Using stable isotopes analysis to understand ontogenetic trophic variations of the scalloped hammerhead shark at the Galapagos Marine Reserve

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Abstract

Changes in life-history requirements drive trophic variations, particularly in large marine predators. The life history of many shark species is still poorly known and understanding their dietary ontogeny is a challenging task, especially for highly migratory species. Stable isotope analysis has proven as a useful method for examining the foraging strategies of sharks and other marine predators. We assessed the foraging strategies and ontogenetic changes of scalloped hammerhead sharks, Sphyrna lewini, at Galapagos Marine Reserve (GMR), by analysing δ13C and δ15N signatures in different maturity stages. Our isotopic results suggest ontogenetic shifts in resource use between sub-adult and adult stages, but not between adult and juvenile stages. Carbon isotopic signatures found in the juvenile stage were enriched in contrast to sub-adults (~0.73‰) suggesting a combination of the maternal input and the use of coastal resources around the Galapagos Islands. Adult female sharks also showed enrichment in δ13C (~0.53‰) in comparison to sub-adult stages that suggest feeding in high primary productivity areas, such as the GMR. This study improves the understanding of the trophic ecology and ontogenetic changes of a highly migratory shark that moves across the protected and unprotected waters of the Eastern Tropical Pacific.

Introduction

The global decline of large predators, such as sharks, has had major impacts on many marine food webs [1, 2], since most shark species are primary or secondary predators controlling trophic relationships and energy flow in the ecosystems they occupy [3]. Understanding foraging strategies of such predators that are highly mobile, provides key information about their basic
ecology and migratory patterns [4, 5]. These life history aspects are very valuable when implementing management strategies aimed at reducing ongoing global shark population declines [6, 7].

The trophic ecology of sharks has been traditionally studied using gut content analysis from fisheries landings [8, 9]. However, this analysis can be challenging when studying threatened large migratory species, particularly in areas where their capture is not permitted, such as fully-protected marine reserves [10]. Stable isotopes of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) in animal tissues are a non-lethal alternative that can provide retrospective information on foraging ecology, since the isotopic composition of the predator reflects the assimilated prey information over time [4, 11]. The isotopic signatures of marine primary producers vary depending on biogeochemical and oceanographic processes. Such differences get spread through local food webs resulting in consumers with isotopic signatures resembling the food webs they feed on [12, 13].

The scalloped hammerhead shark (Sphyrna lewini) is distributed globally in tropical and semitropical waters [14]. The oceanic islands of the Eastern Tropical Pacific (ETP) harbour some of the largest aggregations of this species [15–17] that have become a significant tourist attraction and support lucrative SCUBA diving industries [18, 19]. Sphyrna lewini show seasonal aggregations and migratory patterns within the ETP, covering thousands of miles in their migrations. These regional movements include unprotected waters between the oceanic Marine Protected Areas (MPA) of the Galapagos (Ecuador), Malpelo (Colombia) and Isla del Coco (Costa Rica) where domestic and international fishing fleets operate [20–22]. Despite its ecological and economic importance, this species is heavily fished and caught as bycatch [23, 24], which has greatly decreased their populations [25–29]. Such alarming decrease has led to its listing as ‘Critically Endangered’ (CR) by the IUCN Red List in 2019 and its inclusion in the Annex II of the Convention on International Trade in Endangered Species (CITES) [24, 30].

Sphyrna lewini shows sexual segregation in the ETP, with aggregations found in waters around oceanic island skewed towards large females which likely migrate to coastal waters to give birth [20, 21, 31, 32] after mating in open waters [33]. In contrast, male sharks are thought to migrate more widely in open waters [34, 35]. Juvenile S. lewini sharks are commonly found in shallow waters, which function as nursery grounds along the ETP, and are often caught in coastal artisanal fisheries [36–40]. Although the life history of S. lewini sub-adult stage is noticeably less understood than adult and juvenile stages, it is known that sub-adult female sharks migrate from nursery areas sooner than sub-adult male sharks [31, 41]. However, sub-adult sharks are less commonly found in aggregations where large female sharks are dominant [17, 42] and this apparent disappearance of the transitory sub-adult stage hinders the understanding of this highly migratory species’ ecology.

The Galapagos Marine Reserve (GMR) has afforded over 20 years of protection from industrial fishing for many species of sharks, including S. lewini [15, 17, 43], though many endangered species are often caught as bycatch in artisanal longline fisheries [44]. Studies on residency and migratory patterns using telemetry show that S. lewini is semi-resident in oceanic islands of the ETP and has complex movement patterns around them. During daytime, S. lewini stays close to the islands and disperses into deep and open water during night-time, likely to forage on deep-water prey [17, 22, 45, 46]. Stomach content analyses from several coastal locations across the ETP suggest this species forages on a variety of prey. Generally, cephalopods are the main prey for adult sharks, while crustaceans and bony fish are the primary prey of juvenile sharks [40, 47, 48]. Particularly, adult female hammerhead sharks seem to prefer Octopus spp. and Histiototeuthis spp., while the squids Dosidicus gigas and Mastigoteuthis spp. are the most common prey in juvenile individuals [40, 49]. Despite this knowledge
of *S. lewini* in Ecuadorian waters, nothing is known about their trophic ecology at the GMR which is a concern given ongoing population declines in the region [25, 26].

Here, we present the first study of foraging strategies of *S. lewini* at the GMR, establishing a baseline of the trophic ecology of an endangered species inhabiting an almost-pristine biodiversity hotspot. We hypothesized there would be differences in the isotopic niches of *S. lewini* throughout its ontogeny as a result of variance in foraging strategies and environmental variability in the region.

**Materials and methods**

**Ethics statement**

This research was approved by the Galapagos National Park Directorate (GNPD) on the research permits granted to Pelayo Salinas-de-León from the Charles Darwin Foundation (CDF) (Research Permits: PC-27-17, PC-46-18 and PC-53-19), and Diego Páez-Rosas from the University San Francisco de Quito (USFQ) (PC-24-17, PC-69-18 and PC-86-19). The methods described here were reviewed and approved by the GNPD, CDF and USFQ committees responsible for assessing animal welfare in research activities.

**Study site**

The Galapagos archipelago (Fig 1) is located approximately 1,000 km west from mainland Ecuador and comprises 15 major islands [50]. The archipelago lays within an upwelling system due to the convergence of major ocean currents that characterize the ETP [51–53]. Variations in the strength of these currents throughout the year produce two distinct seasons: the warm season from January to May and the cool season from July to November, while June and December are considered transitional months [51, 54].

**Sample collection**

Skin samples from large pregnant female sharks (>230 cm TL, *n* = 64) and the smallest individuals (<150, *n* = 29, referred to as subadults hereafter) that could be located among the large groups aggregating at Darwin Island [15] were collected during August 2017 and October 2019. These samples were taken using sterile 6-mm biopsy punches to remove ~10 mg of skin tissue close to the first dorsal fin, using a biopsy probe (Pneudart Inc., USA) attached to a Hawaiian sling pole while freediving at Darwin Island (Fig 1). During May 2017 and August 2019, skin samples from the first dorsal fin (fin clips) from juvenile sharks (44 to 89 cm TL, *n* = 33) were collected in shallow mangrove bays of Santa Cruz Island. For such sampling, juvenile sharks were considered when the size is ≥100 cm TL and had a visible and closed umbilical cord wound [56, defined as USS level 4 by 57]. Juvenile shark catch-and-release procedures were based on a standard protocol for monitoring live sharks in shallow bays developed by the GNPD [58]. All collected samples were stored at -20˚C.

**Sample processing**

In the laboratory, shark skin samples were rinsed with deionized water to eliminate residues that could alter their isotopic signatures. Samples were placed in glass vials, which were previously treated for 24 h with a chromic acid mixture prepared from sulfuric acid and potassium dichromate [59]. Samples were then dried in a desiccator at 80˚C for 12 h to remove all moisture and a lipid-extracted protocol was applied via three sequential 24 h soaks in a 2:1 chloroform:methanol solution, then rinsed with deionized water and dried again [5]. This process was applied because lipids incorporate less δ¹³C (i.e. more negative δ¹³C) which, in sufficiently
large quantities, could negatively skew the results of $\delta^{13}$C [60]. Samples were homogenized with an agate mortar to obtain a very fine powder, of which ~1 mg was weighed by means of an analytical microbalance with a precision of 0.001 mg and transferred into a tin capsule for isotopic analysis.

Stable isotope ratios of $\delta^{13}$C and $\delta^{15}$N were determined by a PDZ Europa 20–20 continuous flow isotope-ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility of the University of California, Davis (CA, USA). The precision of the isotopic analyses was 0.15, based on internal reference samples. Ratios of heavy to light isotopes were expressed in $\delta$ notation using the equation: $\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$, where $X$ is the heavy isotope, $R_{\text{sample}}$ is the ratio of heavy to light isotope in the sample, and $R_{\text{standard}}$ is the ratio of heavy to light isotope in the reference standard. The standard reference material for carbon was Pee Dee Belemnite (PDB) and for nitrogen was atmospheric N$_2$. Units are part per thousand (per mil, ‰). Within-run analytical precision was estimated via analysis of two proteinaceous internal reference materials, which was ±0.2‰ for both $\delta^{13}$C and $\delta^{15}$N values. We also measured the weight percentage of carbon and nitrogen concentration of each sample and used the C/N ratio as a proxy of evaluating the extent of lipid content that is likely to affect the results of $\delta^{13}$C values [61].

**Data analysis.** Data were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene test, respectively. The statistical significance in $\delta^{13}$C and $\delta^{15}$N values by life
stage and by years was determined using parametric (t-test and ANOVA tests) or non-parametric (Mann-Whitney and Kruskal-Wallis test) analysis and reported at a significance level of $p < 0.05$. All statistical analyses were performed using R software. Bayesian standard ellipse areas (SEAb) were used to estimate isotopic niche width and overlap among samples collected using the package SIBER (Stable Isotope Bayesian Ellipses in R) [62]. This Bayesian method provides a measure of the isotopic niche area at the population level, expressed as the SEAb in units of area ($\text{‰}^2$), the niche area containing 95% of the data for each group was chosen. We used Monte Carlo simulations to correct the bivariate ellipses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) surrounding the data points in the 95% confidence interval for the distributions of both stable isotopes [62]. These Bayesian ellipses are corrected using posteriori randomly replicated sequences (SEAc = standard ellipse area correction) [62]. The magnitude of the isotopic overlap ($\text{‰}^2$) were estimated using the estimations of the ellipses via maximum likelihood methods [62].

**Results**

**Isotopic comparison between maturity stages**

The $\delta^{13}\text{C}$ values of *S. lewini* varied between -16.58‰ and -11.79‰, with an average of -13.38(-14.96 to -11.79‰) for juvenile, -14.11 (-16.58 to -13.19‰) for sub-adult, and -13.59‰ (-14.93 to -12.54 ‰) for adult sharks. While the range for $\delta^{15}\text{N}$ values varied between 11.03‰ and 13.91‰, with an average of 12.48‰ (10.77 to 13.91‰) for juvenile, 12.46‰ (10.77 to 13.69‰) for sub-adult, and 12.28‰ (10.77 to 15.05‰) for adult sharks (Table 1, S1 Appendix). The C:N values ranged from 2.65 to 3.44 with an average of 2.9 + 0.17 (Table 1), falling within the theoretical range established for the assimilation of protein from a predator’s diet [62, 63]. The comparison showed that juvenile sharks have slightly enriched $\delta^{13}\text{C}$ values compared to adult and sub-adult stages. All three maturity stages had a similar trophic level, although adults had slightly depleted $\delta^{15}\text{N}$. The comparison also showed that sub-adult sharks had wider values for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 1 and Fig 2).

The $\delta^{15}\text{N}$ values did not vary when comparing years in all maturity stages (t-test adults $p = 0.68$, sub-adults $p = 0.80$, juveniles $p = 0.56$) but there was a significant difference in $\delta^{13}\text{C}$ values between years for juvenile sharks (Mann-Whitney test adults $p = 0.122$, sub-adults $p = 0.30$, juveniles $p = 0.001$). No significant difference was found in $\delta^{15}\text{N}$ values among maturity stages (ANOVA test $p = 0.28$), although juveniles showed the highest (enriched) values and adults showed the lowest (depleted). For $\delta^{13}\text{C}$ values, sub-adult sharks had the lowest (depleted) values and showed a significant difference (Kruskal-Wallis test $p = 0.01$) when compared to adult and juvenile sharks (Table 2).

Table 1. Summary of isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean ±SD ‰) and C:N ratio (for *Sphyrna lewini* at the Galapagos Marine Reserve (TL = total length in cm).

| Year | Maturity stage | Site        | n  | Size       | $\delta^{13}\text{C}$ (%) | $\delta^{15}\text{N}$ (%) | C:N Ratio (Mean ± SD) |
|------|----------------|-------------|----|------------|---------------------------|---------------------------|-----------------------|
| 2017 | Sub-adults     | Darwin Island | 13 | <150 cm    | -13.86 (0.66)            | 12.43 (0.61)              | 2.85 (0.14)          |
| 2017 | Adults         | Darwin Island | 30 | >230 cm    | -13.49 (0.48)            | 12.32 (0.60)              | 2.78 (0.13)          |
| 2017 | Juveniles      | Santa Cruz   | 14 | 58–82 cm   | -13.09 (0.79)            | 12.54 (0.49)              | 3.01 (0.06)          |
| 2019 | Juveniles      | Santa Cruz   | 19 | 44–89 cm   | -13.67 (0.39)            | 12.42 (0.66)              | 2.94 (0.10)          |
| 2019 | Sub-adults     | Darwin Island | 16 | <150 cm    | -14.36 (1.09)            | 12.50 (0.86)              | 2.96 (0.18)          |
| 2019 | Adults         | Darwin Island | 34 | >230 cm    | -13.69 (0.19)            | 12.25 (0.71)              | 3.04 (0.19)          |

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Niche breadth and isotopic overlap

The corrected standard ellipse area (SEAc) showed a similar isotopic niche in adult and juvenile stages, while sub-adult sharks had a larger isotopic niche in both years (Fig 3 and Table 3). The ellipses of sub-adult (SEAc = 1.24‰, credibility interval of 0.75–1.73‰) and juvenile sharks (SEAc = 1.26‰, credibility interval of 0.88–1.64‰) had a low overlap in 2017–2018. While the overlap area of adult and sub-adult sharks in 2017–2018 (0.57) was significant representing 80.1% of the ellipse surface of adult sharks and 52.5% of sub-adult sharks. The juvenile sharks' ellipse in 2017 was larger and show a low isotopic overlap relative to adult and sub-adult sharks (0.44 and 0.38 respectively, Fig 3). In 2019, the ellipse of sub-adult (SEAc = 3.02‰, credibility interval of 2.17–3.87‰) and adult sharks (SEAc = 1.64‰, credibility interval of 0.96–2.32‰) showed a low overlap that represented 74.1% of the ellipse surface of sub-adult and the 37.5% of the ellipse surface of adult sharks. A significant overlap was observed between adult and juvenile sharks in 2019 (0.50, Fig 3).

Table 2. Isotopic values of $\delta^{13}$C of adult, sub-adult, and juvenile S. lewini in the Galapagos Marine Reserve.

| Maturity stage | Adult | Sub-adult | Juvenile |
|----------------|-------|-----------|----------|
| Adult          | X     |           |          |
| Sub-adult      | 0.024 | X         |          |
| Juvenile       | 1.000 | 0.006     | X        |

Significant differences (Kruskal-Wallis test, $p < 0.05$) are shown in bold.

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Discussion

This is the first study addressing the trophic ecology of *Sphyrna lewini* at the Galapagos Marine Reserve. Our results reveal ontogenetic changes from sub-adult to adult stages as observed in other areas across the ETP [31, 32, 41] and suggest a wide range of resource use by this migratory shark through its lifetime [47].

Sub-adult *S. lewini* at the GMR show a wider pattern of resource use than adult and juvenile sharks and had a niche overlap with both adult and juvenile stages. This mixture of isotopic signatures is likely the result from an ontogenetic change in movements of sub-adults, accompanied by use of a wider variety of food sources during this maturity stage. Coastal ecosystems have enriched δ^{13}C signatures compared to pelagic environments due to the high primary
production that subsidize carbon derived from benthic macrophytes in coastal zones [64]. Additionally, the majority of deep ocean areas are homogenous in terms of nitrogen sources (nitrates), so the δ15N values are relatively higher in deeper waters than shallower areas where the primary source is the atmospheric nitrogen [65, 66]. The depleted δ13C and enriched δ15N signatures found in sub-adult sharks suggest they are feeding in oceanic and deeper waters. Particularly, the higher values of δ15N in sub-adult S. lewini suggest that sharks are moving from coastal shallow waters to oceanic and deeper waters through this life stage as observed also in the Mexican Pacific populations [31, 32, 41]. These ontogenetic shifts in resource use where adult and juvenile sharks show coastal strategies, and sub-adult sharks a more pelagic behaviour have also been observed in other species of sharks in Ecuadorian waters, such as smooth hammerhead sharks (S. zygaena) [67] and blue sharks (Prionace glauca) [68].

Juvenile sharks showed generally enriched values of δ13C compared to sub-adult and adult sharks, suggesting the use of coastal environments as nursery areas as reported in other locations of the Pacific Ocean [57, 69]. Surprisingly, juvenile sharks sampled in May 2018 were significantly enriched in δ13C signatures compared to August 2019. The integration of isotopic composition over several months due to the isotopic skin-diet fractionation [3–6 months in elasmobranchs 70] show variations associated with the seasonal changes in marine productivity commonly found within the GMR. The juvenile sharks sampled in May 2018, are likely reflecting the diet consumed during the cold season (October-November) characterized by more productive waters [71], whereas juvenile sharks sampled in August 2019 would reflect foraging during the hot season (February-March) which is characterized by less nutrient-rich waters [51, 54].

All juvenile sharks sampled had a clearly visible and closed umbilical wound indicating they were younger than a year old [57]. The slight enrichment of δ15N signatures in juvenile sharks could be explained by a reminiscence from the mother’s isotopic values assimilated through maternal transfer processes [48, 72, 73]. As pseudo-placental sharks, female S. lewini transfer nutrients directly to their offspring through a yolk-sac [74], thus the isotopic signatures of juvenile sharks are likely a mixture of inherited isotopic signatures resulting in a similar isotopic signature to adult females. Importantly, while juvenile blacktip sharks (Carcharinus limbatus) have been commonly recorded in mangrove bays within the GMR by the GNPD shark monitoring program [58, 75, 76], juvenile S. lewini sharks have been less frequently documented in the central islands of the GMR before this sampling event [77].

Table 3. Degree of isotopic niche overlap between maturity stages of Sphyrna lewini in the Galapagos Marine Reserve.

| Maturity stage | Adult | Sub-adult | Juvenile |
|----------------|-------|-----------|----------|
| 2017           |       |           |          |
| Adult          | X     |           |          |
| Sub-adult      | 0.578 | X         |          |
| Juvenile       | 0.440 | 0.384     | X        |
| 2019           |       |           |          |
| Adult          | X     |           |          |
| Sub-adult      | 0.361 | X         |          |
| Juvenile       | 0.504 | 0.244     | X        |

The degree of trophic niche overlap where a value close to 1 (shown in bold) indicates a large overlap between trophic niches.

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The enriched signatures of $\delta^{13}C$ and the overlap of isotopic niche between adult female and juvenile sharks suggest that both maturity stages are exploiting similar environments. All adult sharks sampled in this study were pregnant females that occur in large aggregations at Darwin Island and show seasonal residency between June to March [15, 17, 22]. There is an increase in blooms from the equator to the Galapagos Islands caused by the divergence in equatorial blooms associated to the wind and equatorial sub-stream intensity [78]. These increase of primary productivity levels generates an increase in phytoplankton biomass and, therefore, its respiration capacity, resulting in high CO$_2$ absorption from the aqueous environment [79, 80]. Phytoplankton blooms tend to enrich the $^{13}C$ signal, even though the high levels of photosynthesis cause a rapid use and decrease of CO$_2$ (enriched in $^{12}C$). In this process, there is discrimination towards $^{13}C$ resulting in the enrichment of $\delta^{13}C$ values in primary producers and higher trophic levels [79, 80]. The enriched signatures of $\delta^{13}C$ in both juveniles and adult female sharks suggest a pattern of residence for adult female $S$. lewini at the GMR where there are high levels of productivity that facilitate their permanence [51, 54].

Telemetry studies of $S$. lewini at the GMR have shown that females at Wolf and Darwin Islands move within the GMR and migrate between oceanic islands in the ETP [17, 22], while genetic studies show that $S$. lewini found in Malpelo Island are related to neonates found in coastal nurseries in mainland Colombia [34]. The presence of various species of sharks in juvenile stages including the $S$. lewini highlights the importance of Galapagos mangrove areas for recruitment of key marine fauna in the GMR [58, 76, 81] and highlights the importance of better understanding coastal-oceanic linkages across the ETP maintained by this highly migratory species [39, 77, 82].

Conclusions

The isotopic signatures of $S$. lewini at the GMR indicate an ontogenetic shift of resource use in sub-adult and adult sharks as observed in other areas within the ETP. The $\delta^{13}C$ and $\delta^{15}N$ signatures range in adult and sub-adult sharks suggest foraging on a variety of prey and ecosystems: coastal and pelagic, shallow and deep waters. These changes coincide with the potential residency of adult female sharks and the long-distance migrations between islands and mainland by sub-adult sharks found by previous studies in the ETP using gut content, microchemistry, and telemetry.

Understanding the foraging strategies of a migratory shark that uses a variety of sources throughout its lifetime and in an area with particular oceanographic conditions such as the GMR can be quite challenging. Identifying prey items within the reserve will help better understanding the trophic ecology of this species and characterize the isoscape over which these sharks move. Combining stable isotope analysis with telemetry data would also be useful to monitor long-term $S$. lewini feeding patterns in relation to variations in prey availability and oceanographic conditions. Understanding how each life stage uses resources and how coastal and oceanic aggregations are interconnected will contribute to guide national management plans, regional conservation initiatives, and international treaties to better protect a critically endangered, highly migratory predator whose ontogenetic migratory patterns occur within the jurisdictional waters of several countries.

Supporting information

S1 Appendix. Values of $S$. lewini N and C stable isotopes at the Galapagos Islands.
(XLSX)
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