0.5$	6.1 (5.6–6.7)
3–4	2	24	$-15.4 \pm 0.1$	$16.4 \pm 0.4$	6.4 (5.9–7.1)
5–6	3	26	$-15.2 \pm 0.1$	$16.6 \pm 0.2$	6.5 (5.9–7.2)
6–7	4	18	$-15.0 \pm 0.1$	$15.7 \pm 0.4$	6.2 (5.7–6.7)
8	5	15	$-15.0 \pm 0.1$	$15.3 \pm 0.5$	5.9 (5.4–6.5)
9	6	14	$-14.7 \pm 0.1$	$14.3 \pm 0.5$	5.5 (5.1–6.1)
10	7	11	$-14.5 \pm 0.2$	$14.6 \pm 0.7$	5.7 (5.3–6.3)
11	8	5	$-14.8 \pm 0.3$	$11.4 \pm 0.3$	4.4 (4.1–4.9)
11	9	3	$-14.4 \pm 0.2$	$14.9 \pm 1.6$	5.9 (5.4–6.4)

CI credibility interval

**Fig. 3** Isotopic enrichment (mean + SE) of  $^{13}\text{C}$  (black) and  $^{15}\text{N}$  (gray) in the *Prionace glauca* vs. vertebral radius, relative to values at the 2-mm sampling location ( $N=16$ )

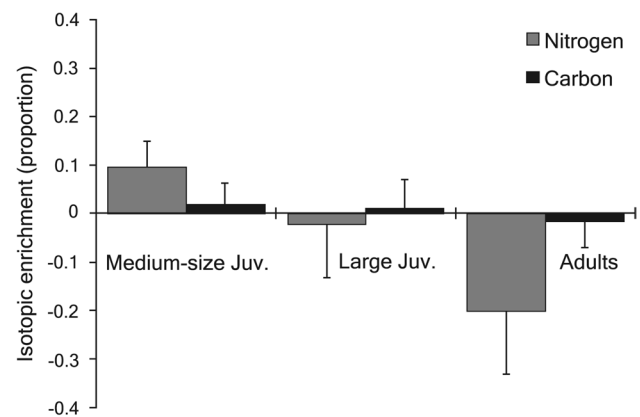
enriched in both  $^{13}\text{C}$  and  $^{15}\text{N}$ , relative to LJuv and adults (Table 1, Fig. 4).

The TL and age, estimated by VR and by maturity stage, are presented in Tables 1, 2, and 3.

### Niche breadth and isotopic overlap

The overall estimated SEAc was  $3.8\%o^2$ , suggesting that *P. glauca* has a broad isotopic niche. A broad isotopic niche was estimated for each sex (in males SEAc was  $3.7\%o^2$ , and in females SEAc was  $3.9\%o^2$ ) and for each maturity stage (Table 4, Fig. 5).

Likewise, a high isotopic overlap was observed between individuals (Table 4), sexes (SIAR's overlap = 2.35), and maturity stages (Table 5, Fig. 5).

**Fig. 4** Isotopic enrichment (mean + SE) of  $^{13}\text{C}$  (black) and  $^{15}\text{N}$  (gray) in the *Prionace glauca* vs. vertebral radius, relative to values at the small juvenile sampling location ( $N=16$ )

## Discussion

This study is the first to use stable isotope analysis of *P. glauca*'s vertebrae. Other studies have been conducted to determine the dietary habits of *Prionace glauca* through stable isotope analysis of muscle (e.g., Estrada et al. 2003; MacNeil et al. 2005; Revill et al. 2009), liver (MacNeil et al. 2005), teeth (Polo-Silva et al. 2012), and cartilage (MacNeil et al. 2005). Our use of hard anatomical structures, such as vertebrae, is highly relevant because they integrate information on the dietary ontogeny of this shark species.

### Food sources

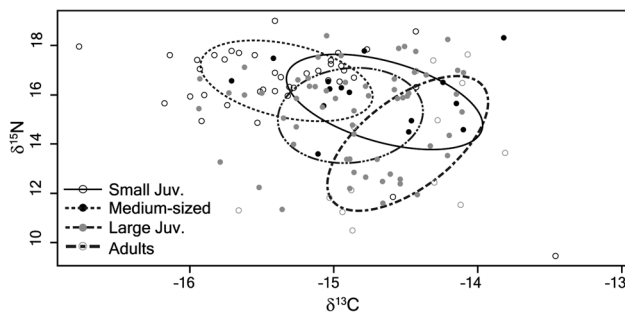
Our analysis of *P. glauca*'s food sources, measuring the  $\delta^{13}\text{C}$  signature of vertebrae, suggests that *P. glauca* prefers to feed in trophic webs that are supported by pelagic phytoplankton (mean  $\delta^{13}\text{C} = -22\%o$ ) and by benthic macroalgae (mean  $\delta^{13}\text{C} = -17\%o$ ) (Peterson and Fry 1987; France 1993) since, in our study,  $\delta^{13}\text{C}$  was  $-14.9 \pm 0.60\%o$  SD ( $N=132$ ). Our results are in agreement with those of MacNeil et al. (2005), who analyzed cartilage samples of *P. glauca* from the northeastern coast of the United States (Atlantic Ocean), reporting an average  $\delta^{13}\text{C}$  of  $-14.9 \pm 0.35\%o$  SD ( $N=14$ ).

The preference of *P. glauca* for feeding in trophic webs supported by pelagic phytoplankton and benthic macroalgae could be explained by the tendency of *P. glauca* to feed on planktivore species (e.g., Myctophidae, Kubodera et al. 2007), Clupeidae (Rosas-Luis et al. 2017), and benthivore species (e.g., Merlucciidae, Looor-Andrade et al. 2017; McCord and Campana 2003). It is also possible that *P. glauca* prefers coastal and oceanic food webs for feeding, which provide different basic carbon sources. The presence in coastal areas of primary production enriched in  $^{13}\text{C}$  (seagrasses and macroalgae) and impoverished in  $^{13}\text{C}$

**Table 4** Isotopic overlap of *Prionace glauca* by individuals, in Ecuadorian waters

Ind.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	SEA <sub>C</sub>
1	–	0	0.4	0	0	0	0.1	0.3	0	0	0	0	0	0	0	0.3	0	0	2.38
2		–	<b>2.7</b>	<b>1.5</b>	0.5	<b>1.5</b>	<b>1.8</b>	<b>2.8</b>	<b>1.5</b>	0	0.8	<b>1.3</b>	<b>1.1</b>	<b>1</b>	0.3	0.6	<b>1</b>	0	2.96
3			–	<b>1.7</b>	0.7	<b>1.2</b>	<b>2</b>	<b>3.5</b>	<b>1.4</b>	0	<b>1</b>	<b>1.3</b>	<b>1.3</b>	<b>1.3</b>	0.4	0.8	<b>1.2</b>	0.3	3.60
4				–	0.6	<b>1.4</b>	0.7	<b>1.8</b>	<b>1.5</b>	0	0.9	0.3	<b>1.3</b>	0.9	0	0	0.9	0.3	1.96
5					–	0.8	0	0.7	<b>1.2</b>	<b>6.9</b>	<b>1.3</b>	0	<b>1.6</b>	0.6	0	0	0.7	0.9	1.69
6						–	0.5	<b>1.3</b>	<b>1.9</b>	0	0.7	0.2	<b>1.1</b>	0.6	0	0	0.7	0.2	2.19
7							–	<b>2.3</b>	0.5	0	0.6	<b>1.8</b>	0.4	0.6	0.9	<b>1</b>	0.9	0	2.63
8								–	<b>1.6</b>	0	<b>1.3</b>	<b>1.6</b>	<b>1.4</b>	<b>1.3</b>	0.7	<b>1</b>	<b>1.5</b>	0.4	4.06
9									–	0	<b>1.1</b>	0.2	<b>1.6</b>	<b>0.8</b>	0	0	0.8	0.6	2.62
10										–	0	0	0.2	0	0	0	0	0	0.73
11											–	0.5	<b>1.7</b>	0.9	0.7	0.4	<b>1.5</b>	<b>1</b>	2.39
12												–	0.2	0.4	<b>1.8</b>	<b>2.4</b>	<b>1.1</b>	0	4.06
13													–	<b>1.1</b>	0.1	0	<b>1.2</b>	<b>1.1</b>	3.10
14														–	0.1	0.1	<b>1.1</b>	0.3	1.34
15															–	<b>2.2</b>	<b>1.1</b>	0.2	2.59
16																–	0.9	0	3.66
17																	–	0.3	2.22
18																		–	1.33

Bold values indicate the significant isotopic overlap between individuals  
*Ind* individual



**Fig. 5** The niche overlap between maturity stages of *Prionace glauca* in the Pacific Ecuadorian and Eastern Tropical Pacific. The ellipses represent the estimated standard ellipse area determined by the SIBER analysis

**Table 5** Niche breadth and isotopic overlap of *Prionace glauca* by maturity stages (combined sexes), in Ecuadorian waters

Category	SJuv	MJuv	Ljuv	Adul	Isotopic niche (SEA <sub>C</sub> [‰ <sup>2</sup> ])
SJuv	–				3.6
MJuv	1.14	–			2.9
Ljuv	1.95	1.01	–		3.0
Adul	1.69	8.40e–07	0.96	–	3.7

*SJuv* small juveniles, *MJuv* medium-sized juveniles, *LJuv* large-sized juveniles, *Adul* adults

(halophytes, mangroves and phytoplankton) can explain this dispersion (Duarte et al. 2018). In keeping with this notion, *P. glauca* has been reported to be a highly mobile species that often migrates from coastal to oceanic areas (Carey et al. 1990). Finally, it is also possible that, as a foraging strategy, this species predate different trophic levels.

Predation at different trophic levels is supported by studies of stomach content analysis in *P. glauca*, which have identified various preys including coastal cephalopods (e.g., Markaida and Sosa-Nishizaki 2010, in Ensenada, México), coastal fish (e.g., McCord and Campana 2003, in Nova Scotia, Canada; Pardo-Gandarillas et al. 2007, in Chile), and oceanic cephalopods (e.g., Preti et al. 2012, in the United States; Galván-Magaña et al. 2013, in the Ecuadorian and Mexican Pacific; Loor-Andrade et al. 2017, in Ecuador).

Here we report a broad range of δ<sup>13</sup>C values in *P. glauca*, which could be related to differences between sexes. For instance, we found a difference in δ<sup>13</sup>C values between males and females. This finding is in agreement with previous reports by McCord and Campana (2003), and Loor-Andrade et al. (2017), who found dietary differences between sexes, through stomach content analysis in Canada and Ecuador, respectively. Thus, according to our results and to the current literature, in the equatorial region, *P. glauca* males spend more time feeding in oceanic areas, while females use coastal areas. Similarly, *P. glauca* females were reported to feed on a neritic-oceanic squid of submarine ridges and



seamounts (*Ancistrocheirus lessueuri*) (Jereb and Roper 2010), where upwelling processes lead to enhanced productivity, such as in the Galapagos Island (Palacios 2002; Palacios et al. 2006). This behavior could explain the  $\delta^{13}\text{C}$  values reported here for adult females ( $-14.3 \pm 0.10\%$  SE) since  $\delta^{13}\text{C}$  values over  $-15.0\%$  are typical of coastal zones or areas of high productivity (e.g., Niño-Torres et al. 2006).

According to our analysis of the  $\delta^{13}\text{C}$  signature of MJuv and LJuv it seems that, at these maturity stages, sharks forage in oceanic areas. In keeping with this finding, stomach content analysis of blue sharks caught in the transition zone of the western north Pacific (geographic coordinates: 3.5–4.5°N, 155–185°E) suggests that these oceanic areas could be used as a feeding area by *P. glauca* at early life stages, and possibly also as a breeding area (Kubodera et al. 2007). Young and sub-adult blue sharks caught in this zone feed mostly on large, inactive cephalopods, with semi-gelatinous bodies and neutral buoyancy that inhabit mesopelagic to bathypelagic areas (Kubodera et al. 2007). On the other hand, we have shown that SJuv and adults prefer to use coastal areas for feeding. These changes in feeding zones preference may be explained by *P. glauca*'s breeding strategy since adult females spend more time in coastal areas during the 9–11 months of gestation (Carrera-Fernández et al. 2010), where food is more abundant and easier to find; these areas provide them with enough food for the development of their offspring, and with safe places for giving birth.

### Trophic position

Our results show that *P. glauca* is a top predator that occupies different trophic levels over its lifetime consuming prey at various trophic levels from trophic webs with different baseline  $\delta^{15}\text{N}$ , which may be changed over time due to environmental factors. For example, upwelling of water that has undergone reduction of  $\text{NO}_3^-$  generates residual nitrates enriched in  $\delta^{15}\text{N}$  (Granger et al. 2008), which would lead to primary production enriched by  $^{15}\text{N}$ , which would result in apparent jumps of one or two units in the trophic chain. This allows *P. glauca* to play different roles in the food chains of the eastern Tropical Pacific and Ecuadorian Pacific, from primary piscivores (TP 3.5–4.5) to tertiary piscivores (TP 5.5–7.0) (Hussey et al. 2015).

The broad range of TP reported here for *P. glauca* (3.9–8.4) may be related to the consumption of organisms near the base of the food chain, such as euphausiids (Harvey 1989; Miller and Brodeur 2007; Preti et al. 2012), myctophids (Markaida and Sosa-Nishizaki 2010), clupeids (Miller and Brodeur 2007), and pelagic crustaceans (Preti et al. 2012; Hernández-Aguilar et al. 2016). On the other hand, the high TP estimated (TP > 8) may be related to the uptake of prey of trophic web with high  $\delta^{15}\text{N}$  values of particulate organic matter (POM) associated with bacterial

and/or decomposition (Macko and Estep 1984), and the consumption of organisms waste with high TP. However, this conclusion should be taken with caution due to the lack information, thus, more studies are necessary to corroborate this hypothesis. In keeping with our results, a wide range of TP can be inferred from previous independent studies that either use stomach content analysis (TP > 4.0; Cortés 1999), or stable isotope analysis (TP 3.8–4.7; Estrada et al. 2003; Hernández-Aguilar et al. 2016; Maya-Meneses et al. 2016). Also in agreement with our findings, Li et al. (2014, 2016) report that, in the mid-east Pacific Ocean (TP 3.9) and northeast Pacific Ocean (TP 4.5), *P. glauca* acts as a primary or secondary piscivore (with a range of TP 4.0–5.0; Hussey et al. 2015).

Here we report a considerable variability in  $\delta^{15}\text{N}$ , which may reflect *P. glauca*'s long-distance migratory movements (Carey et al. 1990; Vandeperre et al. 2014) since  $\delta^{15}\text{N}$  values from different trophic bases would be mirrored in the vertebrae (Revill et al. 2009; Polo-Silva et al. 2013; Kiszka et al. 2014). These  $\delta^{15}\text{N}$  values thus indicate the consumption of food in different habitats during *P. glauca*'s lifetime (Heithaus et al. 2013).

The high variability of  $\delta^{15}\text{N}$  that we report here could be attributed to (1) seasonal feeding changes (MacNeil et al. 2005); (2) the consumption of prey from low trophic positions that may be very close to the trophic base (MacNeil et al. 2005); (3) low  $\delta^{15}\text{N}$  values estimated for the sediments of the study area (5–7‰; Farrell et al. 1995) and the oligotrophic conditions north of the Equatorial Front (Palacios 2002); (4) upwelling of the Equatorial current that could transport bottom material depleted of  $\delta^{15}\text{N}$ , influenced by potential inorganic substrates (Vander-Zanden and Rasmussen 1999); and (5) vertical migration of *P. glauca* to depths over 200 m searching for food (Carey et al. 1990; Vandeperre et al. 2014).

### Dietary ontogeny

Some shark species exhibit ontogenetic changes in their diet (Young et al. 2010; Kim et al. 2012; Preti et al. 2012), which is exactly what we found in *P. glauca* from Ecuadorian waters. The  $\delta^{13}\text{C}$  values of SJuv of both sexes and of adult females (Table 2) suggest that *P. glauca* searches for food mainly in coastal or highly productive areas. Immature sharks, on the other hand, tend to feed in the neritic zone (Pardo-Gandarillas et al. 2007) and in the water column (Harvey 1989), where they consume small coastal fish (McCord and Campana 2003; Pardo-Gandarillas et al. 2007), euphausiids (Harvey 1989; Miller and Brodeur 2007; Preti et al. 2012), and pelagic crustaceans (Preti et al. 2012; Hernández-Aguilar et al. 2016). Adult females spend more time in coastal areas due to reproductive processes (Carrera-Fernández et al. 2010) since these areas provide them with

enough food for the development of their offspring, and with safe places for giving birth.

In our analysis, the  $\delta^{13}\text{C}$  values of medium-sized and large juveniles of both sexes, of adult males, and of sharks 2–5 years old (Tables 2 and 3) suggest a preference for feeding in oceanic areas. This behavior may be explained by the migration of older juveniles to oceanic areas to complement their diet and to explore new ecosystems (Polo-Silva et al. 2012). It is also possible that these areas are being used as breeding areas for juveniles and sub-adults (Kubodera et al. 2007). Tables 2 and 3 show that small juveniles of both sexes, adult females, and sharks 6–9 years old mainly use coastal areas for feeding. It is thus clear that there is a change in habitat related to sexual maturity and age.

It is also possible that high  $\delta^{13}\text{C}$  values (Tables 2 and 3) reflect the mother's isotopic signature acquired through maternal transfer processes (McMeans et al. 2009; Vaudo et al. 2010). As placental trophic sharks, females of *P. glauca* transfer nutrients directly to their offspring through yolk sac placenta, which could be reflected in the  $\delta^{13}\text{C}$  of the early life stages (i.e., small- and medium-sized juveniles). This hypothesis is partially supported by our isotopic enrichment analysis since enrichment in  $^{13}\text{C}$  and  $^{15}\text{N}$  was observed in medium-sized and large juveniles (Table 1, Figs. 3 and 4), relative to small juveniles (Table 1). To confirm this hypothesis, a careful comparison of the isotopic signature of mothers and their embryos and/or offspring is needed.

The isotopic enrichment found in this study suggests that the maternal  $\delta^{13}\text{C}$  signature is “erased” by the offspring between 8 and 9 mm VR (Table 1), and the maternal  $\delta^{15}\text{N}$  “erased” between 7 and 8 mm VR (Table 1). These findings show that blue sharks begin to reflect the isotopic signature of their prey at 7–8-mm VR. The isotopic signature of *P. glauca* juveniles could, therefore, be used as an indicator of the food sources used by their mothers and their trophic level, as has been suggested for other top predators, such as pinnipeds (Elorriaga-Verplancken et al. 2013). Thus, the isotopic signature of juveniles of *P. glauca* should be interpreted with caution when making dietary inferences.

### Niche breadth and isotopic overlap

The SEAc values obtained in this study (for individuals (Table 4), sexes (male =  $3.7\text{‰}^2$ ; female =  $3.9\text{‰}^2$ ), and all maturity stages (Table 5]) suggest that *P. glauca* has a broad isotopic niche. It is worth mentioning that SJuv, LJuv, and adults had the broadest isotopic niche, probably reflecting the use of a broader range of resources (e.g., habitat and food). By comparison, the trophic niche breadth of MJuv was slightly reduced, suggesting the use of a slightly narrower range of resources. These results indicate an ontogenetic change in trophic niche breadth, and suggest that *P. glauca* is a predator with a broad dietary spectrum, using different

food sources and consuming prey at different trophic levels. Our SEAc values are in agreement with previous reports by Li et al. (2016; SEAc  $2.26\text{‰}^2$ ) and Rosas-Luis et al. (2017; SEAc  $1.36\text{‰}^2$ ). In keeping with our results, Miller and Brodeur (2007), and Vaske-Júnior and Rincón-Filho (1998), classified *P. glauca* as an omnivorous and opportunistic species.

Here we report a high variability of  $\delta^{15}\text{N}$  values ( $\sigma_{\text{Male}} = 4.1$ ,  $\sigma_{\text{Female}} = 4.4$ ,  $\sigma_{\text{SJuv.}} = 3.5$ ,  $\sigma_{\text{MJuv.}} = 2.7$ ,  $\sigma_{\text{LJuv.}} = 3.6$ ,  $\sigma_{\text{Adul.}} = 7.0$ ), which may be explained by *P. glauca*'s varied diet that may include crustaceans (Miller and Brodeur 2007; López et al. 2010), birds (Markaida and Sosa-Nishizaki 2010; Rivera et al. 2015), other elasmobranchs (Markaida and Sosa-Nishizaki 2010; López et al. 2012), and even human waste (McCord and Campana 2003; Markaida and Sosa-Nishizaki 2010).

*P. glauca*'s broad trophic niche may be related to (1) the broad range of  $\delta^{13}\text{C}$ , which suggests that blue sharks use different feeding areas (coastal and oceanic) where they have access to different types of prey; (2) *P. glauca*'s movements across spatial gradients, which are reflected in the  $\delta^{15}\text{N}$  values of the trophic base (Revill et al. 2009; Polo-Silva et al. 2013; Kiszka et al. 2014); and (3) seasonal changes in the diet (MacNeil et al. 2005), related to the availability of prey in the different feeding areas.

The high isotopic overlap estimated between sexes and maturity stages suggests that there is a high trophic interaction between the different categories of blue sharks, and that they share prey and feeding areas with the same trophic base. However, the isotopic niche of the medium-sized juveniles did not overlap with that of other maturity stages, which could be explained by (1) seasonal differences in feeding areas (Polo-Silva et al. 2012), (2) maternal transfer processes, and (3) exploratory migrations between coastal and oceanic areas for feeding.

### Conclusions

This study is the first to address the trophic ecology of *P. glauca* based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  patterns of vertebrae. Our findings reveal important ontogenetic changes in the diet of this species over its lifetime. Our results also indicate that *P. glauca* obtains food in both coastal and oceanic environments. We found that *P. glauca* has a broad isotopic niche, consuming prey at both low and high trophic levels, and is a species with generalist habits. Also, enrichment in  $^{13}\text{C}$  and  $^{15}\text{N}$  in vertebrae suggests the existence of maternal transfer processes. Because small juveniles and medium-sized juveniles are enriched in maternally derived  $^{13}\text{C}$  and  $^{15}\text{N}$ , relative to adults, their isotopic signature could be used as an indicator of the food sources used by their mothers and their trophic level. Finally, we found that the  $\delta^{13}\text{C}$  signature

derived from the mother is lost between 8 and 9 mm VR, and that the mother's  $\delta^{15}\text{N}$  signature is lost between 7 and 8 mm VR. After losing the maternal isotopic signature, blue sharks develop their own isotopic signature.

This study provides information that helps to reduce the gaps of knowledge about the diet of *P. glauca* and its dietary changes throughout the eastern Tropical Pacific. The use of hard anatomical structures (vertebrae) is highly relevant because they integrate information on the dietary ontogeny of this shark species.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All sampling and experimental procedures performed in this study comply with the current Ecuadorian legislation.

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