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Using stable isotopes to reveal site-level differences in juvenile lemon shark Negaprion brevirostris (Carcharhiniformes: Carcharhinidae) nursery habitat use in the British Virgin Islands

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Abstract

Shark nursery areas are defined by juvenile site fidelity and by dependence on local resources, but the resource half of that definition is seldom measured directly. Paired plasma and red blood cell (RBC) stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) was used to compare two juvenile lemon shark (*Negaprion brevirostris*) nursery areas in the British Virgin Islands that lie 40 km apart and differ in geological setting: Hans Creek, a volcanic high-island embayment (18°26'N, 64°32'W), and the carbonate lagoons of Anegada (18°43'N, 64°20'W). Twenty two young-of-the-year and juvenile lemon sharks were sampled in May and June 2022 and 2023, in addition primary producers and prey items were sampled. Shark $\delta^{13}C$ signature at both sites corresponded to native seagrass rather than to red mangrove providing evidence for a seagrass-based carbon trophic pathway. The $\delta^{15}N$ signatures differed between sites and seagrass $\delta^{15}N$ at Anegada fell below 0‰, consistent with a phosphorus-limited, oligotrophic carbonate system. PERMANOVA and isotopic niche ellipses separated the two sites in both tissues, with no year effect. Mass-balance mixing models identified mojarra (*Eucinostomus spp*.) as the dominant contributor to plasma values at both sites. The Within shark plasma-RBC offsets followed published elasmobranch turnover rates and were used to provide an isotope offset derived ages that was found to be consistent with length based cohort assignment for young-of-the-year animals. These results provide the first quantitative isotope baseline for lemon shark nursery areas in the region, show that two nearby nurseries operate on distinct nutrient regimes, and establish a reference against which future wastewater-derived nitrogen enrichment can be detected.

Introduction

The core assumption for shark nursery areas is that juvenile sharks exhibit site fidelity to a specific location and are dependent on the available resources for growth and survival (Heupel et al. 2007; Heupel and Simpfendorfer 2011). Potentially prolonged residencies in these areas pose multiple management challenges, as well as opportunities to understand the role of these populations in the ecosystem (Kinney and Simpfendorfer 2009; Heupel et al. 2019). Nursery dependency among young-of-the-year (YOY) and juvenile lemon sharks is ubiquitous throughout their range and creates an opportunity to compare site specific conditions that can influence the long term fitness and survival (Morrissey and Gruber 1993b; Wetherbee et al. 2007; Reyier et al. 2014).

Most of the studies on shark nursery areas are investigations of behavior, occurrence, and movement (e.g., residency, site-fidelity, migration) to classify areas (Morrissey and Gruber 1993a; Wetherbee et al. 2007; Murchie et al. 2010). While these studies comprise a critical first step, they often only allude to the presence of ample resources. However, when investigated directly, prey availability is not always the dominant factor. For example, the behavior and distribution of juvenile blacktip sharks in Terra Ceia Bay, Florida, USA, were driven by predator avoidance and not prey abundance (Heupel and Hueter 2002). To understand how a specific embayment's resources are used for daily activities and growth, we can link the location's resources directly to the shark through the chemical and ecological signature transferred through the fractionation of stable isotopes (Peterson and Fry 1987; Hussey et al. 2012). By quantifying

stable isotopes, it is possible to create a direct connection to the available resources enabling managers to link these habitats to the juvenile sharks that depend on them and, ultimately, to anthropogenic disturbances and environmental changes (Layman et al. 2012; Matich and Heithaus 2014; Dhellemmes et al. 2021).

The lemon shark (*Negaprion brevirostris*) is a coastal species of the western Atlantic, ranging from the southeastern United States to Brazil, including the Gulf of Mexico and Caribbean Sea, with isolated populations in the eastern Atlantic and eastern Pacific (Compagno et al. 2005; Carlson et al. 2021). Sharks that establish residency in a nursery area after parturition draw in the resources from the surrounding environment. Young-of-the-Year (YOY) and juvenile lemon sharks occupy shallow-water nursery areas containing seagrass meadows that are fringed by mangroves where they display site fidelity for foraging and protection from predators (Morrissey and Gruber 1993b; Chapman et al. 2009; Kessel et al. 2014; Reyier et al. 2014; Legare et al. 2015). Juvenile lemon sharks feed primarily on small teleost, such as mojarra (*Eucinostomus spp*.) and on crustaceans that live within seagrass and mangrove habitat (Newman et al. 2010, 2012; Fleischer et al. 2026). In addition to forage, the shallow seagrass and shoreline mangroves provide habitat and structure for protection against predation (Nagelkerken et al. 2008; Guttridge et al. 2012). Using this habitat for both refuge and forage increases survival, but restricts the juvenile sharks to narrow bands of coastal habitat, exposing the species to habitat loss and overfishing; the lemon shark is currently listed as Vulnerable on the IUCN Red List (Carlson et al. 2021, Lapointe et al. 2004; Camacho-Cruz et al. 2020). The lemon shark has served as a model for nursery bound elasmobranchs throughout its range (Gruber et al. 1988; Newman et al. 2010; Dhellemmes et al. 2021) providing foundational information about diet and residency where nuanced studies to identifying isotopic differences can now be confidently be attributed at the site level.

The geological setting constrains the extent of habitat of any kind (reef, seagrass, mangrove) that is in turn available to juvenile sharks (Purdy and Winterer 2001; Harris and Baker 2019; Worthington et al. 2020). In general, the carbonate-platform lagoons in The Bahamas are characterized by expansive shallow seagrass and mangrove habitat (Lopez-Gamundi et al. 2025), allowing lemon sharks to have long, multi-year residency (Chapman et al. 2009). In contrast, the volcanic, high-island embayments of the US Virgin Islands provide limited nearshore shallow (< 2 m) water (Rankin 2002) and residency is limited to typically one year or less for most individuals (Legare et al. 2015). Independent studies across the range of lemon sharks have noted differences between sites from the USVI, the BVI, The Bahamas, and the Turks and Caicos with gradients of residency and site fidelity, which is partially connected to the extent and magnitude of available habitat (DeAngelis et al. 2008; Chapman et al. 2009; Henderson et al. 2010; Legare et al. 2015; Legare et al. in Review). These different geologic settings allow for comparison, as they contain many embayments suitable for juvenile lemon sharks that are distinctly different from one another in their geological foundation and orders of magnitude different in size. Two nursery areas recently identified in the British Virgin Islands are Hans Creek, a typical regional volcanic, high-island embayment, and Anegada, with Bahamian-like carbonate-platform lagoons (Atwater et al. 2017; Kruse et al. 2023; Legare et al. in Review) provide the backdrop to further understand whether these embayments are providing sufficient forage and resources.

Seagrass beds and mangroves are essential fish habitat for juvenile sharks and as primary producers facilitate the availability of local resources that can be traced through $\delta^{13}C$ and $\delta^{15}N$ isotopes signatures aid in understanding the reliance on local resources (Vaudo and Heithaus 2011; Shiffman et al. 2012; Dhellemmes et al. 2021). The use of stable isotopes to examine foraging ecology is is founded in the bioaccumulation of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) that transfer up from primary producers to their consumers, thereby tracing trophic pathways (Peterson and Fry 1987; Post 2002). Carbon stable isotopes are particularly useful for understanding the primary producers of a system, as different primary producers (e.g., seagrasses and mangroves) have distinct chemical pathways characterized by different $\delta^{13}C$ fractionation rates resulting in distinct baseline $\delta^{13}C$ (Cocheret de la Morinière et al. 2003). Seagrasses such as *Thalassia testudinum, Syringodium filiforme*, and *Halophila stipulacea* are know to derive their carbon from the surrounding aquatic environment, generally from available dissolved inorganic carbon (Hemminga and Mateo 1996; Campbell and Fourqurean 2009), whereas mangroves primarily sequester their carbon from the atmosphere (Bouillon et al. 2008).

The two carbon pathways provide different enrichment baselines of stable isotopes, with seagrasses generally between − 10‰ and − 15‰ and mangroves between − 25‰ and − 28‰ (Fourqurean et al. 2005; Bouillon et al. 2008; Campbell and Fourqurean 2009). Using these baseline values, consumers can be linked to their primary producers (higher or lower $\delta^{13}C$ values) to seagrass-based food web or a mangrove-based food web (Layman 2007; Nagelkerken et al. 2008; Dhellemmes et al. 2021). By quantifying $\delta^{15}N$ in animals tissue, the trophic relationships can reveal ecosystem connectivity (Hussey et al. 2014; Nagelkerken et al. 2008; Layman et al. 2012). Specifically, nitrogen stable isotope $\delta^{15}N$ is known to increase with trophic level due to specific metabolic fractionation rates during biochemical processing (Minagawa and Wada 1984; Post 2002). The higher (more enriched) the $\delta^{15}N$ ratio, the higher the trophic position of that individual. The higher nitrogen isotope values of various consumers in a marine ecosystem can be used to distinguish their role as predators, scavengers, grazers, or parasites, when compared to herbivores or primary producers on the lower range of $\delta^{15}N$ ratios (Minagawa and Wada 1984; Post 2002). The carbon values place the consumer relative to the producers (e.g. seagrass vs mangrove range) and the nitrogen stable isotopes place the consumer relative to trophic level identifying the both the energetic and structural context of the consumer in the habitat.

The interpretation of stable isotopes in juvenile sharks is complicated by maternal input during gestation (Olin et al. 2011). The isotopic signature at birth reflects that of the mother and not the local ecosystem (Olin et al. 2011; Belicka et al. 2012; Olin et al. 2018). Even if the mother consumes an identical diet to that of YOY sharks, the signature at birth will have a more enriched $\delta^{15}N$, placing the neonate at a trophic level above the mother. Moreover, the carbon isotope will reflect the carbon source of the adult habitat and not the nursery (Vaudo et al. 2010; Olin et al. 2011, 2013). In the weeks and months after birth, the young sharks feed and their tissues progressively turn over to reflect the locally available forage base at tissue-specific turnover rates (MacNeil et al. 2006; Matich et al. 2010, 2011; Kim et al. 2012). Blood constituents, such as plasma, replace their isotopic content within weeks, whereas red blood cells (RBC) turn over in months, and tissues such as muscle and fin can retain a maternal signature for up to a year (Matich et al. 2010; Olin et al. 2011; Kim et al. 2012; Niella et al. 2021). As a result of sampling more than one tissue a

temporal window into the transition from maternal to local resources can be examined, which can be expressed as an offset, as slow-turnover tissues lag behind fast-turnover tissues (Matich et al. 2014, 2015; Shipley et al. 2021). As lemon sharks are nursery-bound and they consume resources from a fixed location, isotopic ratios can be used as an indicator of impacts on, and fidelity to, the location.

The geological setting constrains habitat extent, and with it resource availability, forage base, and diet. What remains untested is whether nursery areas separated by only 40 km, and differing primarily in geological setting, differ in the resources the sharks use for growth and survival (Weideli et al. 2019; Segura-Cobeña et al. 2021). In this study, we use paired plasma and red blood cell (RBC) stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) of juvenile lemon sharks at Anegada and Hans Creek, as well as concurrent sampling of primary producers and prey, to test three hypotheses: (1) juvenile lemon sharks at both sites depend on locally available resources; (2) site-level differences in isotopic values can indicate ecosystem health and function as a shark nursery area; and (3) within-shark plasma-RBC isotope offsets can be used to account for maternal input for juvenile sharks. Testing these hypotheses provides the first quantitative isotope baseline for shark nurseries in the BVI.

Materials and Methods

Study site

The BVI are located on the eastern portion of the Virgin Shelf in the northeastern Caribbean, as part of a small island chain that stretches from Puerto Rico to Anegada and includes the US Virgin Islands (Rankin 2002; Atwater et al. 2017). The BVI support diverse marine habitats within a regionally biodiverse Caribbean (Miloslavich et al. 2010) and are home to several shark nursery areas (Legare et al. unpubl data), including Hans Creek, on the south side of Beef Islands, and the mangrove lagoon on the south coast of Anegada (Fig. 1).

In the BVI, Hans Creek, located off Beef Island, represents a typical nursery area found in nearby USVI and is a shallow-water lagoon with approximately 800 m of shoreline with dense seagrass beds composed of *Thalassia testudinum*, *Syringodium filiforme*, and the non-native *Halophila stipulacea* (Willette and Ambrose 2012; Fig. 1). This embayment is fringed with red mangroves (*Rhizophora mangle*) and provides nursery habitat for juvenile lemon sharks. The total available habitat in this small embayment is approximately 0.15 km^2. Like the majority of the islands along the Virgin Shelf, Hans Creek is volcanic in origin with limited groundwater and deep (> 10 m) waters directly adjacent to the lagoon (Rankin 2002). Anegada is an atoll and the easternmost island in the territory (Fig. 1). Unlike its neighbors, Anegada has a carbonate base of porous coral and limestone (Atwater et al. 2017; Kruse et al. 2023). The nearshore waters are shallow (< 2 m) and are surrounded by an extensive fringing reef and seagrass systems with shorelines containing mangrove lagoons, salt ponds, and sandy beaches. The shallow waters of Anegada provide available habitat over ten times the size of Hans Creek, with deep waters (> 10 m) located 1 nm from shore adjacent nursery areas.

As a low-lying carbonate island, Anegada is similar to islands in The Bahamas and the Yucatán with subsurface groundwater linking the land to the marine ecosystem (Carruthers et al. 2005; Mutchler et al. 2007; Camacho-Cruz et al. 2020). Carbonate platforms are known to produce groundwater that is phosphorus-limited which is indicative of oligotrophic conditions and can alter the nitrogen stable isotope enrichment (Short 1987; Fourqurean et al. 1992, 2015).

Sampling

Sampling was conducted between 28 May 2022 and 3 June 2022, and 22 May to 27 May 2023, during the peak abundance documented in the adjacent USVI (DeAngelis 2006), as part of a broader long-term monitoring program established in 2022 (Fig. 1; Legare et al. in Review). The majority of sampling occurred between 0700 and 1800 hr using a 10–25 hook modified bottom longline (DeAngelis 2006; Kohler and Turner 2001; Legare et al. 2015). Station positions (Model 78S, Garmin Ltd., Olathe, KS, USA) and times were recorded for each end of the mainline. Sex, fork length (FL), and total length (TL) of sharks were recorded. Sharks were tagged with a red National Marine Fisheries Service (NMFS) Rototag (Dalton-Henly, Nettlebed, Oxford, England) in the first dorsal fin and then released.

For SIA, a total of 4 mL of blood was obtained via caudal venipuncture using a non-heparinized 18-gauge 1.5-inch needle (Stoskopf et al. 1984). On the vessel, blood was then immediately evacuated into 2 ml centrifuge tubes and spun for 10 min at 5000 rpm to separate the red blood cells from plasma. The resulting plasma and red blood cells were then separated into cryotubes placed on ice and immediately frozen at − 20°C when back on shore (< 5 hours) for future processing. All samples were further dried and processed prior to shipping (frozen for 1–1.5 years) to the SIA facility (UC Davis).

To create a contemporary baseline of primary consumers and secondary consumers samples were collected at each site during each year. Specimens collected included two native seagrasses (*Thalassia testudinum* and *Syringodium filiforme*), the non-native seagrass (*Halophila stipulacea*; only present in Hans Creek), macroalgae (*Acanthophora spicifera*), red mangrove (*Rhizophora mangle* leaves), crabs (blue crab *Callinectes sapidus* at Anegada; *Mithraculus sculptus* at Hans Creek), and forage fish (*Eucinostomus* spp.). During tagging events in the broader sampling (Legare et al. in Review), both *Callinectes sapidus* and *Eucinostomus* spp. (mojarra) were regurgitated during handling, validating these constituents as part of the natural diet (Newman et al. 2010, 2012).

Stable isotope sample preparation

All samples were collected, placed in whirl packs placed on wet ice, processed and frozen at − 20°C until they could be dried (< 7 days) in the lab at approximately 60°C for 24 h in a food dehydrator (Cuisinart 5-Tray Food Dehydrator, Stamford, Connecticut, USA). Prior to drying, the samples were cleaned of material (inorganic substrates, epiphytes, decayed leaves). Once dried the samples were ground into a fine powder, weighed (0.5–1.2 mg), and dispensed into tin capsules for shipment to the Stable Isotope Facility at the University of California Davis, where they were analyzed on an elemental analyzer-isotope ratio mass

spectrometer (EA-IRMS) (Sercon Ltd., Cheshire, United Kingdom). Quality assurance for accuracy and precision was quantified using control materials, yielding an overall accuracy of ± 0.18‰ for $\delta^{13}C$ and ± 0.19‰ for $\delta^{15}N$.

Multivariate isotopic comparison

Plasma and RBC isotope signatures were compared to test for differences between sites and years; $\delta^{13}C$ and $\delta^{15}N$ values were used in the construction of Bray-Curtis dissimilarities. This analysis cannot handle negative values, so a constant was added to all values before constructing the Bray-Curtis matrix (Clarke et al. 2006). A nonmetric, multi-dimensional scaling plot with overlaid ellipses (50% and 95% similarities) was used for interpretation through visualization. A PERMANOVA (Anderson 2001) was used to test for Site and Year effects, and ANOSIM (Clarke 1993) was used to quantify the degree of separation between groups; both were implemented in the R package vegan (Oksanen et al. 2022). Pearson's correlation was conducted comparing the mean $\delta^{13}C$ and $\delta^{15}N$ values for both the Plasma and RBC and a significance threshold of $\alpha = 0.05$ was used across tests, and all analyses were performed in R (R Core Team 2022).

Trophic position and niche width

Trophic position (TP) was estimated following Post (2002):

$$TP = \lambda + (\delta^{15}N_{shark} - \delta^{15}N_{baseline}) / \Delta^{15}N$$

where λ is the trophic position of the baseline organism, $\delta^{15}N_{shark}$ is the mean $\delta^{15}N$ of all shark samples, $\delta^{15}N_{baseline}$ is the mean $\delta^{15}N$ of the site-specific forage fish (mojarra, *Eucinostomus* spp.), and $\Delta^{15}N$ is the trophic discrimination factor for sharks (2.3‰; Hussey et al. 2010). Mojarra were used as the baseline rather than a primary producer because they are integrated consumers that smooth the high $\delta^{15}N$ variability seen in seagrass (mean SD across the two sites = 1.4‰), and because they are a well-documented prey item for juvenile lemon sharks (Newman et al. 2010, 2012). Following Post (2002), $\lambda = 3.0$ was assumed for a secondary-consumer baseline; absolute TP values are therefore anchored to that assumption, whereas the between-site difference in TP is not.

TP was calculated separately for plasma and RBC of each shark, and the means and standard deviations were reported across sites, years, and tissues. Isotopic niche width was characterized using maximum-likelihood standard ellipses corrected for small sample size (SEAc; approximately 40% data coverage) implemented in the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011). Ellipses were calculated separately for plasma and RBC and grouped by site and year. Because each site × year group contained only ~ 6 individuals, SEAc values were reported as point estimates of niche breadth and interpreted at the site level rather than as tests of annual change. For every environmental constituent that was sampled the $\delta^{13}C$ and $\delta^{15}N$ were compared between Anegada and Hans Creek using Welch's two-sample *t*-test (unequal variances), with Shapiro-Wilk applied within each site group and Mann-Whitney *U* as a check; taxa recorded at only one site were excluded from between site testing.

Mass-balance mixing model

Published trophic discrimination factors of $\Delta^{13}C$ = + 0.9‰ and $\Delta^{15}N$ = + 2.3‰ were used for the mass-balance modeling (Hussey et al. 2010). Reported $\Delta^{15}N$ varies systematically among shark species and diet compositions (Olin et al. 2013), and the resulting proportions should be read with that variation in mind. Different environmental sources have different contributions to the shark plasma signature, and to estimate the contribution of each, a Monte Carlo mass-balance mixing model was constructed (Phillips and Gregg 2003; Phillips et al. 2014). Priors for each site were obtained from the trophic discrimination factor (TDF) which is a corrected mean and SD of every source. The model relied on 100,000 candidate dietary proportion vectors from a Dirichlet distribution to compute (Parnell et al. 2010) a predicted consumer $\delta^{13}C$ and $\delta^{15}N$ and retained only the vectors within the tolerance window of the observed mean shark plasma values (Phillips and Gregg 2003). The tolerance was broadened in 0.5‰ steps (starting at ± 0.5‰ per axis) until a minimum of 2,000 vectors was achieved. Crabs collected in the two locations occupied distinct trophic values (different species), reducing certainty in pooled trophic positions, so separate tolerance values were used between sites (Anegada: ±2.5‰; Hans Creek: ±4.5‰). Mixing-model proportions were presented as projections rather than literal dietary reconstructions (e.g., sharks do not eat mangroves). RBC was not modeled separately because it is more representative of the maternal transition for the sharks sampled, particularly for Age 0 + individuals (Olin et al. 2011; Niella et al. 2021), while plasma is the more direct indicator of recent local foraging (MacNeil et al. 2006; Kim et al. 2012).

Age class assignment

Each shark was assigned to a cohort (0+, 1+, or 2+) based on capture size (TL) and published age-at-length data (Barker et al. 2005; Tavares et al. 2020). Ages were assigned using ± 1 SD or ± 2 SD criteria, and estimated days since birth were calculated from each shark's TL using growth-per-day estimates (Brown and Gruber 1988) and snapping to the nearest parturition date within the SD tolerance, based on regional peak abundances between May and June (DeAngelis 2006; Legare et al. 2015), resulting in an estimated age as days since birth.

Maternal vs. local signal turnover

The fraction of tissue carbon and nitrogen replacement since birth was calculated using an exponential turnover model (MacNeil et al. 2006; Kim et al. 2012) and provided comparable rates of turnover to understand the shark's stable isotope content as it transitions to local prey with a residual maternal signal. The fraction of tissue carbon and nitrogen that had been replaced since birth was calculated as:

$f_{local}(t) = 1 - e^{(- k \cdot t)}$

where $f_{local}(t)$ is the fraction of the tissue isotope replaced by local diet by time t, bounded 0 to 1 (0 = fully maternal, 1 = fully equilibrated to the local nursery diet), t is estimated days since birth (calculated from age assignment), and k is the tissue-specific turnover rate constant derived from Kim et al. (2012b).

Tissue half-lives of 28 days for plasma (k_plasma) and 180 days for RBC (k_RBC) were used and calculated as a function:

$k_{plasma} = \ln(2)/28 \approx 0.0247\ d^{-1}$

$k_{RBC} = \ln(2)/180 \approx 0.00385\ d^{-1}$

The complementary fraction from the maternal signal is:

$f_{maternal}(t) = 1 - f_{local}(t)$

Where f_maternal(t) is the fraction of the tissue isotope pool still carrying the maternal isotopes. Per-shark estimates of f_local for plasma, f_local for RBC, and f_maternal for RBC were calculated alongside the observed within-shark plasma-RBC isotope offsets calculated from $\delta^{13}C$ and $\delta^{15}N$. The predicted within-shark offset was displayed as a function of age and was modeled assuming a separation of 1.5‰ $\delta^{13}C$ and 2.5‰ $\delta^{15}N$. The resulting expected curves were overlaid with the observed offsets to evaluate whether the timing of the maternal-to-local transition was consistent with the documented elasmobranch turnover framework.

Inverting tissue-turnover offsets to estimate age (bootstrap)

A back-calculated age from the observed plasma-RBC offsets was computed using the turnover framework in Kim et al. (2012b) to compare with age estimates from TL, as the offset is a function of time (days since birth):

$\text{Offset}(t) = \Delta \times [e^{(-\ln 2 \cdot t / 28)} - e^{(-\ln 2 \cdot t / 180)}]$

where Δ is the assumed maternal-to-local dietary contrast in ‰ and e denotes Euler's number (~ 2.71828). The function starts from zero at t = 0, peaks at t* = 89 d, the point of maximal divergence, and then decays to zero as both tissues transition to the local signal. As the model is non-monotonic, an offset can exist on either side of the peak, and each observation was assigned to the early- or late-side value depending on whether the cohort-based age was before or after the peak (89 days). To place the estimations into context and understand their uncertainty, a 1,000-iteration Monte Carlo bootstrap was performed for each shark with R packages dplyr, tidyr, and ggplot2 (Wickham 2016; Wickham et al. 2019).

Results

Stable Isotopes

A total of 22 YOY and juvenile lemon sharks were captured and sampled between Hans Creek and Anegada (11 per site) in May and June 2022 and 2023 (Supplementary Table 1). The mean (± SD) total length was 68.7 ± 8.7 cm at Anegada (n = 11, range 57.5–88.0) and 63.9 ± 3.1 cm at Hans Creek (n = 11,

range 60.5–72.0). Overall, plasma $\delta^{13}C$ ratios ranged from − 14.26 to − 12.45‰, with a mean at Anegada of − 13.27 ± 0.44‰ (n = 11) and at Hans Creek of − 13.30 ± 0.57‰ (n = 11) (Fig. 2; Table 1). For plasma $\delta^{15}N$, ratios ranged from 8.08 to 10.67‰, with an Anegada mean (± SD) of 8.95 ± 0.87‰ and Hans Creek mean of 9.76 ± 0.38‰. RBC $\delta^{13}C$ ratios ranged from − 13.65 to − 11.81‰, with an Anegada mean of -12.81 ± 0.36‰ (n = 11) and Hans Creek mean of -12.61 ± 0.39‰ (n = 11). RBC $\delta^{15}N$ ratios ranged from 8.64 to 10.35‰, with an Anegada mean of 9.34 ± 0.45‰ and Hans Creek mean of 10.02 ± 0.31‰

Table 1
Juvenile lemon shark tissue isotope summaries by site, year, and tissue (plasma, RBC). N is the number of individual samples. Mean, SD, and SE are reported for $\delta^{13}C$ and $\delta^{15}N$ (in ‰).

Site	Year	Tissue	N	Mean $\delta^{13}C$	SD $\delta^{13}C$	SE $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	SE $\delta^{15}N$
Anegada	2022	Plasma	5	−13.34	0.64	0.29	8.70	0.77	0.34
	2022	RBC	5	−12.77	0.31	0.14	9.35	0.42	0.19
	2023	Plasma	6	−13.22	0.23	0.09	9.15	0.96	0.39
	2023	RBC	6	−12.85	0.42	0.17	9.34	0.51	0.21
	All	Plasma	11	-13.27	0.44	0.04	8.94	0.87	0.14
	All	RBC	11	-12.81	0.36	0.03	9.34	0.45	0.16
Hans Creek	2022	Plasma	6	−13.12	0.75	0.30	9.80	0.38	0.15
	2022	RBC	6	−12.45	0.47	0.19	10.15	0.12	0.05
	2023	Plasma	5	−13.50	0.11	0.05	9.72	0.43	0.19
	2023	RBC	5	−12.81	0.07	0.03	9.87	0.41	0.18
	All	Plasma	11	-13.30	0.57	0.05	9.76	0.38	0.11
	All	RBC	11	-12.61	0.39	0.04	10.02	0.31	0.09

Environmental constituents from each location spanned a wide isotopic range and multiple trophic levels, with red mangrove leaves as the most depleted in $\delta^{13}C$ at both sites (Anegada: -27.03 ± 3.28‰; Hans Creek: -27.43 ± 1.49‰) and Hans Creek crabs (*Mithraculus sculptus*) the most enriched (− 5.53 ± 0.83‰) (Fig. 2; Table 2, Supplementary Table 2). Of the six taxa sampled at both sites, $\delta^{13}C$ differed significantly in three (Welch two-sample t-test), with Hans Creek enriched relative to Anegada in *Thalassia testudinum* (5.91‰; $t_{7.67} = -11.30$, *P_BH* < 0.001), *Syringodium filiforme* (6.53‰; $t_{5.28} = -4.28$, *P_BH* = 0.012) and *Eucinostomus* spp. (3.56‰; $t_{6.68} = -15.33$, *P_BH* < 0.001). $\delta^{15}N$ differences were smaller and directionally inconsistent: *Syringodium* was 3.45‰ higher at Hans Creek (*P_BH* = 0.039) whereas *Eucinostomus* spp. was 0.60‰ higher at Anegada (*P_BH* = 0.039), and the Hans Creek enrichment in *Thalassia* and *Acanthophora spicifera* did not survive FDR correction (both *P_BH* = 0.055). *Rhizophora mangle* did not differ between sites in either isotope (Table 2). Forage fish (*Eucinostomus* spp.) presented divergent $\delta^{13}C$ values (Anegada: -13.14 ± 0.53‰; Hans Creek: -9.58 ± 0.22‰) between sites and a small $\delta^{15}N$ offset

(Anegada: 5.68 ± 0.38‰; Hans Creek: 5.08 ± 0.26‰), indicating that the baseline isotope environment differed between sites and that the two food webs were built on different carbon sources. A single marine leech (*Pontobdella muricata*.) was found on a shark in Hans Creek and was processed ($\delta^{13}C$ =-9.71‰, $\delta^{15}N$ = 6.37‰) but not included in the model.

Table 2
Environmental isotope summaries by site and source group. N is the number of samples per group.

Source groups include; Blue Crab (*Callinectes sapidus*, Anegada); Crabs (other) (*Mithraculus sculptus*, Hans Creek); Teleost (mojarra, *Eucinostomus* spp., both sites); Macroalgae; *Thalassia testudinum* and *Syringodium filiforme*; Halophila (non-native *Halophila stipulacea*, Hans Creek only); Mangrove (*Rhizophora mangle*); and Leech (Hans Creek only, n = 1, reference only). Mean and SD reported for $\delta^{13}C$ and $\delta^{15}N$ (‰).

Site	Source group	N	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$
Anegada	Blue Crab	5	-12.52	1.87	4.06	0.68
Anegada	Macroalgae	5	-13.89	1.76	2.35	0.14
Anegada	Mangrove	5	-27.03	3.28	-0.76	1.62
Anegada	*Syringodium*	6	-14.04	1.39	-3.46	2.38
Anegada	Teleost (*Eucinostomus*)	6	-13.14	0.53	5.68	0.38
Anegada	*Thalassia*	6	-12.82	1.14	-0.79	1.25
Hans Creek	Crab	9	-5.53	0.83	1.2	0.41
Hans Creek	*Halophila*	5	-7.52	0.34	-0.19	0.55
Hans Creek	Leech	1	-9.71		6.37	
Hans Creek	Macroalgae	5	-14.02	1.07	2.62	0.2
Hans Creek	Mangrove	6	-27.43	1.49	0.21	0.6
Hans Creek	*Syringodium*	5	-7.52	3.17	-0.01	0.48
Hans Creek	Teleost (*Eucinostomus*)	6	-9.58	0.22	5.08	0.26
Hans Creek	*Thalassia*	8	-6.90	0.68	0.64	0.64

Multivariate site and year effects

Site differences for RBC were significant in the PERMANOVA analysis (R^2 = 0.299, F = 8.80, P = 0.002) which explained approximately 30% of the variation. Year as a factor was non-significant (R^2 = 0.054, F = 1.59, P = 0.202). ANOSIM also indicated significant site separation (R = 0.356, P = 0.002) and no effect by year (R = 0.009, P = 0.324). Plasma was significant between sites (R^2 = 0.223, F = 5.43, P = 0.013; ANOSIM R = 0.321, P = 0.007) but not year (R^2 = 0.038, P = 0.384).

Trophic position and niche width

Standard ellipse areas (SEAc) reinforced the site separation indicated by the PERMANOVA (Fig. 3A, B), with Anegada plasma occupying the lower-$\delta^{15}N$ portion of the biplot relative to Hans Creek. In plasma, SEAc was 0.75‰² (2022) and 0.42‰² (2023) at Anegada and 1.11‰² (2022) and 0.16‰² (2023) at Hans Creek; in RBC it was 0.47‰² and 0.69‰² at Anegada and 0.20‰² and 0.11‰² at Hans Creek. RBC ellipses were narrow at Hans Creek in both years, and narrower than plasma at Anegada in 2022, consistent with the lower variance expected of this longer-integrating tissue (Hans Creek RBC $\delta^{15}N$ SD = 0.31‰; Table 1); the exception was Anegada in 2023, where the RBC ellipse (0.69‰²) exceeded the plasma ellipse (0.42‰²). Niche breadth varied between years within a site (e.g. Hans Creek plasma, 1.11 to 0.16‰²) despite no year effect on ellipse position, a pattern we attribute to the sensitivity of SEAc to individual sharks at these small group sizes (n = 5–6) rather than to a true annual shift in niche width.

The estimated trophic position (TP) of the juvenile lemon sharks ranged from 4.32 to 5.21 across site, year, and tissue combinations (Table 3). The Hans Creek sharks consistently occupied a higher trophic position than Anegada sharks in tissues and years, with a mean TP difference of approximately 0.5–0.7 trophic levels. The Anegada plasma TP averaged 4.32 ± 0.33 in 2022 (n = 5) and 4.51 ± 0.42 in 2023 (n = 6), while RBC TP was slightly higher and stable across years at 4.59 ± 0.18 (2022) and 4.59 ± 0.22 (2023). At Hans Creek, plasma TP was 5.06 ± 0.16 in 2022 (n = 6) and 5.02 ± 0.19 in 2023 (n = 5), and RBC TP was 5.21 ± 0.05 (2022) and 5.09 ± 0.18 (2023). RBC-derived TP was slightly higher than plasma-derived TP within each site-year combination (mean offset of 0.07–0.27), consistent with RBC integrating a longer dietary window that retains more of the maternal signal. The between year variation was minimal at both sites (≤ 0.19 TP units) showing temporal stability pointing to site level trophic signals, while site level differences were consistent and pronounced for both tissues and years.

Table 3
Estimated trophic position (TP) of YOY and juvenile lemon sharks by site, year, and tissue. TP was calculated following Post (2002) as $TP = \lambda + (\delta^{15}N_consumer - \delta^{15}N_baseline) / \Delta^{15}N$, with mojarra (*Eucinostomus* spp.) $\delta^{15}N$ used as the baseline, $\lambda = 3$ for a secondary-consumer baseline, and $\Delta^{15}N = 2.3‰$ (Hussey et al. 2010).

Site	Year	Tissue	N	Mean TP	SD TP
Anegada	2022	Plasma	5	4.32	0.33
Anegada	2022	RBC	5	4.59	0.18
Anegada	2023	Plasma	6	4.51	0.42
Anegada	2023	RBC	6	4.59	0.22
Hans Creek	2022	Plasma	6	5.06	0.16
Hans Creek	2022	RBC	6	5.21	0.05
Hans Creek	2023	Plasma	5	5.02	0.19
Hans Creek	2023	RBC	5	5.09	0.18

Mass-balance source contributions

The Monte Carlo mass-balance mixing models determined that forage fish (*Eucinostomus* spp.) were the dominant contributors to plasma isotopic signatures (Anegada mean = 56.5%, *n*_feasible = 2,017 at ± 2.5‰ tolerance; Hans Creek mean = 52.5%, *n*_feasible = 2,005 at ± 4.5‰ tolerance) (Table 4; Fig. 3C). At Anegada, *Callinectes sapidus* (blue crab) contributed a smaller secondary proportion (mean = 21%) of the proposed diet, with all other sources (macroalgae, mangrove, native seagrass) contributing ≤ 8.4% on average. At Hans Creek, contributions were broadly distributed across crabs (*M. sculptus*: 6%), *Halophila stipulacea* (5%), macroalgae (15%), mangrove (8.7%), *Syringodium* (6%), and *Thalassia* (6%), with no single secondary source dominating.

Table 4
Monte Carlo mass-balance model summaries per site. For each source: posterior mean, median, SD, and 2.5%/97.5% credible interval bounds of the feasible dietary proportion. N feasible is the number of retained simplex draws. Tol. (‰) is the tolerance required for convergence (iteratively widened from ± 0.5‰ in 0.5‰ steps; see Methods).

Site	Source	Mean	Median	SD	2.5%	97.5%	N feasible	Tol. (‰)	In polygon
Anegada	Macroalgae	0.084	0.064	0.072	0.002	0.265	2,017	2.5	No
Anegada	Mangrove	0.052	0.039	0.047	0.001	0.174	2,017	2.5	No
Anegada	*Syringodium*	0.043	0.029	0.054	0.001	0.163	2,017	2.5	No
Anegada	*Thalassia*	0.051	0.036	0.048	0.001	0.181	2,017	2.5	No
Anegada	Blue Crab	0.206	0.149	0.186	0.005	0.684	2,017	2.5	No
Anegada	Teleost	0.565	0.601	0.187	0.098	0.834	2,017	2.5	No
Hans Creek	Macroalgae	0.152	0.124	0.122	0.006	0.449	2,005	4.5	No
Hans Creek	Mangrove	0.087	0.075	0.061	0.005	0.234	2,005	4.5	No
Hans Creek	*Halophila*	0.054	0.04	0.049	0.001	0.187	2,005	4.5	No
Hans Creek	*Syringodium*	0.056	0.041	0.052	0.002	0.193	2,005	4.5	No
Hans Creek	*Thalassia*	0.062	0.046	0.056	0.003	0.207	2,005	4.5	No
Hans Creek	Crab	0.064	0.047	0.057	0.002	0.207	2,005	4.5	No
Hans Creek	Teleost	0.526	0.534	0.122	0.264	0.745	2,005	4.5	No

Cohort and age structure

The age distribution was dominated by 0 + animals at both locations (Fig. 4). Using the ± 1 SD assignment, 12 of 22 sharks were assigned to Age 0+, 3 to Age 1+, 1 to Age 2+, and 6 individuals (TL 57.5–69.0 cm) fell between cohort bins and were left unassigned. Using the ± 2 SD assignment, all 22 sharks were assigned, with the six previously unassigned individuals all reclassified as 0+ (18 Age 0+, 3 Age 1+, 1 Age 2+). Estimated days since birth ranged from 0 to 693 days old, with a clear gap between the 0+ (mean 27 d, SD 9) and 1+ (mean 332 d, SD 5) cohorts. The cohort separation and size at capture provided gaps that were reasonable to assign sharks to different cohorts using the ± 2 SD criterion.

Maternal vs. local signal turnover

The shark turnover modeling based on Kim et al. (2012b) indicated that 0 + animals at the time of capture had replaced 36–56% of their plasma isotope values at the ± 1 SD and 0–56% at the ± 2 SD and a smaller proportion (7–12% @ ±1 SD, 0–12% @ ±2 SD) of their RBC isotope values. Age 1 + animals (n = 3) had fully replaced their plasma ($f_{local} \approx 1.00$) and replaced 71–73% of their RBC, leaving 27–29% of the RBC pool maternal. The single 2 + shark (Tag 78957, 88 cm TL, age 693 d) effectively replaced all of its tissue isotopes from maternal to local (RBC $f_{maternal} = 7\%$).

Shark plasma-RBC offsets were negative for both isotope types (mean $\Delta\delta^{13}C$ =-0.57‰, range-1.55 to + 0.45‰; mean $\Delta\delta^{15}N$ =-0.33‰, range-1.10 to + 0.74‰) and indicating that plasma was depleted relative to RBC. The magnitude of offset (unsigned) followed the expected cohort pattern in nitrogen but not in carbon: mean $|\Delta\delta^{15}N|$ declined from 0+ (0.45‰) to the 2 + individual (0.06‰), while mean $|\Delta\delta^{13}C|$ was effectively flat (0+: 0.69‰; 2+: 0.71‰). The $\delta^{13}C$ and $\delta^{15}N$ turnover curves were visually inspected (Fig. 5C), with predicted peaks at 60–90 days after birth and decay toward zero by 500–1000 days as both tissues converted to the local isotope signal.

Independent age estimates from two tissue isotope offset

The bootstrapped analysis indicated that offset-derived ages tracked closely to the independently assigned length-derived ages (Pearson correlation, $r = 0.98$, $N = 22$, $P < 0.001$; Spearman rank correlation, $r_s = 0.59$, $N = 22$, $P = 0.004$; Fig. 6). Agreement between the two estimates was within 14 d for 14 of 18 0 + sharks (78%) and within 30 d for 18 of 18 (100%). Per-shark bootstrap distributions for the 0 + cohort had standard deviations of 5.5–12.3 d (mean 10.1 d), compared with a the between individual standard deviation of 7.8 d in length-derived age within the same cohort. All 18 0 + animals were evaluated on the pre-peak branch of the offset function and all four 1 + and 2 + animals on the post-peak branch; branch assignment followed the cohort-based age. The bootstrapped results at the 95% credible interval averaged 37 d (range 21–46 d) for 0 + sharks. For the four older sharks (1 + and 2+), the credible intervals widened considerably to a mean of 707 d (range 516–780 d) because of the gradual slope of the post-peak curve. The sharks classified as 0+ (n = 18) were within ~ 10 days of the cohort-snapped age on average, with the offset method tending to return a slightly younger estimate. Calculated values were on average 79 days younger (for all sharks) than cohort estimates for sharks estimated between 330–338 d

(length-based estimates) and 299–455 d (offset-based estimates), well within the ± 516 to ± 780 d 95% confidence intervals produced by bootstrapping.

Model-compatibility scores were generally high across all sharks and returned a feasible solution 67% of the time on average. Eighteen of 22 sharks had greater than 50% feasible iterations, and four 0 + sharks had compatibility scores lower than 50% (Tags 78917 = 5%, 78919 = 25%, 78920 = 35%, and 78905 = 45%), all driven by $\delta^{13}C$ offsets larger than the assumed $\Delta^{13}C = 1.5 \pm 0.5‰$. In each of these cases, the $\delta^{15}N$ inversion still performed with greater agreement (> 96% iterations). These four individuals were interpreted as sharks for which the maternal and local nursery carbon signals differed by more than the assumed $\Delta^{13}C$. Three of the four were captured at Hans Creek, raising the possibility of a shared mother, or of mothers foraging in the same adult habitat (Feldheim et al. 2002, 2014).

Discussion

Results of our study showed that young-of-the-year and juvenile lemon sharks occupied distinct isotopic niches at nursery areas around Anegada and at Hans Creek. The differences expressed by the stable isotope analysis, particularly for $\delta^{15}N$ provides a baseline against which to make comparisons to future impacts to the habitats. The different isotopic signatures point to site-specific differences that stem from primary producers in their respective geological settings. Implications for stable isotope work that does not consider contemporary primary producers and relies strictly on published values run the risk of creating false baselines that cannot decipher ecological, geological, or anthropogenic effects. Here we discuss how stable isotope signatures, even from small sampling efforts, can link juvenile sharks to the biogeological ecosystem if sampled properly, and if factors such as maternal contribution are accounted for.

Seagrass carbon pathway

Lemon sharks are dependent on shallow seagrass meadows fringed with mangrove stands for protection from predators and to provide ample forage for growth (Brown and Gruber 1988; Morrissey and Gruber 1993b; Chapman et al. 2009; Newman et al. 2010). Seagrass utilizes dissolved inorganic carbon resulting in relatively enriched seagrass tissue $\delta^{13}C$, while mangroves draw on atmospheric CO_2 which is more depleted $\delta^{13}C$ (Hemminga and Mateo 1996; Bouillon et al. 2008; Fourqurean et al. 2005; Campbell and Fourqurean 2009). At both locations the mean shark $\delta^{13}C$ corresponded more with native seagrass species and not the red mangroves, supporting the interpretation that the isotopes are derived from a seagrass-based pathway. Although mangroves do not contribute a direct food source, the physical structure does provide protection from predators and stabilizes the shoreline, allowing healthy seagrass meadows to persist (Nagelkerken et al. 2008; Heithaus et al. 2008).

Depleted seagrass $\delta^{15}N$ at Anegada signals an oligotrophic regime

Depleted nitrogen at Anegada relative to Hans Creek matched the local geological settings, but the Anegada $\delta^{15}N$ values fell at the low end of the global seagrass nitrogen range. Geologically, Hans Creek is volcanic in origin and Anegada is a carbonate atoll. Anegada is geologically more analogous to the Bahamas Bank or the Yucatán platform, whereas Hans Creek is representative of other embayments in the Lesser Antilles. Given these results, the parsimonious explanation for the depleted nitrogen at Anegada is the geological setting that created an oligotrophic, phosphorus-limited system (carbonate sediments bind phosphate, leaving the system P-limited rather than N-limited; Short 1987; Fourqurean et al. 1992). In a phosphorus-limited system, N_2 fixation by cyanobacteria contributes a more substantial fraction of the seagrass nitrogen budget which derives its Nitrogen from the atmosphere thus depleting the seagrass $\delta^{15}N$ a lower baseline (Capone and Taylor 1977; Welsh 2000). Phosophuros-limited systems derived from the geological setting have been documented on the Bermuda Platform (Fourqurean et al. 2015) and the Yucatán (Mutchler et al. 2007; Camacho-Cruz et al. 2020) and further elevate the importance of site-specific isotopic sampling. Hans Creek $\delta^{15}N$ values were more enriched than those at Anegada and more typical of broader Caribbean systems. As such, these differences in the baseline lead us to treat trophic position independently and not pooled across sites, providing a cautionary note against the use of regional literature values instead of study-specific baselines.

Understanding the biogeochemical influence on seagrass values has direct ecological and management implications. Seagrass values below 0‰ are considered indicative of oligotrophic conditions where wastewater has not yet penetrated the system (Lapointe et al. 2004; Mutchler et al. 2007). This matches Anegada, which has limited development; groundwater discharge through the limestone aquifer (Carruthers et al. 2005; Paytan et al. 2006) will be vulnerable to future disturbance. Any future development that increases terrestrial nitrogen, particularly from wastewater, could be traced in the seagrasses and the shark's plasma within months and into tissues with slower turnover rates over the course of the year (Camacho-Cruz et al. 2020). Given Anegada’s current low values (below 0‰), any change will be detectable in future sampling. The immediate ecological repercussion is that any increase in nitrogen would degrade the seagrass meadows through epiphyte and macroalgal growth and through light attenuation (Lapointe et al. 2004; Waycott et al. 2009). As the values allow for change detection, the baseline created here changes this study from strictly a description of the study sites to potentially be a basis for monitoring wastewater intrusion into the system as development proceeds.

Trophic position offsets are partially confounded by baseline differences

Although the Hans Creek trophic position was approximately 0.6 TP higher than at Anegada, the offset was consistent across tissues and years and should not be interpreted as evidence that Hans Creek sharks are higher on the trophic ladder. The results were confounded by the strongly depleted seagrass nitrogen at Anegada (as seagrasses presented the highest discrepancy compared to macroalgae or *Eucinostomus*), and by higher maternal input not fully accounted for, as Hans Creek samples contained fewer older sharks. In addition, resource availability around Anegada is likely more abundant due to the greater extent of shallow (< 2 m) habitat accessible to the sharks compared to Hans Creek. For these

reasons, we interpreted TP at the site level rather than rescaling the framework that would require extensive sampling and a higher-resolution food web model (Hussey et al. 2014).

Resolving the maternal-to-local transition

Using the plasma-RBC isotope offsets proved an effective mechanism for linking the sharks to the habitat as they shift from maternal to local input when compared to the known turnover rate (Fig. 5C; Kim et al. 2012). Modeling of 0 + animals at the time of capture indicated that they had replaced 36–56% of plasma but only 7–12% of RBC with locally-derived values, whereas the model indicates that older sharks had effectively completed plasma turnover and 71–73% RBC turnover during their residency. Comparing the published values from Kim et al. (2012b) to the observed values validated the shift from maternal to the local system. Although the sample size was moderate (n = 22), the predicted offset and the pattern observed support two important conclusions: first, the majority of our captures occurred early in nursery residency, when accumulation of local isotopes was actively in progress. Second, the plasma-RBC offset is a useful proxy for nursery residency through measuring the isotope offsets was useful in validating the cohort assignment, as the patterns in 0 + to 2 + individuals aligned with the modeled turnover, which from length-based estimations can be uncertain when encountering a runt or extra-large individual. This finding corresponds to recent telemetry-validated isotope work on other coastal elasmobranchs (Matich and Heithaus 2014; Matich et al. 2015; Shipley et al. 2021) and supports the broader concept that paired plasma and RBC sampling in a single capture can aid movement and habitat use metrics.

Statistical utility and ecological interpretation of bootstrap-derived ages

Bootstrap-derived ages agreed well with the length-based assignment across the 0 + cohort (Fig. 6a). This is the resolution the paired-tissue approach delivers, and it is enough to place a 0 + shark within the correct month of its first summer. The remaining scatter reflects the noise expected of the approach, since the model rests on a monthly parturition window. The resulting uncertainty is of the same order as the between-individual variation within the 0 + cohort, so the method cannot rank littermates in birth order. It does estimate young-of-year age to within roughly a week and a half of the length-based value. It does not independently separate 0 + from 1 + animals, because the branch of the offset curve used for each individual was selected using the cohort-based age; the agreement between the two estimates is therefore a consistency check rather than an independent validation.

Bootstrap reconciliation was most informative for 0 + animals, where the 95% intervals averaged 37 d (range 21–46 d). It was far less so for 1 + and 2 + animals, whose intervals averaged 707 d (range 516–780 d), and for which the offset method returned ages on average 79 d younger than the length-based estimates. This follows from the shape of the offset curve: after the modelled peak in plasma to RBC divergence near 89 d the relationship flattens, so a single offset value maps to a wide range of candidate ages. All 18 young-of-year animals were evaluated on the pre-peak branch and all four older animals on the post-peak branch. This is the limitation of single-snapshot two-tissue isotope analysis (Vaudo et al.

2010; Shipley et al. 2021): once both tissues have equilibrated toward the same local signal, the method has little left to read.

Two independently constructed estimates, one built from published turnover rates (Kim et al. 2012) and the other from age-at-length relationships (Barker et al. 2005; Tavares et al. 2020), converge for young-of-year animals. That convergence supports the assumption that BVI juvenile lemon sharks have plasma and RBC turnover comparable to rates reported elsewhere (MacNeil et al. 2006; Matich et al. 2010; Kim et al. 2012). It does not independently validate those rates, since agreement at this coarse a scale is compatible with a range of turnover parameters.

Anegada is a Bahamas-scale nursery; Hans Creek is a USVI-scale nursery

The geological setting and habitat extent of Anegada and Hans Creek create a unique opportunity to test resource use in two different sites. Anegada is a low-lying carbonate atoll with ample resources that support a multi-year cohort of lemon sharks (Legare et al. in Review) and is analogous to the Bahamas Bank, whereas Hans Creek, with its volcanic origin, is representative of the broader Virgin Islands, which comprise smaller, deeper areas with limited resources and relatively truncated residencies (DeAngelis et al. 2008; Chapman et al. 2009; Feldheim et al. 2014; Legare et al. 2015). The differences in habitat extent and geological setting allude to the plasticity juvenile lemon sharks exhibit for nursery area use. As the residences occur in the same region and within 40 km of each other, one would expect them to occupy a similar ecological niche, but trophic position offsets, carbon signatures within the seagrass community, and niche separation (SIBER) all suggest that the two sites should be considered as ecologically distinct but functioning nurseries. These findings indicate that the BVI are home to a network of nurseries differing in ecological scale and nutrient regime, with each component of this network playing a different role in supporting the regional juvenile lemon shark population (Heupel et al. 2007; Chapman et al. 2015; Olinger et al. 2017).

Management implications

As juvenile lemon sharks have high site attachment, particularly during the first year of life, this system also provides opportunities for strategic and effective management tools, including the creation of Marine Protected Areas and legislation that limits undue harm to the population. Several management recommendations are supported by this study. First, both of the nursery areas are seagrass-dependent, and seagrass meadow protection (against dredging, anchor damage, propeller scarring, and water-quality degradation) should be a priority. Second, the depleted seagrass $\delta^{15}N$ signature identifies Anegada as oligotrophic and can be used as a baseline against which future enrichment from wastewater nitrogen can be detected. Third, the geological contrast between Hans Creek (small, USVI-scale) and Anegada (Bahamas-scale, > 2 km^2) suggests that protection efforts need to be location-specific based on conditions rather than uniform between sites (Simpfendorfer et al. 2011; Knip et al. 2012).

Conclusion

The British Virgin Islands support juvenile lemon shark nurseries with seagrass meadows as the foundation, yet differences in nutrient regimes and isotopic niches indicate that they are ecologically distinct. The depleted seagrass $\delta^{15}N$ at Anegada goes hand in hand with the geological setting and provides a baseline for future wastewater detection. The Hans Creek nursery exhibits a more typical Caribbean nutrient regime, but the limited seagrass habitat exposes it to what could be considered spatially acute impacts. Using the plasma-RBC offsets, we gained confidence with the cohort assignments and a garnered a better understanding of the transition from maternal nutrition to local prey. Compared to documented elasmobranch turnover rates, these findings support the use of paired-tissue isotope analysis as a tractable proxy for nursery residency duration. This study provides the first quantitative isotope baseline for nursery bound lemon sharks in the region, garners insight into their distinct ecological pathways, and supports targeted, location-specific management.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

Animal handling was conducted under the Government of the Virgin Islands Ministry of Environment, Natural Resources and Climate Change permit ENV.E1/17 and approved by UMass Amherst IACUC protocol #6635. Field methods followed Stoskopf et al. (1984) for caudal-vein blood sampling.

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Author Contributions

BJL conceived and led the study, conducted fieldwork, analyzed data, and drafted the manuscript. CJ conceived and conducted fieldwork. BJL, CJ, and KB acquired funding. AM contributed seagrass sampling design and biogeochemical interpretation. AEC facilitated permits and field logistics through the Government of the BVI. GBS provided methodological guidance and contributed to manuscript revision. AJD supervised analysis and contributed to manuscript revision. All authors reviewed, edited, and approved of the final manuscript.

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Data availability

All raw stable isotope data (shark plasma and RBC $\delta^{13}C$, $\delta^{15}N$; environmental sources) are supplied as an Electronic Supplementary Material file.

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Figures

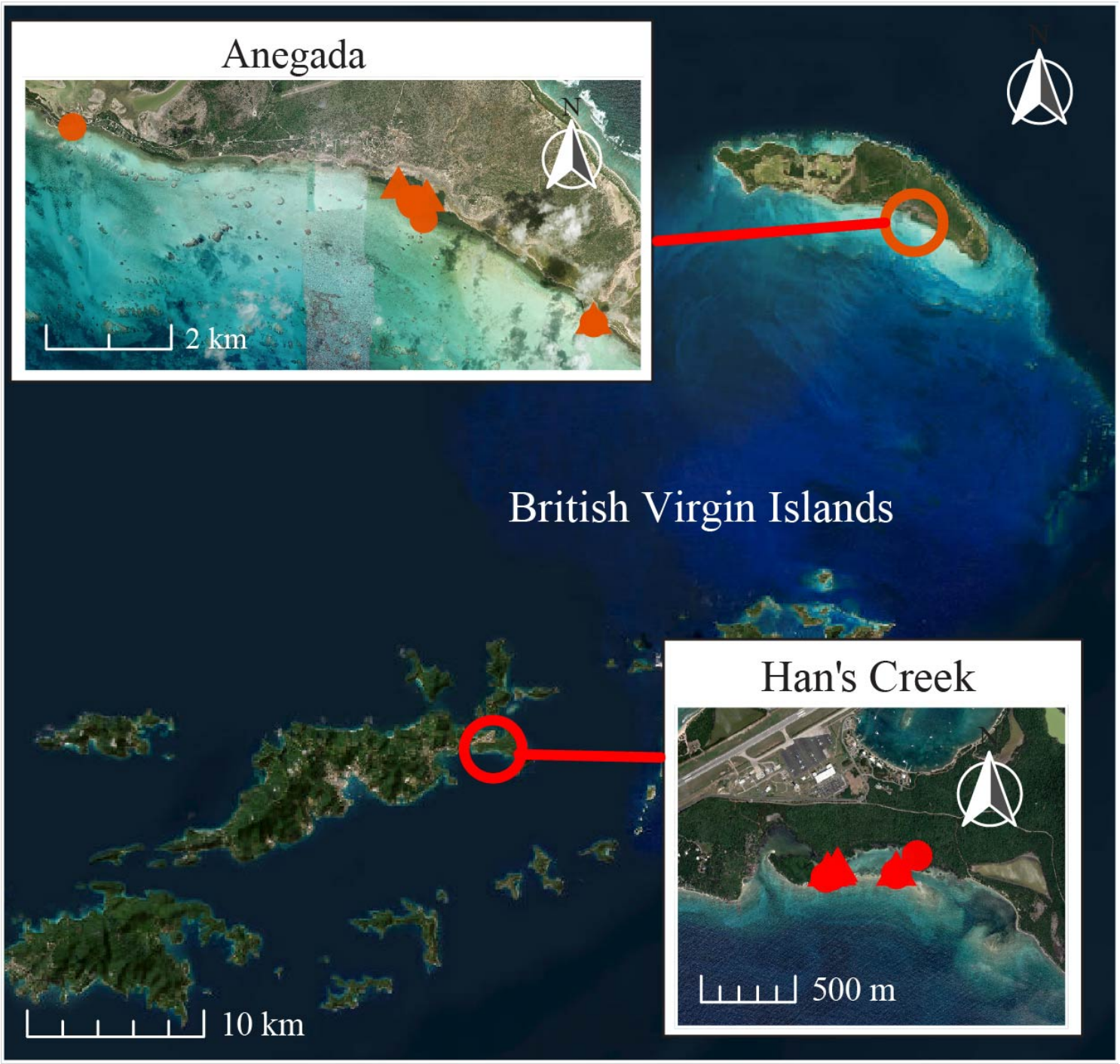


Figure 1

Study sites in the British Virgin Islands and locations of juvenile lemon shark (*Negaprion brevirostris*) captures in 2022 and 2023.

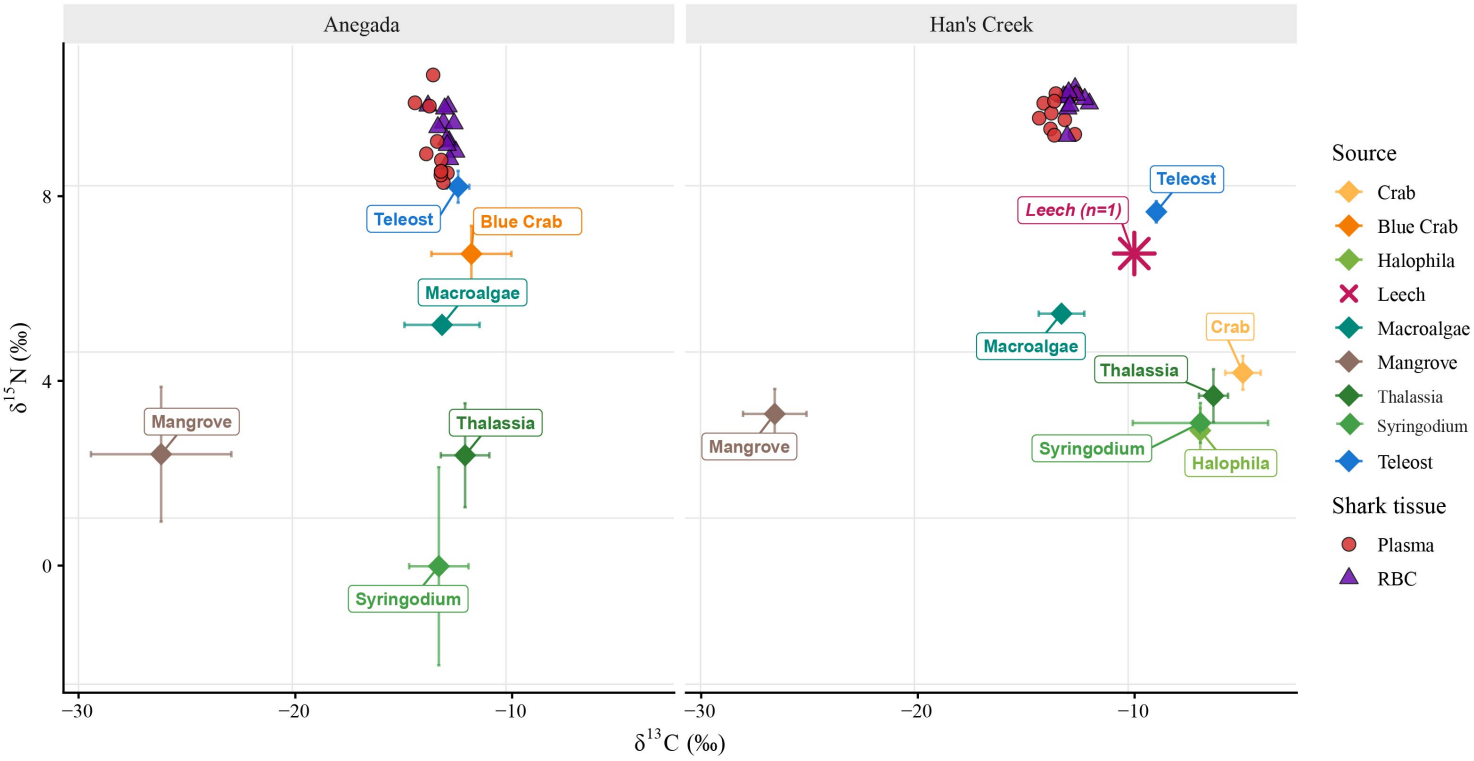


Figure 2

Isotopic biplot of juvenile lemon shark tissues and samples from the environment from Anegada and Hans Creek, British Virgin Islands. Mean values for shark plasma (●) and red blood cells (RBC; ▲) are shown uncorrected; environmental source means (diamonds) are TDF-corrected using lemon shark trophic discrimination factors of +0.9‰ δ¹³C and +2.3‰ δ¹⁵N (Hussey et al. 2010). Error bars around means are ±1 SD. A single marine leech (Leech, pink asterisk) is shown raw (no TDF correction) as a reference only and was not included in modeling.

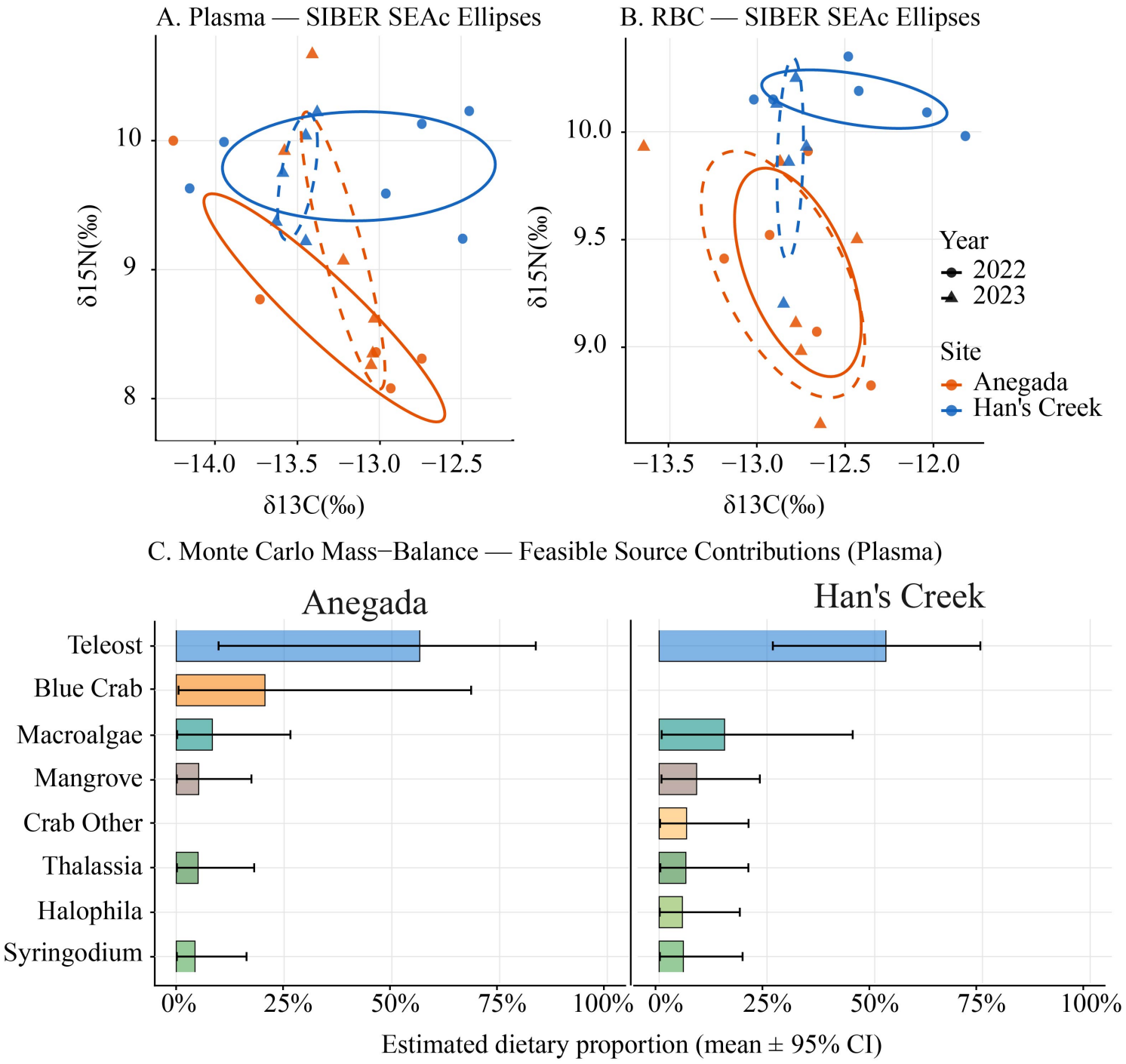


Figure 3

Isotopic niche and isotope source contributions of YOY and juvenile lemon shark. SIBER maximum-likelihood ellipses (SEAc) for plasma (A) and RBC (B) by site (Anegada: orange, Hans Creek: blue) and year (solid: 2022, dashed: 2023). Monte Carlo mixing model (C) of the dietary contributions to plasma at Anegada (left) and Hans Creek (right). Mean contribution with 95% credible intervals. Each site used its own sampled sources.

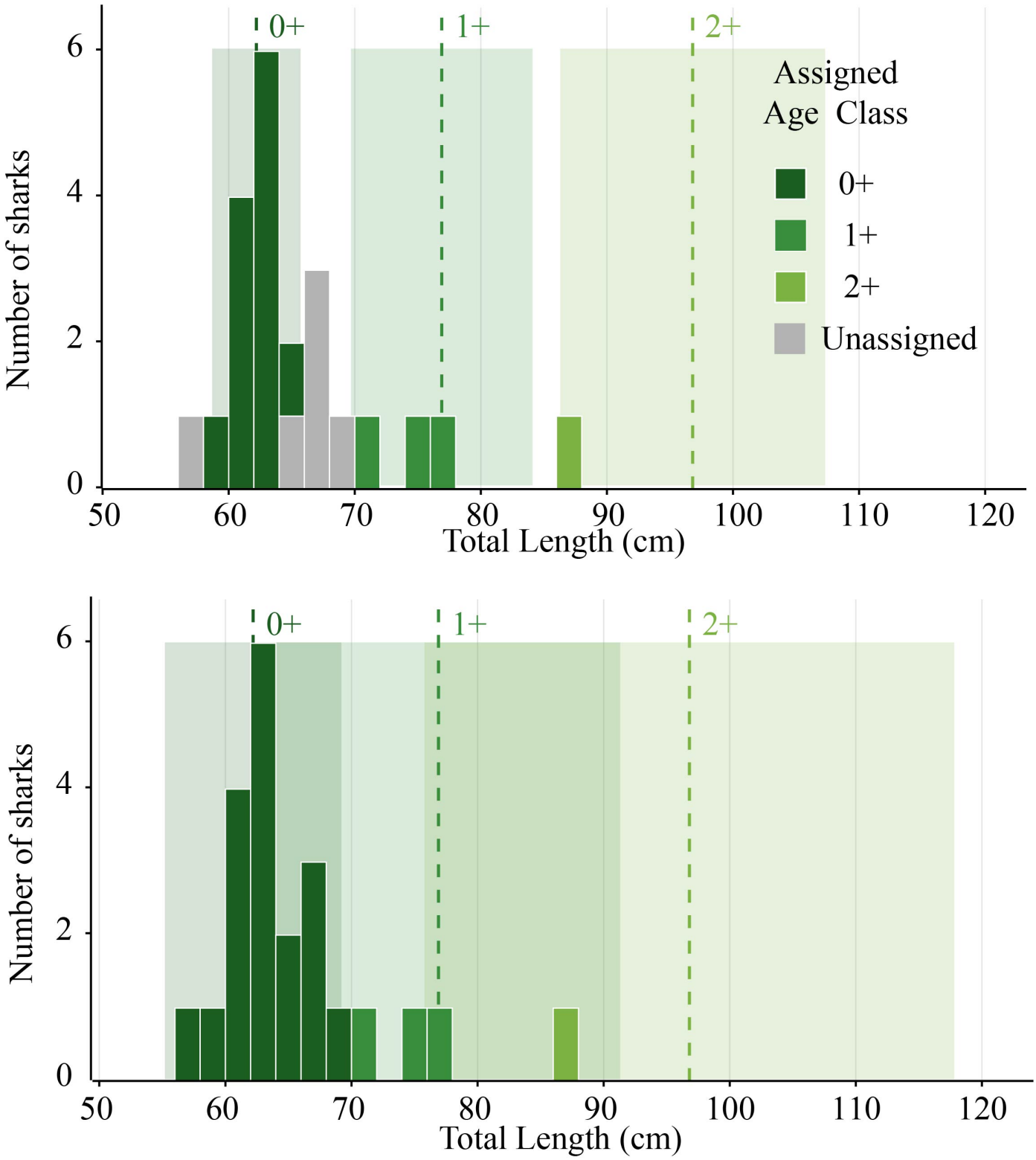


Figure 4

Age estimate for size at capture for juvenile lemon sharks using the Tavares et al. (2020) size at total-length (TL) for age 0–2 individuals. Histogram displays number of sharks in each size bin (TL; 2-cm bins) and shades them by assigned cohort. Age assignments were based on ±1 SD (top) or ±2 SD assignment (bottom). Dashed vertical lines mark the Tavares cohort means (0+ = 62.2 cm, 1+ = 76.9 cm, 2+ = 96.8 cm TL).

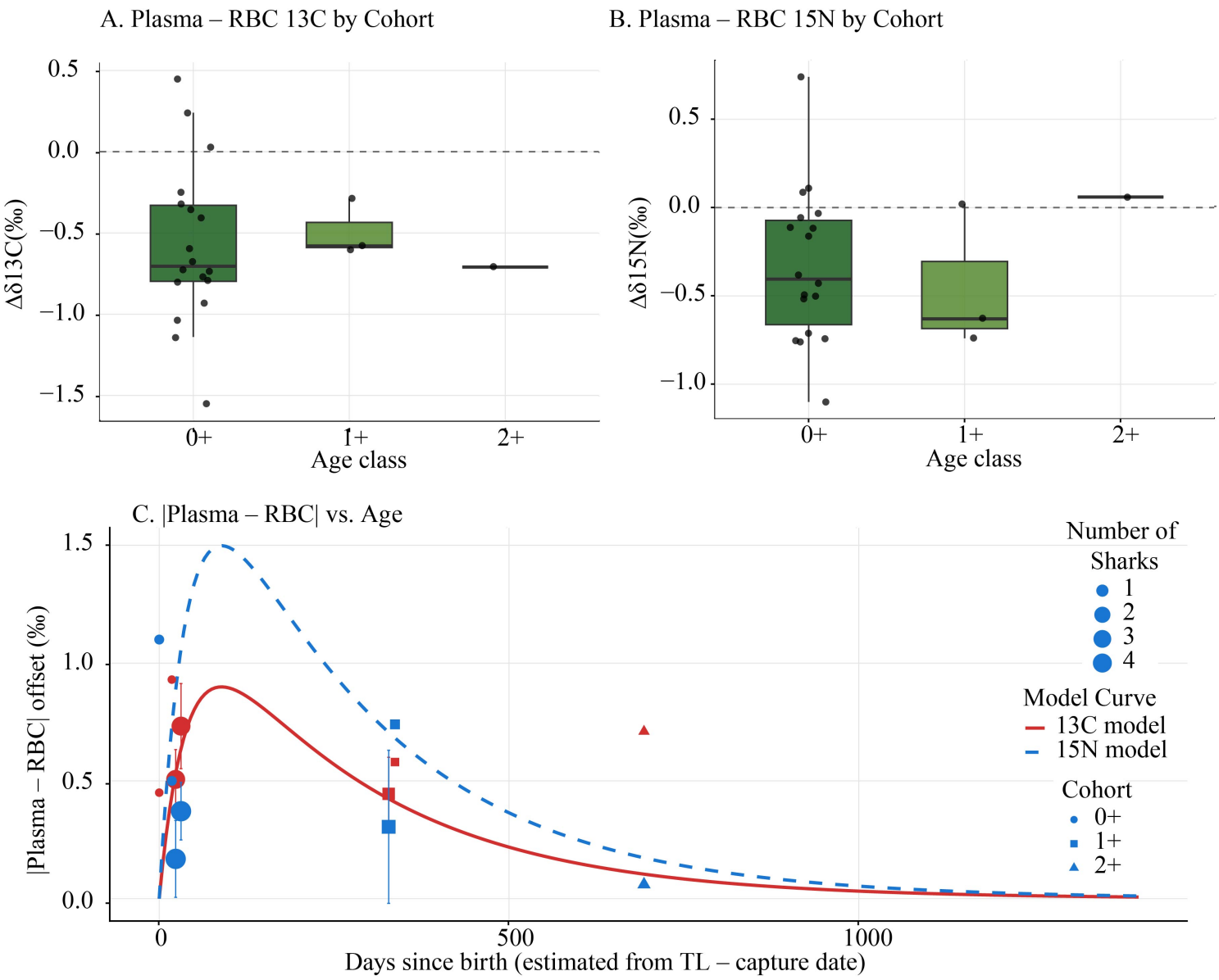


Figure 5

Plasma-RBC offsets and tissue turnover in YOY and juvenile lemon sharks pooled across sites and year. Shark plasma-RBC offsets for δ¹³C (A) and δ¹⁵N (B) plotted by cohort (0+, 1+, 2+) with individual sharks (●). Absolute value of tissue offsets |Plasma- RBC| are plotted against estimated days since birth (TL-derived, cohort-snapped to the May 1-June 30 parturition window), and against the published (Kim et al. 2012) turnover model for δ¹³C (red solid) and δ¹⁵N (blue dashed), for a maternal-to-local change of 1.5‰ in δ¹³C and 2.5‰ in δ¹⁵N (C).

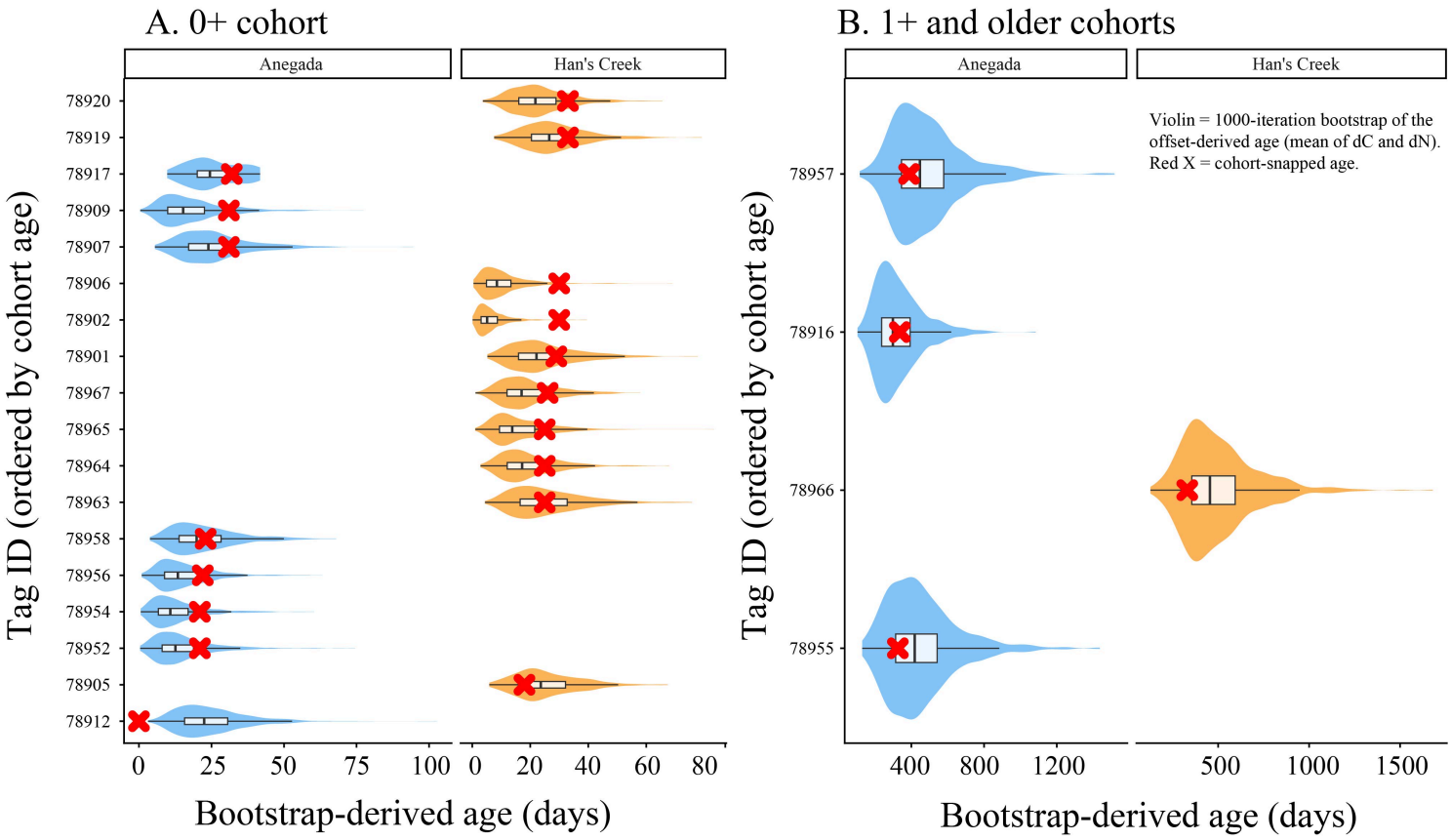


Figure 6

Bootstrap age distributions per shark from the offset inversion for the 0+ cohort (panel A) and the ≥1+ cohorts (panel B). Each violin shows the distribution of offset-derived age estimates from a 1,000-iteration Monte Carlo bootstrap drawing maternal-to-local isotope values variances derived from contrasts, plasma and RBC half-lives, and analytical precision. Boxplots indicate the median and interquartile range. Red X = the independent total length derived age.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ElectronicSupplementaryMaterial.docx