



Ontogenetic feeding ecology of the scalloped hammerhead shark *Sphyrna lewini* in the Colombian Eastern Tropical Pacific

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ABSTRACT: Changes in feeding habits during ontogeny show that organisms can present shifts in foraging behavior during their life cycle, which can alter local trophic dynamics. Therefore, describing diet across species ontogeny clarifies the ecological niche and ecosystem role of marine predators. In this study, diet tracers (stable isotope analysis) were analyzed in 16 scalloped hammerhead sharks *Sphyrna lewini*, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of collagen in vertebral cross-sections to reconstruct diet across their ontogeny. Our results suggest that *S. lewini* occupies a broad isotopic niche due to the consumption of prey belonging to different trophic levels ($\delta^{15}\text{N}$: 7.6–13.0‰) of the food chain in both coastal and oceanic zones ($\delta^{13}\text{C}$: –17.2 to –14.1‰) during their lifetime. Accordingly, ontogenetic changes in diet and habitat use were suggested by differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across age groups, indicating high consumption of coastal prey at 0–2 yr, oceanic prey at ~2–4 yr, a shift to high coastal prey at >4 yr, and a shift to high coastal prey, along with the consumption of prey from multiple trophic levels through feeding ontogeny (estimated trophic position: 2.9–6.5). This study showed migration from coastal to oceanic zones in juvenile *S. lewini*, and their return to coastal habitats as adults, potentially related to the use of coastal zones (i.e. mangroves) in the Eastern Tropical Pacific, both as important feeding areas for neonates and as feeding and breeding grounds for adults.

KEY WORDS: Ontogeny · Stable isotopes · Vertebrae · Maturity stages · Maternal transfer · Southeast Pacific

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1. INTRODUCTION

Ontogenetic changes in diet suggest shifts in foraging behavior during a species' life cycle (Werner & Gilliam 1984, Newman et al. 2012), which could cause modifications in their habitat use (e.g. coastal

vs. oceanic). These trophic patterns may alter community structure (Estrada et al. 2006) and local trophic dynamics (Polis et al. 1996, Polis & Strong 1996). For this reason, quantitative diet changes in the ontogeny of a species are important, as they provide new information about the niche and ecological

role of a species in an ecosystem (Braga et al. 2012), along with the effects of predator–prey relationships on ecosystem structure (Juanes et al. 2001, Newman et al. 2012). The current study addresses information regarding early stages of life and habitat changes of *Sphyrna lewini*.

Studies of trophic level and ontogenetic diet shift of different species of sharks have based their methodology on stomach content analysis (SCA) and stable isotope ratios (Torres-Rojas et al. 2010, 2014). While SCA generates information about the prey digested in the recent past (i.e. in the last hours or days), the isotopic analysis of a tissue sample (e.g. muscle, teeth, or liver) reveals the food synthesized by the predator over different time and spatial scales (Kim et al. 2012), providing additional information about habitat use, migration, feeding preferences, ecological niche, sharing resources, and trophic position (TP) of an individual species (e.g. Post 2002, Graham et al. 2010, Jackson et al. 2011). The osteological sections formed by accretion (e.g. fish otoliths and shark vertebrae) show fluctuations in diet and habitat use of sharks and other species during their lives (Best & Schell 1996, Kim et al. 2012, Carlisle et al. 2015, Estupiñán-Montaño et al. 2019). Despite the important record that vertebrae preserve, these structures have received less attention from researchers, which could be related to the greater time and energy demands necessary for tissue extraction and processing, since these structures must be systematically sampled with the help of micro-drills (Estupiñán-Montaño et al. 2019) and processed for several days for the adequate extraction of carbonates (Kim et al. 2012), unlike soft tissues such as muscle, which have less complex treatment processes (Kim et al. 2012, Tamburini et al. 2019, 2020).

The scalloped hammerhead shark *S. lewini* has a broad distribution throughout the Eastern Tropical Pacific (ETP), including around oceanic islands and seamounts (Klimley 1981, Klimley & Nelson 1981, Compagno 1984), which is partly related to large-scale migratory movements among various oceanic islands of the ETP (Malpelo, Colombia; Cocos Island, Costa Rica; Galapagos Islands, Ecuador; Bessudo et al. 2011a,b). This characteristic makes *S. lewini* vulnerable to overexploitation, which has led to its classification as a Critically Endangered species (Rigby et al. 2019) and its inclusion in Appendix II of CITES (CITES 2013). In spite of this, and its ecological importance as a top predator in the ETP (Torres-Rojas et al. 2010, 2015, Estupiñán-Montaño et al. 2017), detailed studies about the species and its biology are still lacking. Few studies have been carried out to

investigate the changes in diet that occur during its life cycle.

SCA of *S. lewini* suggests that the species consumes a high diversity of prey, including crustaceans, teleosts, and cephalopods (coastal and oceanic), and even other chondrichthyans (Bethea et al. 2004, Estupiñán-Montaño et al. 2009, Torres-Rojas et al. 2010, 2015, Bornatowski et al. 2014, Galván-Magaña et al. 2013, Flores-Martínez et al. 2016, Rosende-Pereiro et al. 2020). Nonetheless, *S. lewini* has shown a preference for the consumption of oceanic squids (e.g. Ommastrephidae; Estupiñán-Montaño et al. 2009, Galván-Magaña et al. 2013), which has led some to consider the species to be a predator with a narrow trophic niche at the population level (Levin's index, $B_i < 0.35$; Estupiñán-Montaño et al. 2009, Torres-Rojas et al. 2010, 2014, Bornatowski et al. 2014), but with wide trophic niches at specific ontogenetic levels. For example, the juveniles of this species consume a high variety of pelagic and benthic teleosts (i.e. Albulidae, Arridae, Carangidae, Centropomidae, Haemulidae, and Lutjanidae), coastal squids (i.e. Loliginidae), and some crustaceans (i.e. Penaeidae) (Flores-Martínez et al. 2016, Rosende-Pereiro et al. 2020), suggesting a higher use of food resources (Rosende-Pereiro et al. 2020) in different habitats. Adults of this species occupy narrower trophic niches, with preferential consumption of oceanic squid (Estupiñán-Montaño et al. 2009).

Similarly, stable isotope analysis (SIA) of $\delta^{13}\text{C}$ in muscle samples of *S. lewini* (Loor-Andrade et al. 2015, Li et al. 2016, Estupiñán-Montaño et al. 2017, Rosende-Pereiro et al. 2020) have suggested that the species has a wide trophic niche due to the use of both coastal (Torres-Rojas et al. 2014, Estupiñán-Montaño et al. 2017) and oceanic zones (Loor-Andrade et al. 2015, Li et al. 2016, Estupiñán-Montaño et al. 2017, Rosende-Pereiro et al. 2020), considering changes in $\delta^{13}\text{C}$ that occur from coastal areas to oceanic areas (Niño-Torres et al. 2006). In this context, the use of diverse trophic levels (TP: 3.8–5.9; Li et al. 2016, Estupiñán-Montaño et al. 2017) is a reflection of individual specialization over a diverse range of habitats (Loor-Andrade et al. 2015, Li et al. 2016) and a high degree of trophic plasticity (Torres-Rojas et al. 2014).

The objective of this study was to analyze ontogenetic changes in the diet and habitat use of *S. lewini* in the Colombian ETP, using stable isotope ratios of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in vertebral collagen. This study generated information about the ecological patterns of *S. lewini* throughout its feeding ontogeny in order to understand the role that the

species plays in potential nursery areas (e.g. Colombian mangroves) and the coastal and oceanic food web of the southeastern tropical Pacific.

2. MATERIALS AND METHODS

2.1. Collection of samples

During 2013, a total of 16 illegally fished *Sphyrna lewini* were confiscated by Colombian National Park authorities in the 'Fauna and Flora Sanctuary Malpelo, Colombia' (Fig. 1). Although sex was not registered due to the lack of pelvic fins and viscera from these illegal captures, the total length (TL, cm) was measured for each specimen. Vertebrae were sampled close to the head of each individual (Estupiñán-Montaño et al. 2019), stored in previously labeled plastic bags, and transported to the Fundación Alium Pacific facilities for further processing.

The neural arc and the connective tissue were removed from all vertebrae, leaving the body of the vertebra completely clean; afterwards, the tissue samples were dehydrated at ambient temperature (Estupiñán-

Montaño et al. 2019). The samples were then transferred to the Laboratorio de Ecología de Peces del Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (La Paz, Mexico).

2.2. Sample preparation and analysis

Subsamples were obtained from each vertebra using a microdrill and a 0.5 mm diameter bit to obtain 2 sets of samples. We drilled twice in each sample location along the entire vertebra from the center to the periphery. In order to remove the inorganic carbon, the first set of samples was weighed (0.48–0.98 mg) and stored in a desiccant with HCl vapor at 37% for 12–24 h (Hedges & Stern 1984). The first set of samples registered %C values between 0.08–14.6% ($7.68 \pm 3.1\%$); while the second set of samples weighed between 0.22 and 0.71 mg and was analyzed without any treatment in order to avoid any distortion of the $\delta^{15}\text{N}$ values (Christiansen et al. 2014), resulting in %C values between 10.6 and 19.3% ($14.1 \pm 1.7\%$). Both sets of vertebral collagen samples were stored in 3.2×4 mm tin capsules.

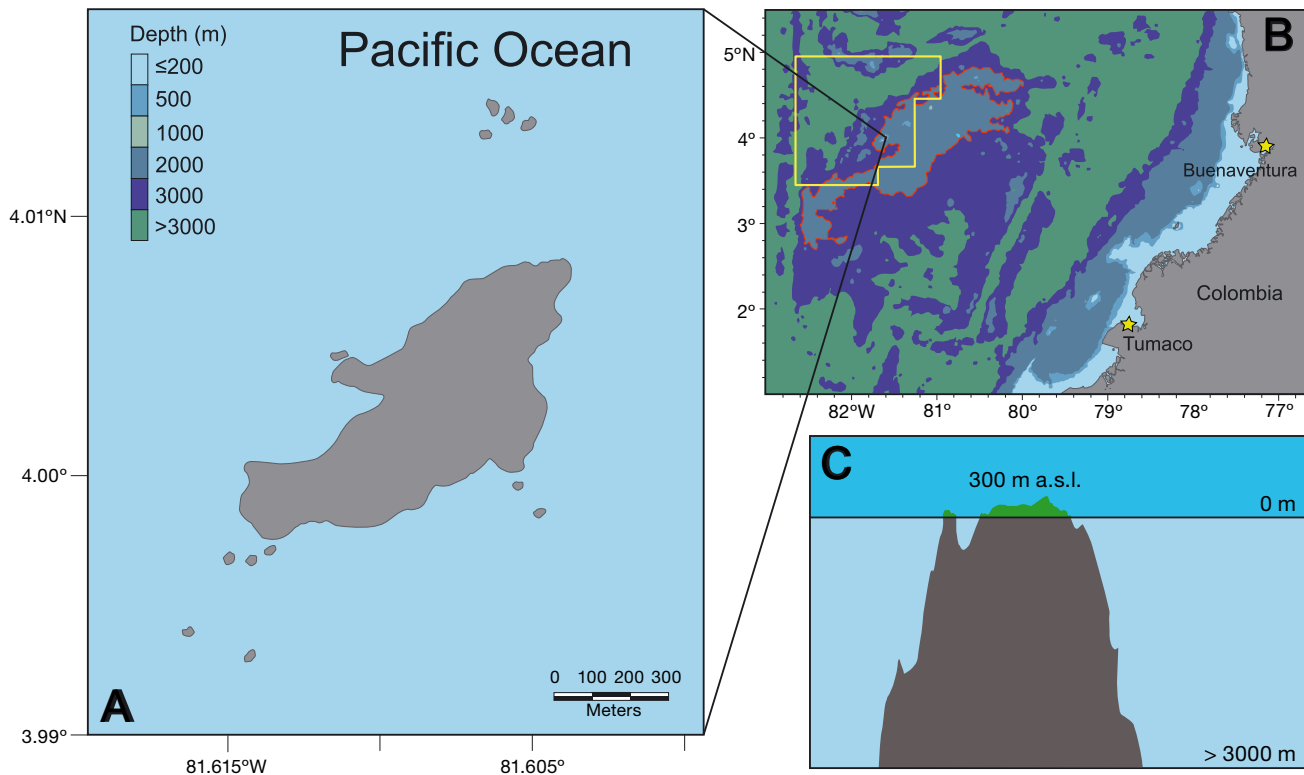


Fig. 1. Study area in the Eastern Tropical Pacific Ocean. (A) Malpelo Island, Colombia; (B) marine protected area of the Fauna and Flora Sanctuary (yellow) and the Malpelo dorsal (red); and (C) lateral view of the bathymetric profile of Malpelo Island obtained from the management plan of the protected area. m. a.s.l.: m above sea level

SIA was carried out in the Laboratorio de Biogeoquímica de Isótopos Estables del Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC-UGR), Spain. The C and N isotopic composition was determined using a Carlo Erba NA 1500 NC elemental analyzer with a ConFlo III interface to a Delta Plus XP mass spectrometer (EA-IRMS; ThermoQuest). The isotopic ratio was represented as δ values per thousand (‰) as follows:

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

where R_{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 (Vienna-Pee Dee Belemnite [V-PDB] limestone for carbon and atmospheric N_2 , [AIR] respectively). Sample internal standards for $\delta^{13}\text{C}$ (−30.63 and −11.65‰; V-PDB) and $\delta^{15}\text{N}$ (−1.0 and +16.0‰; AIR) were used for the tests on the sample tissues (vertebral collagenous) to calibrate the results to the international reference materials (NBS-21, USGS-24, and IAEA-C6 for carbon; IAEA-N1, IAEA-N2, and IAEA-N3 for nitrogen), and were combined every 10 tissue samples to take into account possible instrument errors. The instrumental precision was calculated, after correction of the instrumental daily drift, from standards systematically interspersed in analytical batches; variability was ± 0.1 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

2.3. TP

The TP of *S. lewini* was estimated by individual, across estimated ages associated with vertebral radius (VR), by the implementation of a Bayesian method within the R statistical environment (R Core Team 2018), employing the 'tRophicPosition' package v.0.7.5 (Quezada-Romegialli et al. 2018). Analyses of TP were calculated using the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *S. lewini* and isotopic values of brown algae *Padina* sp. from Malpelo Island (mean \pm SD: $\delta^{13}\text{C} = -18.7 \pm 1.58\%$, $\delta^{15}\text{N} = 5.2 \pm 0.45\%$; C. Estupiñán-Montaño et al. unpubl. data) as an isotopic baseline. A Bayesian model of one baseline and 2 trophic discrimination factors (TDFs) was run with 2 Markov Chain Monte Carlo (MCMC) and 20 000 adaptive interactions, assuming a λ of the baseline taxa = 1. Accordingly, *S. lewini*'s TP value was determined using a TDF for the tissue-specific (vertebrae) fractionation of phylogenetically similar species to *S. lewini* as well as lemon shark *Negaprion brevirostris* (Vélez-Zuazo & Agnarsson 2011) with values of $\Delta^{13}\text{C} = 3.75 \pm 0.44\%$ and $\Delta^{15}\text{N} =$

$1.45 \pm 0.61\%$ (Hussey et al. 2010) for carbon and nitrogen, respectively.

The estimated TPs were classified into 5 functional groups: primary carnivores (level 3), intermediate (primary–secondary) carnivores (levels 3.5–4), secondary carnivores (level 4), intermediate (secondary–tertiary) carnivores (levels 4.5–5), tertiary carnivores (level 5 and greater) (Mearns et al. 1981).

2.4. Bayesian mixing models and prey selection

In order to make inferences about the dietary preferences of *S. lewini*, we applied Bayesian mixing models, based on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of vertebrae, to determine the species' overall diet during its life cycle (e.g. Kim et al. 2012). The mixing model used 4 steps: (1) selection of potential prey of *S. lewini*, based on SCA (e.g. Estupiñán-Montaño et al. 2009, Torres-Rojas et al. 2010, 2015, Bornatowski et al. 2014) and potential prey within Colombian mangrove ecosystems (Medina et al. 2018), as mangroves are important breeding areas for this species (Quintanilla et al. 2015); (2) grouping the potential prey into 5 categories according to their biological characteristics (Table 1) (Phillips et al. 2014); (3) as there are no species-specific TDFs for *S. lewini*, we selected estimated TDFs for other shark species such as the lemon shark *N. brevirostris* ($\delta^{13}\text{C} = 3.75 \pm 0.44\%$, $\delta^{15}\text{N} = 1.45 \pm 0.61\%$; Hussey et al. 2010), a species which is phylogenetically similar to *S. lewini* (Vélez-Zuazo & Agnarsson 2011) and has approximately 'equivalent' food preferences (e.g. squid, shrimp, fish, elasmobranchs; Cortés & Gruber 1990, Wetherbee et al. 1990, Newman et al. 2012). The mixing model adjustment was run with 10^3 iterations with a 95% probability for the mixing polygon (Smith et al. 2013). The model was considered adequate if the isotopic values were within 1% of the mixing model polygons (Reum et al. 2020).

In order to make inferences about ontogenetic habitat use, we estimated the relative contribution of habitat-specific prey to *S. lewini* diet, through the package 'simmr' v.0.3 of the isotope mixing model 'SIAR' (stable isotope analysis in R; Parnell 2016) v.3.4.3 in the R statistical platform (R Core Team 2018). This model uses a Bayesian isotopic framework to estimate the proportional contribution of prey to a consumer's diet (Parnell et al. 2013), including variability in model inputs, such as TDF values for sharks. To run the SIAR model, we used the mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *S. lewini* (i.e.

Table 1. Previously published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) and trophic position of potential prey of scalloped hammerhead shark *Sphyrna lewini* in the southeast Pacific Ocean, used in mixing model analyses. Prey selection was based on stomach content studies of *S. lewini* in regions closest to the study area (e.g. Estupiñán-Montaño et al. 2009), potential prey of nurseries area at Colombian Pacific coastal zones (Medina et al. 2018), and in the eastern Pacific Ocean (e.g. Calle-Morán 2010, Rosas-Luis et al. 2017)

Potential prey	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Trophic position
Oceanic cephalopods	-18.0 \pm 0.6	10.2 \pm 1.4	4.14
<i>Dosidicus gigas</i> ^{a,b}	-17.8 \pm 0.4	10.1 \pm 1.3	4.14 ^g
<i>Sthenoteuthis oualaniensis</i> ^a	-18.0 \pm 0.2	10.4 \pm 0.2	4.09 ^g
<i>Ommastrephes bartramii</i> ^c	-18.1 \pm 0.4	10.0 \pm 0.4	4.20 ^g
Coastal cephalopods	-16.9 \pm 0.4	12.5 \pm 0.2	3.90
<i>Lolligunculla (Loliolopsis) diomedea</i> ^a	-16.9 \pm 0.4	12.5 \pm 0.2	3.90 ^g
Oceanic fish	-16.8 \pm 1.1	12.3 \pm 3.7	4.16
<i>Auxis thazard</i> ^d	-16.6 \pm 0.1	11.0 \pm 0.1	4.33 ^g
<i>Auxis</i> spp. ^d	-15.5 \pm 0.9	12.1 \pm 1.7	4.33 ^g
<i>Katsuwonus pelamis</i> ^d	-17.1 \pm 0.5	12.3 \pm 3.0	4.30 ^g
<i>Thunnus albacares</i> (Ecuador) ^d	-17.2 \pm 0.2	13.3 \pm 1.1	4.30 ^g
<i>Thunnus albacares</i> (Malpelo) ^f	-17.5 \pm 0.4	13.7 \pm 0.7	4.30 ^g
<i>Scomber japonicus</i> ^c	-16.8 \pm 0.1	11.6 \pm 0.5	3.38 ^g
Mangrove fish ^e	-18.3 \pm 1.9	11.1 \pm 1.1	3.56 ^e
<i>Spheroides rosenblatti</i>	-17.3 \pm 0.1	11.8 \pm 0.1	3.90
<i>Lutjanus argentiventris</i>	-17.2 \pm 0.9	13.5 \pm 0.5	4.40
<i>Daector dowii</i>	-18.7 \pm 0.4	11.1 \pm 0.1	3.70
<i>Bathigobius andrei</i>	-18.7 \pm 0.4	9.4 \pm 0.2	3.20
<i>Ctenogobius sagittula</i>	-21.1 \pm 1.3	8.1 \pm 0.6	2.80
<i>Microgobius tabogensis</i>	-17.9 \pm 0.8	11.3 \pm 0.2	3.80
<i>Larimus argenteus</i> ^b	-17.4 \pm 0.5	12.6 \pm 0.7	3.10 ^g
Coastal crustaceans ^e	-20.5 \pm 2.5	6.5 \pm 1.3	2.48 ^e
<i>Petrolishes zaca</i>	-15.7 \pm 0.7	6.6 \pm 0.8	2.40
<i>Panopeus chilensis</i>	-19.3 \pm 0.8	9.4 \pm 1.1	3.20
<i>Macrobranchium panamensis</i>	-20.5 \pm 1.7	8.4 \pm 1.3	2.90
<i>Alpheus colombiensis</i>	-22.2 \pm 0.9	6.4 \pm 0.7	2.30
<i>Armases occidentale</i>	-24.8 \pm 0.3	3.8 \pm 0.1	1.60

^aBolaño-Martínez (2009); ^bRuiz-Cooley et al. (2010);
^cCalle-Morán (2010); ^dRosas-Luis et al. (2017);
^eMedina et al. (2018); ^fThis study; ^gPauly & Zeller (2015)

individuals and age groups), potential prey species (Table 1), and tissue-specific (vertebrae) fractionation ($\Delta^{13}\text{C}_{N. brevisrostris} = 3.75 \pm 0.44\text{‰}$ and $\Delta^{15}\text{N}_{N. brevisrostris} = 1.45 \pm 0.61\text{‰}$; Hussey et al. 2010) as mixture, sources, and correction factors, respectively. The model was run with 10^6 iterations, 10 000 burn-in, 100 thinning period, and 4 MCMC.

2.5. Feeding ontogeny

The patterns of isotopic enrichment along the ontogeny of *S. lewini* were inferred from the initial point of sampling located 2 mm from the center of the vertebrae. The relative enrichment of ^{13}C and ^{15}N were calculated using the algorithm proposed by Estrada et al. (2006):

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

where Y is the element of interest (^{13}C and ^{15}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).

As it was not possible to determine the sex for each analyzed individual, TL and age were estimated from the average of the regression parameters for both sexes. Thus, the TL for every VR sample was estimated using the following equation (Anislado-Tolentino et al. 2008):

$$\text{TL} = 12.65 + 214 \times \text{VR} \quad (3)$$

where VR is the vertebral radius distance (in cm). Additionally, the age (t) from each vertebral collagen sampling point (i.e. every mm) was estimated in accordance to the von Bertalanffy growth function:

$$\text{TL} = L_{\infty} (1 - e^{-K(t-t_0)}) \quad (4)$$

where TL is the predicted length at age t , L_{∞} is the asymptotic mean length, K is the growth rate (yr^{-1}), and t_0 is the theoretical age at which the shark had zero length. Likewise, the year in each mm (location of vertebral collagen sample point) was estimated using the following formula:

$$t = -\frac{\ln\left(1 - \frac{L_t}{L_{\infty}}\right)}{K} + t_0 \quad (5)$$

where the parameters of the von Bertalanffy growth function were: $L_{\infty} = 370$ cm TL; K was 0.11 yr^{-1} , and t_0 was -1.17 yr (Anislado-Tolentino et al. 2008).

2.6. Niche width and isotopic overlap

Isotopic niche was quantified for individuals and age groups, using stable isotopic Bayesian ellipses method in R (SIBER; Jackson et al. 2011). This analysis is based on calculated ellipses from a covariance matrix, which defines its forms and areas (Jackson et al. 2011) to estimate the width of the isotopic niches (standard ellipse corrected area, SEA_C).

Isotopic overlap was estimated using the 'niche-ROVER' package in R (Lysy et al. 2014), which is a Bayesian method that calculates the probability of overlap between niche pairs using multidimensional information as niche indicators (e.g. stable isotopes, environmental variables). The probabilistic density of niche overlap was calculated by running 10^4 iterations and 95% of the data from each species or group occurring within their respective isospace, providing directional niche overlap estimates (e.g. x vs. y and y vs. x), according to the distributions of a specific species in the multivariate niche space (Lysy et al. 2014).

2.7. Statistical analysis

The normality and homoscedasticity of the isotopic information was tested by a Shapiro-Wilks test and a Levene's test, respectively. Non-parametric analyses of variance (Kruskal–Wallis test, Wilcoxon signed-rank test) were used to test the isotopic differences among categories (individuals and VR [ages]). A post hoc test of multiple comparisons (Dunn's test) was then performed to identify the specific differences among categories.

3. RESULTS

Cross-sectional sampling and analysis of vertebrae of 16 *Sphyrna lewini* (LEW#: individual ID) ranged between 145.3 and 193.2 cm TL (Table 2), with an

estimated age of 0.1–5.5 yr (Table 3). A total of 101 vertebral collagen samples were analyzed.

3.1. Feeding sources

The values of $\delta^{13}\text{C}$ ranged from -17.2 to -14.1‰ (mean \pm SE: $-15.2 \pm 0.06\text{‰}$, V-PDB) (Shapiro, $p = 0.001$; Levene, $p = 0.44$). The individual analysis showed that LEW8 ($-15.6 \pm 0.23\text{‰}$), LEW12 ($-15.7 \pm 0.36\text{‰}$), and LEW15 ($-15.7 \pm 0.10\text{‰}$) had the lowest $\delta^{13}\text{C}$ values, while LEW3 ($-14.7 \pm 0.11\text{‰}$), LEW11 ($-14.6 \pm 0.1\text{‰}$), and LEW13 ($-14.7 \pm 0.12\text{‰}$) showed the highest $\delta^{13}\text{C}$ values (Table 2, Fig. A1 in the Appendix).

We obtained $\delta^{13}\text{C}$ values of 7 age groups (VR; Table 3): the age group 0.1–0.6 (2 mm in VR) showed the lowest $\delta^{13}\text{C}$ compared to other estimated age groups (Fig. 2). Nonetheless, no statistical differences were apparent among age groups (Kruskal–Wallis, $p = 0.10$; Table 3).

3.2. TP

The values of $\delta^{15}\text{N}$ in *S. lewini* specimens ranged from 7.6 – 13.0‰ ($11.3 \pm 0.09\text{‰}$, AIR) (Shapiro, $p < 0.01$; Levene, $p = 0.31$). Individual analyses showed that LEW1 ($9.3 \pm 0.40\text{‰}$) and LEW2 ($9.4 \pm 0.79\text{‰}$) had the lowest $\delta^{15}\text{N}$ values, while LEW3 ($12.2 \pm 0.19\text{‰}$) and LEW4 ($11.9 \pm 0.08\text{‰}$) had the highest $\delta^{15}\text{N}$ (Table 2, Fig. A1). The $\delta^{15}\text{N}$ values obtained

Table 2. Isotopic analyses and trophic estimates of scalloped hammerhead *Sphyrna lewini* in the Eastern Tropical Pacific and around Malpelo Island (Colombia), represented in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, total length (TL), estimated age, trophic position, and isotopic niche (area of the corrected standard ellipse [SEAC]). **Bold:** individuals that showed statistically significant differences according to the multiple comparisons test (Dunn's test; see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m663p127_supp.pdf)

Individuals	n	TL (cm)	Age (years)	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		Trophic position		SEAC (‰ ²)
				Range	Mean \pm SE	Range	Mean \pm SE	95% CI	Mode	
LEW1	4	145.3	3.4	-15.8 to -14.6	-15.3 \pm 0.26	8.3 to 10.2	9.3 \pm 0.40	3.3–4.5	3.9	2.40
LEW2	5	176.8	4.7	-16.1 to -14.6	-15.3 \pm 0.28	7.6 to 12.2	9.4 \pm 0.79	2.9–4.9	4.9	3.30
LEW3	7	162.1	4.1	-15.1 to -14.4	-14.7 \pm 0.11	11.5 to 13.0	12.2 \pm 0.19	5.1–6.5	5.8	0.62
LEW4	6	165.8	4.2	-16.2 to -14.7	-15.5 \pm 0.24	11.6 to 12.2	11.9 \pm 0.08	5.0–6.3	5.6	0.24
LEW5	7	176.8	4.7	-16.7 to -14.3	-15.2 \pm 0.64	11.1 to 12.1	11.7 \pm 0.12	4.9–6.2	5.5	0.28
LEW6	6	162.1	4.1	-15.5 to -14.6	-15.1 \pm 0.15	10.4 to 12.0	11.5 \pm 0.26	4.8–6.1	5.4	0.68
LEW7	6	193.2	5.5	-15.8 to -14.4	-15.2 \pm 0.19	10.1 to 12.1	11.0 \pm 0.29	4.4–5.6	5.0	1.89
LEW8	4	193.2	5.5	-16.8 to -15.0	-15.6 \pm 0.23	11.2 to 12.3	11.7 \pm 0.18	4.8–6.1	5.4	0.37
LEW9	6	151.1	3.6	-16.3 to -14.4	-15.1 \pm 0.27	11.1 to 12.1	11.6 \pm 0.16	4.8–6.1	5.4	0.32
LEW10	7	165.8	4.2	-17.2 to -14.3	-15.3 \pm 0.38	11.3 to 11.9	11.7 \pm 0.08	4.9–6.1	5.4	0.30
LEW11	6	191.5	5.5	-15.3 to -14.3	-14.6 \pm 0.15	10.8 to 11.7	11.3 \pm 0.15	4.6–5.8	5.2	0.34
LEW12	6	151.1	3.6	-16.8 to -14.6	-15.7 \pm 0.36	10.7 to 12.2	11.4 \pm 0.21	4.7–5.9	5.2	0.92
LEW13	7	186.0	5.2	-15.2 to -14.3	-14.7 \pm 0.12	10.7 to 12.1	11.4 \pm 0.18	4.7–6.0	5.2	0.50
LEW14	7	171.2	4.5	-15.8 to -14.1	-15.0 \pm 0.19	10.9 to 11.9	11.5 \pm 0.14	4.7–6.0	5.4	0.20
LEW15	7	191.8	5.5	-16.0 to -15.2	-15.7 \pm 0.10	9.1 to 11.5	10.7 \pm 0.36	4.2–5.6	4.8	0.68
LEW16	7	184.1	5.1	-15.2 to -14.6	-15.0 \pm 0.08	10.7 to 12.1	11.4 \pm 0.20	4.7–6.0	5.3	0.22

Table 3. Isotopic analyses and trophic estimates of scalloped hammerhead *Sphyrna lewini* in the Eastern Tropical Pacific and around Malpelo Island (Colombia), represented in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, trophic position, isotopic niche (area of the corrected standard ellipse [SEAC]), and estimated lengths (TL, cm) and ages (years). VR: vertebral radius

VR	n	TL _{Estimated} (range)	Age _{Estimated}		$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		Trophic position 95% CI	SEAC (‰ ²)	
			Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE			
2	18	47.3–65.7	0.1–0.6	0.3 \pm 0.04	–16.8 to –14.5	–15.6 \pm 0.16	7.6 to 12.5	11.3 \pm 0.26	4.3–6.0	5.2	2.40
3	15	66.2–87.3	0.6–1.3	1.0 \pm 0.06	–17.2 to –14.4	–15.2 \pm 0.22	8.4 to 12.0	11.3 \pm 0.22	4.5–6.0	5.2	2.53
4	17	88.6–107.9	1.3–2.0	1.7 \pm 0.05	–16.4 to –14.1	–15.0 \pm 0.15	9.3 to 13.0	11.6 \pm 0.18	4.8–6.2	5.4	1.55
5	15	110.4–128.0	2.0–2.7	2.4 \pm 0.05	–15.8 to –14.4	–15.1 \pm 0.12	9.6 to 12.4	11.6 \pm 0.17	4.7–6.1	5.4	0.86
6	19	131.8–151.1	2.8–3.6	3.1 \pm 0.05	–16.2 to –14.4	–15.1 \pm 0.13	8.3 to 12.2	11.0 \pm 0.28	4.1–5.8	4.9	2.08
7	13	152.6–171.2	3.7–4.5	4.1 \pm 0.08	–15.9 to –14.3	–15.1 \pm 0.14	9.6 to 12.0	11.1 \pm 0.18	4.5–5.8	5.1	1.07
8	4	183.9–191.8	5.1–5.5	5.2 \pm 0.09	–15.6 to –14.4	–15.0 \pm 0.26	10.7 to 11.3	11.0 \pm 0.12	4.5–5.6	5.0	0.59

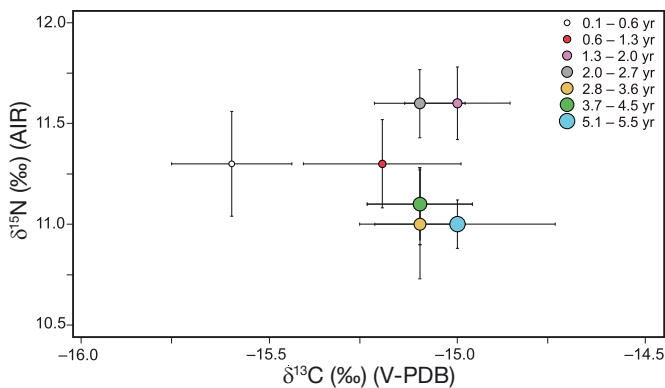


Fig. 2. Mean (\pm SE) values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for estimated age groups of the scalloped hammerhead shark *Sphyrna lewini*. Each vertebral radius represents an estimated age group

from 7 distinct age groups did not reveal statistical differences among the different age groups (Kruskal-Wallis, $p = 0.12$; Table 3, Fig. 2).

The TPs estimated for *S. lewini* had a mode of 4.8 (95% CI = 2.9–6.5); with TPs estimated by individuals producing modes that varied between 3.9 and 5.8 (95% CI = 2.9–6.5; Table 2); when estimated by age groups, the mode varied between 4.9 and 5.4 (95% CI = 4.1–6.1; Table 3), respectively.

3.3. Bayesian mixing models and prey selection

The fitting model (i.e. mixing polygons, subsequent predictive validations; Fig. S1A in the Supplement at www.int-res.com/articles/suppl/m663p127_supp.pdf), suggested that these results explained the uncertainty of the TDFs and of the isotopic values of the 5 groups of potential prey (Fig. S1B). Therefore, the application of the mixing model was adequate to estimate the selection of potential feeding habits of *S. lewini* during its life cycle. Additionally, the mixing

model produced a total of 3600 posterior distributions and Gelman-Rubin (Rhat) convergence diagnostic statistics between 1.00 and 1.03 for all parameters, suggesting that there was convergence.

The mixing model suggested a high negative correlation (>0.50) between the estimated relative contribution of mangrove fish with respect to coastal cephalopods (-0.51) and coastal crustaceans (-0.55) as well as oceanic cephalopods with respect to coastal cephalopods (-0.63) and coastal crustaceans (-0.67). Coastal cephalopods and coastal crustaceans showed a high positive correlation (0.71). The correlation between the other potential groups of prey showed low negative and positive values (<0.31) (Fig. S2).

The estimated relative contribution of the potential prey groups of *S. lewini* indicated that the main dietary contribution was provided by coastal crustaceans (median [95% CI] = 30.3% [24.5–37.0%]), followed by oceanic cephalopods (28.0% [12.1–40.5%]), mangrove teleosts (26.4% [14.6–36.8%]), coastal cephalopods (9.7% [2.1–22.9%]), and oceanic teleosts (4.8% [1.1–12.0%]). The relative contribution of the groups of potential prey for each individual *S. lewini* indicated different degrees of contribution to the diet at the population level (Fig. 3).

The mixing model by age group indicated that coastal crustaceans, oceanic cephalopods, and mangrove teleosts contributed to the diet of *S. lewini* throughout its life cycle (Fig. 4A). Of these 3 prey groups, coastal crustaceans contributed a greater proportion to the diet at 0.1–0.6 yr (37% [24.3–50.1%]), 0.6–1.3 yr (36% [20.5–45.4%]), 3.7–4.5 yr (34% [20.6–43.6%]), and 5.1–5.5 yr (35% [7.5–52.7%]), while the relative contribution of oceanic cephalopods was highest at 1.3–2.0 yr (22% [3.3–44.5%]), 2.0–2.7 yr (22% [3.9–48.2%]), and 2.8–3.6 yr (39% [5.2–57.9%]) (Fig. 4B).

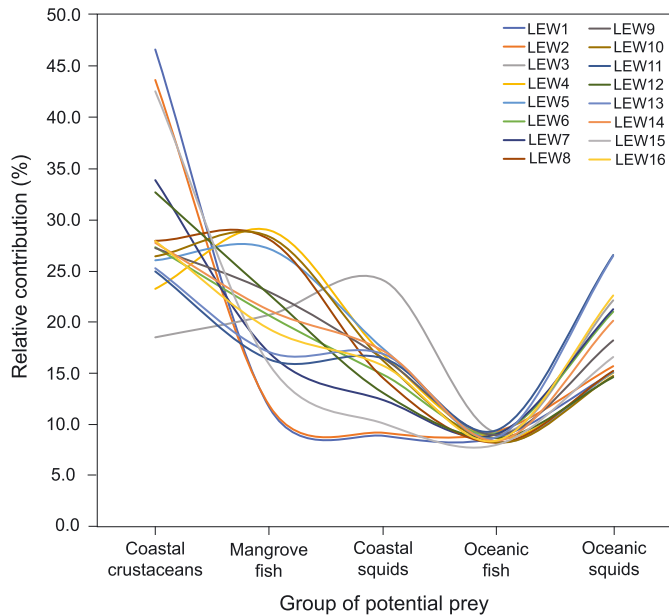


Fig. 3. Individual feeding preferences of the scalloped hammerhead shark *Sphyrna lewini*, represented by the estimated relative contributions of potential prey groups, reported in contribution percentage values obtained from stable isotope mixing models. LEW#: identification code of each individual

3.4. Feeding ontogeny

Vertebral collagen $\delta^{13}\text{C}$ of *S. lewini* decreased along the vertebra (from 3–8 mm VR) (Fig. 5), while $\delta^{15}\text{N}$ increased between 3 and 5 mm VR from the

center of the vertebra, and then decreased at >6 mm (Fig. 5). The estimated ages are presented in Table 3 according to each VR.

3.5. Niche width and isotopic overlap

The SEA_C estimated for *S. lewini* was 1.80‰^2 at the population level, while at the individual level SEA_C ranged between 0.20 and 3.30‰^2 , suggesting that 50% of the individuals had broad isotopic niches ($>0.50\text{‰}^2$) while the rest of the individuals had narrow niches ($<0.50\text{‰}^2$; Table 2).

Alternatively, the results for isotopic niche by estimated age suggested that the individuals between 5.1–5.5 yr had the narrowest niches, followed by intermediate niches for individuals between 2.0–2.7 and 3.7–4.5 yr, and broad isotopic niches between 0.1–0.6 and 0.6–1.3 yr (Table 3).

Based on the isotopic overlap, the individual LEW1 showed the lowest probabilities of overlap in both directions (LEW1 vs. all specimens; $<50\%$), followed by the individual LEW2 (LEW2 vs. all specimens; $<60\%$); while the other overlap combinations showed the highest probabilities of isotopic overlap ($>60\%$) (Fig. 6, Fig. S3).

Meanwhile, the isotopic overlap between ages suggested that individuals of 5.1–5.5 yr showed an overlap probability $<40\%$ with respect to all age groups while the other ages displayed overlap prob-

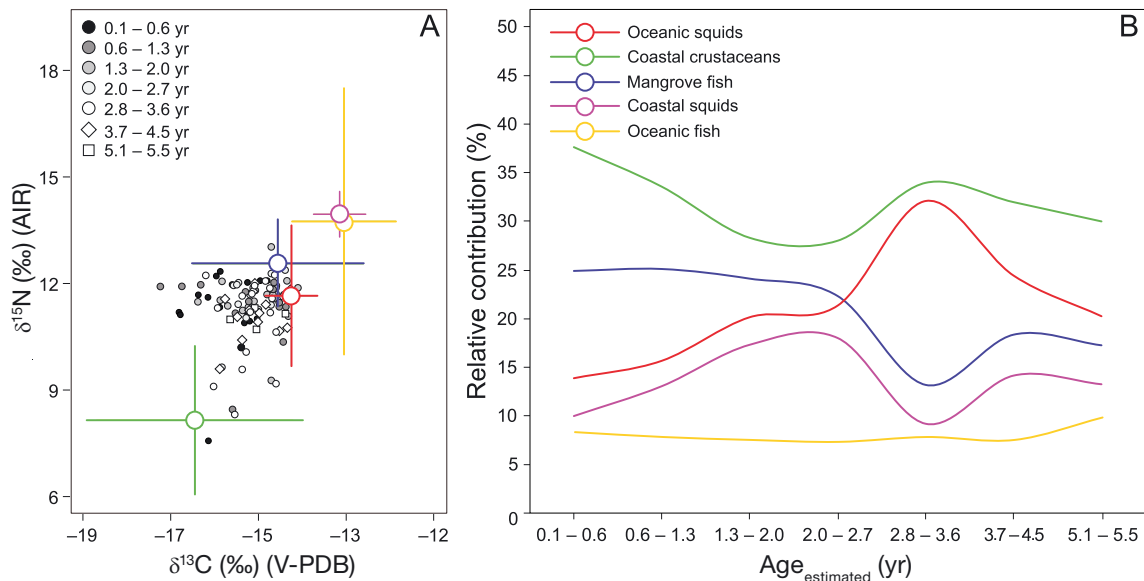


Fig. 4. (A) Isotopic values by age groups in scalloped hammerhead shark *Sphyrna lewini* (gray symbols) and their potential prey (mean \pm SD; colors as in Fig. 4B) used in mixing models. (B) Estimated prey group contribution to diet of scalloped hammerhead shark *Sphyrna lewini* during its life cycle, represented by relative contribution from stable isotope mixing models, and reported in percentage values for estimated age groups

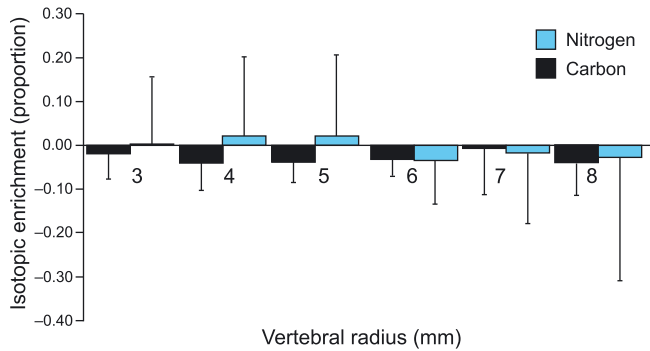


Fig. 5. Mean (\pm SD) isotopic enrichment of ^{13}C and ^{15}N in the scalloped hammerhead shark *Sphyrna lewini* by vertebral radius, related to the values of the sample points relative to the 2 mm base point ($n = 101$)

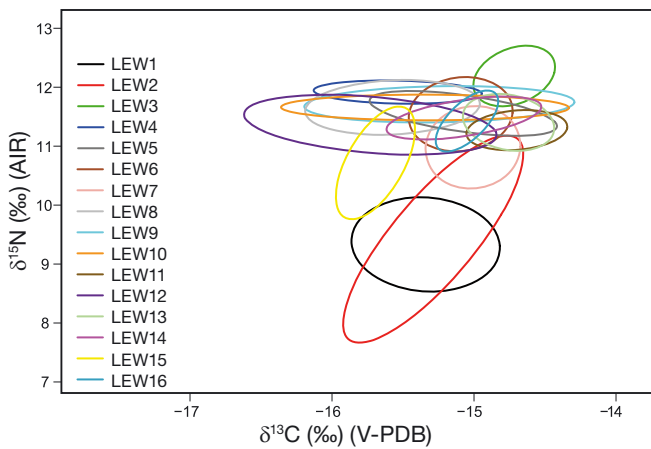


Fig. 6. Isotopic overlap between individuals of the scalloped hammerhead shark *Sphyrna lewini* around Malpelo Island, Colombia. LEW#: identification code of each individual

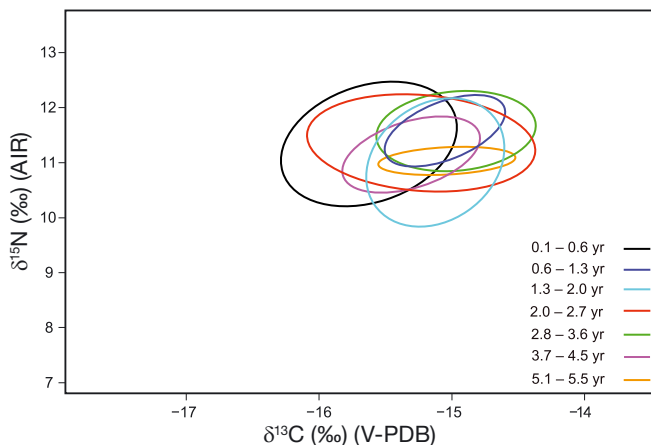


Fig. 7. Isotopic overlap between estimated ages of the scalloped hammerhead shark *Sphyrna lewini* around Malpelo Island, Colombia

abilities $>50\%$ (Fig. 7, Fig. S4). The probability of overlap between all ages generated the highest probabilities of overlap ($>70\%$) (Fig. 7, Fig. S4).

4. DISCUSSION

The investigation of feeding ontogeny using hard anatomic structures has allowed the integration of dietary information throughout the life cycle (Estrada et al. 2006, Kim et al. 2012), which is typically difficult to determine in highly migratory species. The scalloped hammerhead *Sphyrna lewini* uses diverse coastal and oceanic feeding areas, which indicates a broad isotopic niche ($\text{SEA}_C = 1.80\text{‰}^2$), occupying different TLs from primary carnivore ($\text{TP} = 3.0$) to tertiary carnivore ($\text{TP} > 4.0$) (Mearns et al. 1981). These results demonstrate an ontogenetic change in the use of habitat (from coastal to oceanic zones) and prey types during their life cycle.

4.1. Feeding sources

Different food webs make up the coastal and oceanic areas used by *S. lewini* as foraging areas along the ETP. Juveniles of the species mainly forage in coastal areas where they consume crustaceans (e.g. Penaeidos), planktivorous fish (e.g. Engraulidae, Carangidae, Haemulidae), benthic fish (e.g. Merluccidae, Paralychthidae, Synodontidae), and coastal cephalopods (e.g. Loliginidae, Octopodidae) (Estupiñán-Montaño et al. 2009, Bornatowski et al. 2014, Torres-Rojas et al. 2014, Flores-Martínez et al. 2016, Rosende-Pereiro et al. 2020). Adults prefer oceanic areas where they have access to larger prey (i.e. Scombridae, Ommastrephidae; Estupiñán-Montaño et al. 2009, Galván-Magaña et al. 2013).

These food preferences could explain the origin of different sources of basal carbon, reflected in a depletion of ^{13}C associated with migrations from coastal areas to oceanic, pelagic, or demersal areas (Cherel et al. 2008), which causes the ontogenetic changes. This is especially true at low latitudes, where C_4 biomass abounds on the continent and seagrasses are present, contributing to higher $\delta^{13}\text{C}$ values at the base of the food chain (Pereira et al. 2007).

Our results suggest that the food sources of *S. lewini* correspond to trophic webs of the ETP and Malpelo Islands, supported mainly by pelagic phytoplankton ($\delta^{13}\text{C} = -22$ to -18‰) and benthic macroalgae (-17‰) (Peterson & Fry 1987, France 1993). The latter is supported by the values of $\delta^{13}\text{C}$ from

–17.2 to –14.1‰, which, after being corrected for the TDF of the vertebrae ($\Delta^{13}\text{C} = 3.75\text{‰}$, Hussey et al. 2010) showed $\delta^{13}\text{C}_{\text{adjusted}}$ values from –20.9 to –17.8‰, similar to the basal sources of Malpelo Island (zooplankton: –21.9 to –17.8‰ and macroalgae *Padina* spp.: –21.0 to –17.1‰; C. Estupiñán-Montaña et al. unpubl. data).

Isotopic studies in oceanic sharks that are highly migratory and that frequent oceanic islands (i.e. Galapagos Islands, Ecuador) along the ETP, such as the blue shark *Prionace glauca* (Carey et al. 1990, Vandeperre et al. 2014), showed $\delta^{13}\text{C}$ values from –16.8 to –13.1‰ (Estupiñán-Montaña et al. 2019). These values suggest that the base of the trophic chain demonstrates low latitudinal variation for the zooplankton of Malpelo Island, Colombia ($\delta^{13}\text{C} = -20.7 \pm 1.48\text{‰}$; C. Estupiñán-Montaña et al. unpubl. data) and the Galapagos Archipelago ($-21.6 \pm 0.52\text{‰}$; Páez-Rosas et al. 2012; difference = 0.9‰), and/or the use of similar feeding zones in the life cycle of both species along the ETP.

The similarities in the values of $\delta^{13}\text{C}$ of *S. lewini* and *P. glauca* could reflect the use of a similar type of feeding area around Malpelo Island and the Galapagos Archipelago. The latitudinal differences in the basal $\delta^{13}\text{C}$ between both top predators could reflect (1) migratory processes of *S. lewini* (Bessudo et al. 2011a,b) and *P. glauca* (Carey et al. 1990, Vandeperre et al. 2014) and (2) the timeframe represented by vertebral rings (e.g. Kim et al. 2012). On the other hand, the differences in $\delta^{15}\text{N}$ between both species was 4.2‰ (*S. lewini* = $11.3 \pm 0.09\text{‰}$, *P. glauca* = $15.5 \pm 0.20\text{‰}$; Estupiñán-Montaña et al. 2019) which suggests (1) the use of distinct ecosystems that correspond to different values of basal $\delta^{15}\text{N}$, with the difference related to oceanographic processes (e.g. upwelling, nitrification/denitrification, assimilation, currents, oxygen minimum layer; Olson et al. 2010, Casciotti et al. 2013, Martin et al. 2019); (2) greater migratory routes (in distance) by *P. glauca* with respect to *S. lewini*, which permits the use of different geographical areas along the ETP; and (3) the use of different nursing areas (*S. lewini*: coastal zones, Quintanilla et al. 2015, Zanella et al. 2019; *P. glauca*: oceanic zones, Kubodera et al. 2007).

On the other hand, $\delta^{13}\text{C}$ values in muscle samples suggest that *S. lewini* frequently uses oceanic zones to feed ($\delta^{13}\text{C}_{\text{muscle}} = -15.9\text{‰}$, Looor-Andrade et al. 2015 and -16.3‰ , Li et al. 2016, Estupiñán-Montaña et al. 2017). These $\delta^{13}\text{C}$ results are similar to those obtained for the interval of 0.1–0.6 yr (-15.6‰), which reflect the maternal signature and diet (McMeans et al. 2009, Vaudo et al. 2010, Estupiñán-Montaña et al.

2019). These similarities could be supported by the rate of renewal of muscle tissue (488 and 555 d; MacNeil et al. 2005, Logan & Lutcavage 2010) as well as the gestation period of *S. lewini* (10–11 mo; Torres-Huerta et al. 2008).

Finally, our results of $\delta^{13}\text{C}$ (–17.2 to –14.1‰) from different age groups of *S. lewini* are supported by 2 factors: (1) migratory patterns of *S. lewini* between oceanic islands and the ETP (Bessudo et al. 2011a,b, Nalesso et al. 2019) as aggregation and feeding zones; and (2) the use of coastal zones as nursery areas (Quintanilla et al. 2015, Zanella & López-Garro 2015), ecosystems which are characterized by the presence of mangroves and seagrass (Zanella et al. 2019) and continental C_4 biomass contributions. Both factors are confirmed by genetic connectivity between individuals of *S. lewini* samples from Malpelo Island and the provinces of Choco and Nariño, Colombia (Quintanilla et al. 2015), which represent 22.9 and 52.3% of the mangroves in the Colombian Pacific (282 835 ha; Ulloa-Delgado et al. 2004). These results exemplify the role of Colombian mangroves in the ontogeny of *S. lewini*.

4.2. TP

Similar to other sharks, *S. lewini* is considered to be a species that occupies high positions in the trophic networks of the ETP (3.8–5.9; Li et al. 2016, Estupiñán-Montaña et al. 2017). These observations are consistent with the wide range of TPs (2.9–6.5) estimated in this study.

The different TPs that juveniles of *S. lewini* occupy throughout their life cycle are reflected in the consumption of prey from low trophic levels (e.g. penaeids, engraulids, clupeids, hemiramphids; Estupiñán-Montaña et al. 2009, Torres-Rojas et al. 2014, Flores-Martínez et al. 2016) and some mid-level predators (e.g. Scombridae, Lutjanidae, Ommastrephidae; Estupiñán-Montaña et al. 2009, Galván-Magaña et al. 2013, Torres-Rojas et al. 2014, Flores-Martínez et al. 2016). In contrast, the high TPs (TP > 6.0) are related to the feeding behavior of *S. lewini* adults, which consume other elasmobranchs (Bethea et al. 2004, Bornatowski et al. 2014), along with the consumption of prey from different trophic webs with a $\delta^{15}\text{N}$ -enriched baseline (Vanderklift & Ponsard 2003, Graham et al. 2010, Tamburin et al. 2019) that could be reflected in the chemical composition of the vertebrae (Estupiñán-Montaña et al. 2019); however, more studies would be necessary to obtain more precise diet information.

The results of this study suggest that *S. lewini* plays different trophic roles (from primary consumer to tertiary consumer) throughout its life cycle, consuming prey from trophic webs with different $\delta^{15}\text{N}$ baselines; these can change over time due to environmental and/or oceanographic factors (e.g. upwelling, currents), and feeding zones (e.g. oceanic zones, mangroves, reefs). Relatively anoxic zones experience a reduction of NO_3^- generating residual nitrates enriched in ^{15}N (Granger et al. 2008) and/or upwelling oceanic areas that could reflect $\delta^{15}\text{N}$ values of 5–8‰ (Sigman et al. 1997). In the case of the Malpelo Islands, upwelling during the whole year (Rodríguez-Rubio & Stuardo 2002) would favor ^{15}N -enrichment from deep waters rich in nutrients (Bauersachs et al. 2009). Nonetheless, the absence of fractionation due to assimilation (by the phytoplankton, not depleting nitrates) and the abundance of herbivorous prey would explain the relatively low values of $\delta^{15}\text{N}$ in the vertebrae of *S. lewini* (7.6–13.0‰).

In contrast, the use of habitat occupied by diazotrophic cyanobacteria communities ($\delta^{15}\text{N}$ range: -5 to $+2$ ‰; Bauersachs et al. 2009), such as the mangrove zones, present depleted $\delta^{15}\text{N}$ values (i.e. detritus: -0.9 ± 0.5 ‰; leaves: 1.8 ± 0.6 ‰; sediment: -0.1 ± 0.5 ‰; seston: 1.8 ± 1.1 ‰; Medina et al. 2018). The Colombian mangroves are used as nursery areas by *S. lewini* (Quintanilla et al. 2015), where they spend their first 2 yr of life (Zanella et al. 2019). The use of mangroves as nursery areas by *S. lewini* supports the low values of $\delta^{15}\text{N}$ in the vertebrae, which is a reflection of the consumption of coastal prey from low trophic levels, i.e. coastal crustaceans (Estupiñán-Montaño et al. 2009, Flores-Martínez et al. 2016). For example, after being corrected for TDF, individuals LEW1 and LEW2 showed $\delta^{15}\text{N}_{\text{corrected}}$ values of 7.8 and 7.9‰, respectively, which are relatively similar to the snapping shrimp *Alpheus colombiensis* (mean \pm SD: 6.4 ± 0.7 ‰; Medina et al. 2018). These values could be reflecting the use of mangroves as feeding areas for this species. Therefore, the consumption of prey from low trophic levels, the use of mangroves as nursing areas, the consumption of oceanic prey from high trophic levels, and the individual foraging strategies of the species (Fig. 4) allow *S. lewini* to have a diversity of roles in coastal and oceanic trophic chains of the ETP. However, more studies concerning the baseline throughout the ETP are necessary given the oceanographic conditions of these zones (e.g. upwelling, currents, etc.; Rodríguez-Rubio & Stuardo 2002, Rodríguez-Rubio et al. 2007) which may demonstrate higher variability in basal isotopic signals.

4.3. Bayesian mixing models and prey selection

Some crustaceans, teleosts, and cephalopods of coastal zones are important prey for *S. lewini* juveniles (Torres-Rojas et al. 2010, Flores-Martínez et al. 2016) and adult females (Estupiñán-Montaño et al. 2009). Our results support these conclusions, because this study found that coastal crustaceans made the highest dietary contribution, followed by oceanic cephalopods, mangrove fish, and coastal cephalopods (Figs. 3 & 4). This highlights the importance of coastal crustaceans and mangrove fish in the diet and habitat selection of *S. lewini*. Accordingly, the input of these 2 prey groups (crustaceans and mangrove fish) in the diet of *S. lewini* could be related to (1) feeding behavior of neonates and small juveniles that feed in coastal zones (Flores-Martínez et al. 2016) and nursing areas (mangrove zones), in which they spend their first 2 yr of life (Zanella et al. 2019); (2) availability of accessible prey for juveniles that are adjusting their ability to capture prey; and (3) adult females consuming coastal and benthic prey (Estupiñán-Montaño et al. 2009). These results demonstrate the importance of the Colombian mangrove ecosystems in the ontogeny of *S. lewini*.

The low values of $\delta^{15}\text{N}$ observed in the vertebrae and the isotopic enrichment observed between the first millimeters of the VR (3–5 mm VR; Fig. 6) could be a reflection of maternal transfer processes (i.e. inherited proteins; Estupiñán-Montaño et al. 2019) and the scavenger consumption of high trophic prey hunted by adult females. One explanation about the enrichment of ^{15}N could be maternal (Fuller et al. 2004) or embryonic (Vander Zanden et al. 1998) changes during 2 stages of the gestation period (10–11 mo; Torres-Huerta et al. 2008) of *S. lewini*. Thus, the first stage would correspond to the initiation of the gestation period, in which pregnant females spend more time in oceanic areas, consuming prey with high protein content (e.g. *Dosidicus gigas*: 78.3–78.5%; Ochoa-Tepetla 2014). In the second stage of gestation period, pregnant females move towards coastal areas (e.g. nursing areas), where there is a higher availability of prey and easier access for feeding (Estupiñán-Montaño et al. 2009, Torres-Rojas et al. 2010, Flores-Martínez et al. 2016), which may allow them to increase foraging efficiency. In this way, conserved energy would be routed to development and nutrition of the final stage of the embryos, reflected in the depleted ^{15}N from the food webs of the mangrove zones (Medina et al. 2018). Another explanation for the depleted ^{15}N is their strategy of favoring small, normally herbivorous prey from lower trophic levels.

The mixing model applied to individuals did not show an individual feeding pattern in *S. lewini*, unlike those shown in Ecuadorian waters (Loor-Andrade et al. 2015). The observed discrepancies between studies could be related to (1) the integration of the maternal isotopic signature in the vertebrae of each individual (inherited proteins), reflecting the consumption of coastal prey (i.e. crustaceans and mangrove fishes); (2) migratory movements between coastal and oceanic zones, where they consume prey with a high caloric content (i.e. oceanic cephalopods, Ochoa-Tepetla 2014); and (3) high isotopic overlap between individuals, suggesting the consumption of similar prey and feeding areas.

4.4. Feeding ontogeny and habitat use

Studies of the trophic ecology of *S. lewini* suggest changes in the consumption of prey and habitat use that are associated with different life cycle stages (Loor-Andrade et al. 2015). Neonates and small juveniles of *S. lewini* prefer to consume small and medium sized prey associated with coastal areas (e.g. Torres-Rojas et al. 2014, Rosende-Pereiro et al. 2020). These observations are confirmed by $\delta^{13}\text{C}$ values (-17.2 to -14.1‰) of ages 2.0–2.7 and 5.1–5.5, which suggest that *S. lewini* searches for food mainly in highly productive areas, such as mangrove zones (Fig. 4B) along the Colombian Pacific coast. On the contrary, intermediate ages (2.8–5.0 yr) were associated with the use of zones of lesser productivity (i.e. oceanic, pelagic, and benthic; Fig. 4B), where they consume larger prey (Estupiñán-Montaño et al. 2009, Galván-Magaña et al. 2013).

Accordingly, the preferences in habitat use and prey consumption of *S. lewini* highlighted changes in their trophic ecology in relation to age, supported by SCA (e.g. Estupiñán-Montaño et al. 2009, Rosende-Pereiro et al. 2020) and the findings of mixing models (Fig. 4) which suggest that such ontogenetic change is carried out at approximately 2 yr of age (Figs. 4B & 5). The explanation for the changes in the use of habitat and consumption of prey of *S. lewini* could be due to (1) the use of nursery areas for a 2 yr period (Zanella et al. 2019); (2) abundance and availability of prey; (3) body development and prey-capture skills (Lowe et al. 1996); (4) multiple visits at intermediate ages (1.3–4.5 yr) to the nursery areas (Bessudo et al. 2011a,b); and (5) pregnant females spending more time in coastal zones (see Section 4.3), because these areas provide them with sufficient food for the development of their pups as well as safe zones for giving birth (Estupiñán-

Montaño et al. 2019). Therefore, our results suggest that *S. lewini* has a habitat cycle. Individuals between 0–2 yr old use coastal zones as important feeding areas, where they have easy access to small coastal prey (benthic and pelagic). From approximately 2 yr of age they migrate to oceanic areas, changing their dietary preferences from small prey to large squid (e.g. Ommastrephidae). Finally, they return to coastal areas as adults (>4 yr; Anislado-Tolentino et al. 2008), which is potentially related to the search for important areas to ensure giving birth to, feeding, and protecting their young (i.e. mangrove areas).

We found similar contributions of crustaceans to the diet of individuals of 0.1–1.3 and 3.7–5.5 yr (Fig. 4B) that could be the reflection of maternal transfer processes (McMeans et al. 2009, Vaudo et al. 2010). This hypothesis is partially supported by the isotopic enrichment observed between the 3 and 5 mm VR (Fig. 5). The isotopic enrichment analysis did not show a clear pattern for ^{13}C along the vertebrae (Fig. 5). The reason for these results could be related to (1) the use of the same feeding areas by juveniles younger than 2 yr of age and pregnant females during the second phase of the gestation period; and (2) sporadic visits of juveniles over 2 yr of age to the nursery areas (Zanella et al. 2019). This would cause the maternal $\delta^{13}\text{C}$ present in *S. lewini* juvenile vertebrae to remain combined (maternal transfer); however, it is possible that the metabolic turnover result in the maternal carbon isotopic signal is being 'diluted' between 3.1–4.5 yr (6–7 mm VR; Fig. 5). In contrast, $\delta^{15}\text{N}$ values were higher between 0.6–2.7 yr (3–5 mm VR; Fig. 5). These higher values reflect prey from high trophic levels (e.g. Ommastrephidae) consumed by the mothers during the gestation period (see Section 4.2). The results for adult individuals indicate a turnover in vertebrae proteins, which results in primary signals of maternal $\delta^{15}\text{N}$ being 'diluted' between 2.0–3.6 yr (5–6 mm VR; Fig. 5), suggesting that *S. lewini* would reflect the isotopic signal of the life cycle of its prey and its age when it was consumed, which is also consistent with the time they spend in the breeding areas (~2 yr; Zanella et al. 2019).

4.5. Niche and isotopic overlap

SEA_C estimates indicated that a high number of individuals presented broad isotopic niches ($\text{SEA}_C > 0.70\text{‰}^2$; Tables 2 & 3). These results, along with the low probability of individual isotopic overlap (Fig. 6), suggest low interspecific competition and a high degree of specialization. This behavior was observed

for *S. lewini* in Ecuadorian waters (Loor-Andrade et al. 2015), as well as for the white shark *Carcharodon carcharias* (Kim et al. 2012). These characteristics can be attributed to (1) changes in prey preference and foraging locations (Kim et al. 2012) in relation to ontogeny (Kim et al. 2012, Loor-Andrade et al. 2015); (2) consumption of the same prey in the same area (Kim et al. 2012); and (3) the consumption of the same prey in the same area or the combination of different types of prey in different localities (Kim et al. 2012). These explanations are confirmed by the high contribution of coastal crustaceans, mangrove fish, and oceanic cephalopods in individuals (Fig. 3), associated with vertical and horizontal migratory movements (Bessudo et al. 2011a,b).

Moreover, isotopic niches estimated by age group suggest that juveniles (2.0–2.5 yr) and adults (>4.0 yr; Anislado-Tolentino et al. 2008) occupy narrow niches. These results are a reflection of the migration by pregnant females towards coastal areas to consume prey that are easier to capture and have high protein content (i.e. shrimp: 82–86%; Rivas-Vega et al. 2001), as well as the use of nursery zones, as these are places where neonates can have access to abundant and easily captured prey (e.g. crustaceans, mangrove fish; Fig. 5). Conversely, the intermediate ages (2.7–5.0 yr) occupied broad isotopic niches ($SEA_C > 0.90$; Table 3). These results suggest the exploration and use of new feeding areas and the consumption of a higher diversity of prey. This behavior is associated with morphological changes and the ability to capture potential prey.

5. CONCLUSIONS

This investigation is the first study to describe in detail the ontogenetic trophic ecology of *Sphyrna lewini* of Malpelo Island based on vertebral $\delta^{13}C$ and $\delta^{15}N$ values, demonstrating ontogenetic changes in diet and habitat use. The results of the study showed that *S. lewini* occupies a wide trophic niche as a result of the use of different habits (coastal and oceanic) and the consumption of prey from different trophic levels throughout its life cycle. These changes reflect a migration from coastal to oceanic zones in juveniles (~2–4 yr old) and their return to coastal habitats as adults (>4 yr), potentially related to the use of coastal zones (i.e. mangroves) in the ETP, both as important feeding areas for neonates and feeding/pupping grounds for adults.

Finally, the enrichment of ^{15}N in the vertebrae reflects the maternal transfer of nutrients during preg-

nancy, particularly in the first stages of life. Thus, the isotopic signal could be an indicator of the trophic level of their mothers. In contrast, $\delta^{13}C$ values cannot be used in a similar way due to the use of similar feeding zones by neonates and adults. Metabolic turnover processes contribute to a loss of the original isotopic signal. Maternal ^{13}C could be diluted between 3.6–4.5 yr and maternal ^{15}N diluted between 2.0–3.6 yr of age (Fig. 6). Accordingly, the isotopic signal of neonate and juvenile *S. lewini* could also be an indicator of prey sources used by their mothers, and maternal trophic level (Estupiñán-Montaño et al. 2019). Therefore, isotopic values of *S. lewini* juveniles should be interpreted with caution when making dietary inferences for this species.

Additionally, the estimates of relative contribution (mixing models) and TPs must be applied with caution due to the uncertainty that can be produced when using diverse TDFs (Parnell et al. 2013, Hussey et al. 2014), particularly when the species demonstrates ontogenetic changes (Hussey et al. 2014). In future research we suggest the use of TDFs in shark species with 'equivalent' food habits and phylogenetically similar species.

The results of this study have provided additional information to reduce knowledge gaps for *S. lewini* in the ETP, and in particular highlight the importance of the mangrove areas of the Colombian Pacific as significant feeding sites for neonates, juveniles, and adults of the species. The use of anatomically hard structures (i.e. vertebrae) is of particular use, as it integrates information on the dietary ontogeny of a shark species throughout its life cycle and provides a better understanding of the trophic characteristics of the species, as well as the variety of roles they can play in different marine ecosystems.

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Appendix.

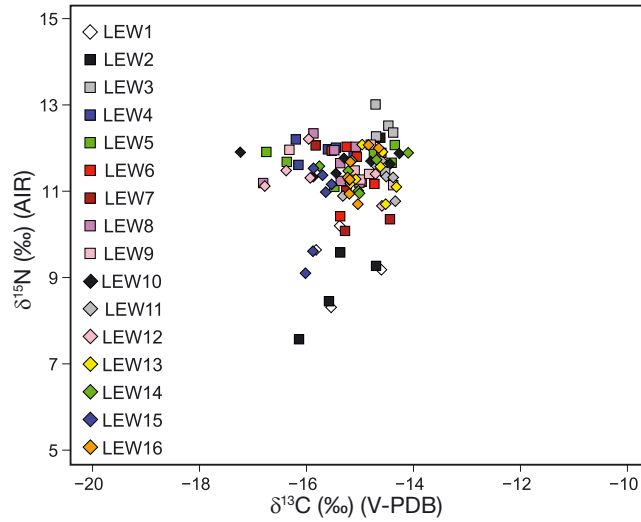


Fig. A1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all vertebral samples analyzed ($n = 101$) from scalloped hammerheads *Sphyrna lewini* captured around Malpelo Island, Colombia. LEW#: identification code of each individual

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