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ORIGINAL PAPER

Sequential scute growth layers reveal developmental histories of hawksbill sea turtles

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Abstract

Understanding the basic life history patterns of highly migratory species is important for effective management. For sea turtles, evidence of developmental biogeography and discrete life stage residency provides key information for understanding resource use and population threats and defining conservation priorities. Resolving gaps in these knowledge areas is not straightforward, however. Inaccessible habitats, low survivorship, late maturity ages, and technology limitations all complicate monitoring individuals continuously throughout their life span. Here, we expand on previous studies and document a near-complete tissue record in the ultimate posterior marginal scutes of hawksbill sea turtle (*Eretmochelys imbricata*) carapace. Stable isotope analysis (SIA) of ventral scute surfaces reveals differences between three geographically isolated populations in the Pacific and Atlantic basins. Additionally, sequential sampling and SIA along growth line contours of sectioned scutes reveals developmental movements. Perhaps surprisingly, no clear or general patterns emerge. Bivariate isotope data (stable carbon, $\delta^{13}C$, and nitrogen $\delta^{15}N$) indicate that only one of six Central Pacific hawksbills showed a distinct ontogenetic shift. And while all three Western Pacific individuals showed evidence of ontogenetic shifts, these individuals had three unique patterns. We summarize regional stable isotope values for common hawksbill foraging items, discuss drivers of regional nitrogen structure, and make recommendations for future study.

Keywords Highly migratory species · Biogeography · Sclerochronology · Population structure · Ontogenetic shifts

Introduction

Stable isotope analysis (SIA) is a relatively low-cost diagnostic tool for inferring individual-based ecological information from marine consumers. Isotopic compositions of animal tissues integrate ecosystem and foraging information (Deniro and Epstein 1981; Popp et al. 2007), and thus, when an animal moves among geographically discrete food webs, the stable isotope values of its tissues reflect these habitat shifts (Reich et al. 2007; Hobson and Wassenaar 2008; Ramirez et al. 2015). Known as stable isotope tracking, this method carries some advantages over traditional population monitoring via mark-recapture or biotelemetry. One, SIA does not require an initial marking of individuals to obtain subsequent data, but rather provides information on prior experiences and behavior. Two, if the sampled tissues provide a developed chronology, then SIA may provide a time series and not simply a snapshot of ecological information (Becker et al. 1991; Grottoli and Eakin 2007; Trueman et al. 2012). Three, unlike most biotelemetry

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studies that focus on geographic locations, SIA also has the potential to reveal foraging niche and trophic position (Seminoff et al. 2012; Clyde-Brockway et al. 2022). Four, SIA and other diagnostic tools can be applied to both living organisms and dead tissues, and therefore may incorporate samples from natural history archives to expand datasets and derive novel historical records (Gagné et al. 2018b; Miller et al. 2020; Smith et al. 2021).

As distinct isotopic patterns have been described across marine regions ("isoscapes"), stable isotope tracking has been broadly applied to understand the life history of sea turtles. Researchers have analyzed soft high-turnover tissues like skin and blood (Seminoff et al. 2006, 2012; Wedemeyer-Strombel et al. 2021; Clyde-Brockway et al. 2022), as well as hard tissues with sequential layering like scute and bone (Reich et al. 2007; Avens et al. 2013; Van Houtan et al. 2016a; Turner Tomaszewicz et al. 2017). When time-specific growth layers in these hard tissues are serially sampled, researchers can measure isotope values across an organism's distinct life stages. Pioneering work by Reich et al. (2007) performed SIA of keratin plugs of old and new scute tissues from green turtles (*Chelonia mydas*) in the western North Atlantic to reveal a transition from oceanic to neritic habitats during early juvenile development. However, Reich et al. (2007) only sampled two points in each individual's life history, and thus were unable to validate the duration of transition age of these discrete stages. SIA of scute plugs has since been conducted on successive scute growth layers to study habitat use by green and hawksbill (*Eretmochelys imbricata*) sea turtles (Vander Zanden et al. 2013; Wedemeyer-Strombel et al. 2021), yet still lack the contiguous sampling necessary for a more complete life history record.

SIA has also been examined in the growth layers of humerus bones from loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), green, and hawksbill turtles to document chronologies of habitat use (Avens et al. 2013, 2020; Ramirez et al. 2015; Turner Tomaszewicz et al. 2017, 2022). While these studies have provided new and important life history insights, one limitation of this approach is that complete life history records are frequently precluded by the loss of early growth layers due to inner bone resorption (Snover 2002; Van Houtan et al. 2014a); analogous to scute sloughing (Caine 1986; Palaniappan 2007). An ideal tissue for SIA chronology study in sea turtles would sequentially deposit layers, retain early life stage layers, and provide a full life record. Known as tortoiseshell in international trade (Donnelly 2008; Miller et al. 2019), the robust keratin deposits in hawksbill carapace scutes present a good candidate for study. Van Houtan et al. (2016a) advanced earlier studies of hawksbill carapace scutes (Tucker et al. 2001; Palaniappan 2007) by discovering a near-complete chronology in the ultimate posterior marginal (PM) scutes from hawksbill carapaces, tabulating internal growth lines, and using bomb radiocarbon ($\delta^{14}C$) to estimate tissue age.

Here, we expand on previous approaches by examining SIA in the ventral surface of central scutes and internal layers of PMs in hawksbill turtles. We first source hawksbill scutes through a variety of pathways and institutional partnerships (see Methods) to obtain scutes from all demographic stages and spanning four marine regions. Then, we compare SIA results from the most recently deposited ventral surface keratin tissues to examine patterns across ontogeny within and between geographic regions. Next, we perform SIA on sequential scute growth layers for six hawksbills from Hawaii and three hawksbills from the Western Pacific to reveal details from the cryptic early life history phase. Lastly, we collect stable isotope values and compile published records for common hawksbill forage items across the Pacific as a comparative reference.

Materials and methods

Specimen collection

We obtained hawksbill carapace samples from strandings, museum collections, and US federal repositories in accordance with US Endangered Species Act guidelines (US Fish &Wildlife Service permit #TE-72088A-0). Originating institutions provided sample metadata including location of origin, date of death or receipt, morphometrics, and sex. Specimens arrived in a variety of dispositions: whole organisms (frozen, taxidermized), whole carapaces (dried), and disintegrated scutes. Strandings were from NOAA's ongoing sea turtle stranding program at the Pacific Islands Fisheries Science Center in Honolulu, Hawaii (see: Work et al. 2004; Van Houtan et al. 2010; Balazs et al. 2015; Brunson et al. 2022). The Bernice Pauahi Bishop Museum provided samples from their collections and the US Fish and Wildlife Service, Office of Law Enforcement (Clark R. Bavin National Fish and Wildlife Forensics Laboratory, and National Wildlife Property Repository) provided seized specimens. Hatchling scutes came from emerged or partially emerged, deceased hatchlings during nest excavations on Maui and Hawaii Islands in conjunction with nest monitoring programs (e.g., Seitz et al. 2012; Gaos et al. 2021). Table S1 provides more details and metadata on the hawksbill specimens.

Hawksbill forage item samples (macroinvertebrates and macroalgae) were derived from field surveys and stranded turtles and supplemented with additional data from the published literature. Previous nearshore reef surveys collected macroalgae in the Main Hawaiian Islands (Van Houtan et al. 2014b). We supplemented these collections with surveys of established hawksbills foraging sites on Oahu, Maui, and Hawaii islands in 2012–2014, and at Rose Atoll, American

Samoa in 2012. During necropsy, we obtained additional undigested forage specimens from the upper gastrointestinal tract (i.e., esophagus) of two hawksbills from Kwajalein Atoll, Republic of the Marshall Islands. These turtles died from traumatic injuries in September 1992, were kept in a freezer, and necropsied in July 2012 following established protocols (Work 2000). Published studies provided further isotope values from additional hawksbill forage items collected on Hawaii island in 2007–2008 (Graham 2009) and at Palmyra Atoll in 2008–2010 (Kelly 2012).

Specimen preparation and sample extraction

Following published methods (Van Houtan et al. 2016a; Miller et al. 2019), we prepared all hawksbill scute specimens for imaging, microsampling and diagnostic analysis. We began by separating carapace and marginal scutes from their adjoining tissues through natural tissue degradation. This process enclosed carapaces in perforated heavyweight polypropylene bags and submerged them in seawater for < 7 days. Then, we removed surface algae, epibionts, debris, and cleaned scute surfaces with tap water and mild detergent. We rinsed the cleaned scutes first with deionized water, then with 90% EtOH and air-dried scutes in a fume hood for 24 h. Following previous studies (Dailer et al. 2010; Van Houtan et al. 2014b), we rinsed collected macroalgae in deionized water, patted samples dry with cloth towels, and placed them on aluminum foil in a drying oven at 60 °C until fully desiccated (24–48 h). We repeated this same procedure for additional hawksbill forage items, separating forage items into discrete taxonomic groups.

We first sampled superficial scute surfaces as previous studies examined these tissue sections for patterns of growth (Tucker et al. 2001; Palaniappan 2007) and stable isotope content (Reich et al. 2007; Kelly 2012). Using central carapace scutes from Hawaii, Caribbean, and American Samoa specimens, we examined the newest tissue deposits—the center of the ventral side of the scute that directly contacts the living epidermis (see below, also Palaniappan 2007)—to capture a snapshot of their most recent life history and ecosystem experience (Van Houtan et al. 2016a). With a scalpel, we scraped the exterior of ventral scute surfaces, moving perpendicular to the edge of a No. 21 blade (at < 0.5-mm depth) to create 5 mg of sample material. For hatchlings only, as this demographic has no pronounced scute chronology, we shredded whole scutes using medical grade scissors (Excelta® #364, 1.25" blade). Using a ceramic mortar and pestle, we further homogenized all extracted scute and forage item material into a fine powder storing all sample homogenate in 1.5-mL Nalgene™ cryogenic vials for isotope analysis.

Seeking a more complete life history record, we supplemented these ventral scute surface samples by revealing and sampling sequential growth layers within PM scutes, derived from Western Pacific and Hawaii specimens. Following Van Houtan et al. (2016a), we used a low speed precision cutter (Buehler Isomet™, No. 11–1280-170) with diamond wafering blades (Buehler 15HC, No. 11–4244) to make 1.5-mm-thick sagittal cross sections in ultimate PM scutes. To reveal growth layers, we polished the cross-sectioned wafers (Buehler ECOMET IIITM 800 Polisher, Mark V Laboratory® A/O lapping film) sequentially moving from coarse to finer lapping film. We imaged each polished PM cross section with a brightfield, phase contrast, and darkfield equipped microscope (scope: Olympus BX41™, camera: ImagingPlanet 20MPX ™, adapter: Olympus U-TVO.5XC-3, firmware: IMT i-Solution Lite), using software (Adobe Photomerge®) to stitch a single composite image from multiple sub-field image frames (e.g., Fig. 2B). The variable illumination and contrast capabilities of this microscope were useful for identifying growth lines across variously melanized sections of scute keratin.

Following Van Houtan et al. (2016a), we counted the apparent growth lines on each PM composite image and extracted tissue samples with a Carpenter Microsystems CM2 microsampling system (Avens et al. 2013; Turner Tomaszewicz et al. 2017). Here, we drilled ~ 1-mm paths along PM growth contours (see Figs. 3 and 4), extracting > 1.5 mg of keratin powder for each microsample, repeating this process to capture material representing distinct developmental stages in each PM. Further treatment of scute material for lipid extraction was not required due to low C:N ratios among samples (see below; Turner Tomaszewicz et al. 2015).

Isotope analysis and data visualization

We determined bulk $\delta^{13}C$ and $\delta^{15}N$ stable isotope compositions using an in-line C-N analyzer coupled with an isotope ratio mass spectrometer (Finnigan ConFlo II/DeltaPlus). Approximately, 1.0 mg of each sample was loaded into sterilized tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer at the Light Stable Isotope and Mass Spectrometry Laboratory at University of Florida (Gainesville, Florida, USA). We used a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT) to a DeltaPlus gas isotope-ratio mass spectrometer (Finnigan MAT). The elemental analyzer combusted samples in pure O_2, resultant gases were reduced to N_2 and CO_2 and passed through a series of thermal conductivity detectors and element traps to determine percent compositions. Besides isotopes, this method also provided bulk elemental composition (%) for carbon and nitrogen. Acetanilide (C_8H_9NO: 71.09% C; 10.36% N) was the calibrant. We sent a small subset of additional samples ($n < 20$) to the Biogeochemical Stable Isotope Facility at the

University of Hawaii, where similar analyses and procedures were followed.

We expressed sample stable isotope ratios relative to the isotope standard following conventional delta (δ) notation in parts per thousand (‰), using $\delta = ([R_{sample}/R_{standard}] - 1)*(1000)$, where R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes (e.g., $^{15}N/^{14}N$) in the sample and standard, respectively. $R_{standard}$ for ^{13}C was Baker Acetanilide ($\delta^{13}C = -10.4$) calibrated monthly against the Peedee Belemnite limestone formation international standard. The $R_{standard}$ for ^{15}N was IAEA N1 ammonium sulfate/$(NH_4)_2SO_4$ ($\delta^{15}N = 0.4$) calibrated monthly against atmospheric N_2 and USGS nitrogen standards. All analytical runs included known standards placed every 6–7 samples to calibrate against instrument drift. Hundreds of replicate assays of reference materials indicated measurement errors of 0.06‰ for carbon and 0.12‰ for N for this setup (e.g., Seminoff et al. 2006, 2012).

We generated a series of visualizations from the scute imaging and SIA data, with a few provisions. First, the Caribbean hawksbill scutes alone were disintegrated from the original carapace with no accompanying demographic data. For these scutes, we previously (see Miller et al. 2019) estimated the straight carapace length ("SCL") of the turtle from which they originated from the area of individual scutes. Second, in plotting the stable isotope values from scute microsampling, we recognized that drilled transect paths always exceeded individual growth lines, and at times imperfectly followed growth line contours (range = 2–35 growth lines, mean = 6.1). As a result, we recorded the minimum and maximum growth line number of each transect drill path and plotted SIA results graphically against the median growth line.

Third, to compare isotope trajectories through development between samples, we generated an ensemble model for $\delta^{13}C$ and $\delta^{15}N$ values across development. As we have no telemetry or genetics data to indicate these adjoining regions hold completely distinct populations (Gaos et al. 2020), we conservatively pooled data from all North Pacific turtles (Central and Western Pacific). The resulting ensemble is a locally weighted regression (Cleveland and Devlin 1988) of the average stable isotope values in each 10 growth line wide bin (lines 0–9, 10–19… 190–199, etc.) of the median microsampled position value. We use this not to make population inferences, but only to illustrate a stage-specific stable isotope value reference. To augment sample sizes in each of these bins (range: 1–7 samples, mean 3.1 samples), we added the results from the previous ventral surface scrapings from the Hawaii samples only. For these samples, the growth line number attributed to the sample was the maximum growth line number for that individual. [Here, growth lines were calculated and described in a previous study (Van Houtan et al. 2016a)] We excluded hatchling data as well as data originating from scutes from other ocean sub-basins from these ensemble models.

We previously aged turtles through a validated, bomb radiocarbon $\delta^{14}C$ method or estimated age from a derived von Bertalanffy growth function (Van Houtan et al. 2016a). As the PM for one individual turtle was worn (see below), its early tissue record is absent, and its discernable count of growth lines ($n = 110$) is truncated. As a result, we estimated its total growth line count ($n = 200$) from a derived length-to-growth-line model (Van Houtan et al. 2016a) and plot its isotope data beginning at the difference between that estimate and its documented count (e.g., 90).

Results

Sampling the ventral surfaces of central scutes does not indicate a clear stable isotope pattern throughout development, though it suggests some regional structure. Figure 1 plots the $\delta^{13}C$ and $\delta^{15}N$ values and bulk carbon and nitrogen content from scute surface samples from $n = 106$ hawksbills. Of these samples, 28 originated from Hawaii (4.0–88.7 cm SCL), 60 from Caribbean (38.5–84.3 cm SCL), and 18 from American Samoa (27.7–68.4 cm SCL). Scatter and density plots show somewhat clustered and normally distributed $\delta^{13}C$ values (Fig. 1A, B, mean − 15.8 ± s.d. 1.32 ‰, 95% CI: − 18.3 to − 13.2 ‰), but simple linear regressions reveal no significant trends across development ($F_{1,105} = 0.002$, $P = 0.97$, adjusted $R^2 = -0.01$). The $\delta^{15}N$ plots (Fig. 1 C-D) indicate more spread in the N isotope data (10.0 ± 2.48 ‰, 95% CI: 5.1–14.9 ‰). This is evidenced in the long tail toward heavier N isotopes (Fig. 1D) and as six American Samoa juveniles and two Hawaii adults are heavier than the 95% CI for $\delta^{15}N$ (Fig. 1C). Simple linear regression suggests significant $\delta^{15}N$ changes over development ($F_{1,105} = 4.73$, $P = 0.03$); however, the model's explanatory power is weak (adjusted $R^2 = 0.03$). When regions were considered separately, the $\delta^{13}C$ (-16.1 ± 1.38 ‰) and $\delta^{15}N$ (10.1 ± 2.29 ‰) values from Hawaii are consistent with the pooled results. The American Samoa ($\delta^{13}C$: − 16.9 ± 1.44 ‰; $\delta^{15}N$: 13.7 ± 2.85 ‰) and Caribbean ($\delta^{13}C$: − 15.3 ± 0.95 ‰; $\delta^{15}N$: 8.8 ± 0.86 ‰) also overlap with the pooled results but show more $\delta^{15}N$ structure. Figure 1E, F details the bulk elemental composition, with carbon = 48.9 ± 1.37%, $N = 14.7 \pm 0.63\%$, and the remaining 36.4% arising from H, O, S, and other elements. The C:N ratio for all ventral scute surface samples had a mean of 3.33 ± 0.08 sd. Table S1 provides further details on sample metadata. As the SCL domains differ between regional sample groups, we cannot rigorously model population differences in stable isotopes. However, the stable isotope values from these surface samples show no clear developmental trends.

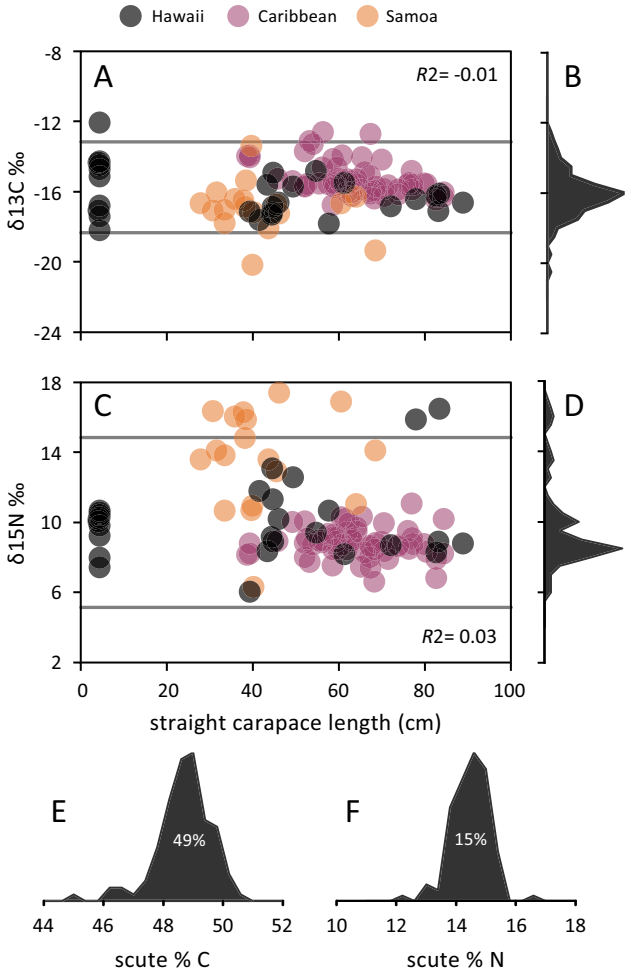

Fig. 1 Bulk stable isotope and elemental composition from the ventral surfaces of central hawksbill carapace scutes. **A** Raw results and **B** density of $\delta^{13}C$ content (− 15.8 ± 1.32 ‰), with **C** raw results and **D** density of $\delta^{15}N$ values (10.0 ± 2.48 ‰). Filled circles are individual turtles comprising ten hatchlings (4–5 cm SCL), 77 juveniles (28–71 cm SCL), and 19 adults (72–89 cm SCL) from Hawaii (gray circles), Caribbean (purple circles), and American Samoa (orange circles) populations. Horizontal gray lines are the normalized 95% interval of all 106 samples. Simple linear regressions of both series (adjusted correlation coefficients listed) show no trend through development. Density plots of **E** carbon and **F** nitrogen composition (expressed as a percentage, median values listed) indicate 36.4% of scute material is exclusive of carbon and nitrogen. Sampled tissue is from the ventral scute surfaces and captures the ecosystem experience preceding each turtle's demise. Hatchlings are ecologically naïve, and their tissues are maternally derived. As we lack samples from 8–28 cm SCL (0–4 years old), these plots do not represent the cryptic early life history phase

Fig. 2 Cross sections of posterior marginal (PM) scutes from hawksbills contain a longitudinal chronology. **A** Dorsal carapace view of a 44.2-cm SCL juvenile Hawaiian hawksbill with the left ultimate PM removed. PM scutes both retain the largest keratin archives on the shell and can be less frequently damaged than carapace scutes. White dashed rectangle indicates the ventral surface region sampled in Fig. 1. **B** PM sagittal cross section with growth line chronology where tissue accretes from left (posterior, old) to right (anterior, new). Cross section polished to 1-mm thickness and imaged under magnification using a combination of reflected and transmitted light. This turtle had 50 growth lines, with every tenth contour labelled and highlighted for clarity

Figure 2 illustrates the general structure that ultimate PM scutes capture growth continuously throughout development, and potentially contain a near-complete life history record. Figure 2A locates the left ultimate PM scute on a dry-archived, juvenile hawksbill carapace, and the ventral surface regions sampled (white dashed line rectangle). When sectioned sagittally and polished, these PM scutes reveal internal incremental growth layers (Fig. 2B). Parallel growth layers occur on either side of the central suture line, where the dorsal carapace and ventral plastron fuse. Here in this 44.2-cm SCL juvenile, the PM contained 50 growth lines. Bomb radiocarbon techniques aged this turtle at 6.8 years, suggesting it deposited an average of seven growth lines annually (Van Houtan et al. 2016a).

Continuous sampling of $\delta^{13}C$ and $\delta^{15}N$ values throughout the life history of six Hawaii hawksbill turtles reveals individual life histories, but no consistent pattern (Fig. 3). Only one turtle shows a clear ontogenetic shift indicated by abrupt coincident changes in the $\delta^{13}C$ and $\delta^{15}N$ values between the early and late growth lines sampled (Fig. 3F). Here, $\delta^{13}C$ values decrease from growth lines 0–60 and then flatten out near -16 ‰ $\delta^{13}C$. By contrast, $\delta^{15}N$ values increase through development, jumping from near 6 ‰ to near 15 ‰ $\delta^{15}N$ between growth lines 40 to 60. When the bivariate isotope data for this turtle is plotted ($\delta^{15}N$ plotted against $\delta^{13}C$), a dramatic dietary (and/or habitat) shift is apparent (highlighted by the orange arrow, Fig. 3F). This pattern suggests a discrete biogeographical and developmental phase shift, perhaps being an early life history shift from pelagic to neritic ecosystems (Reich et al. 2007; Bjorndal and Bolten 2010). The remaining

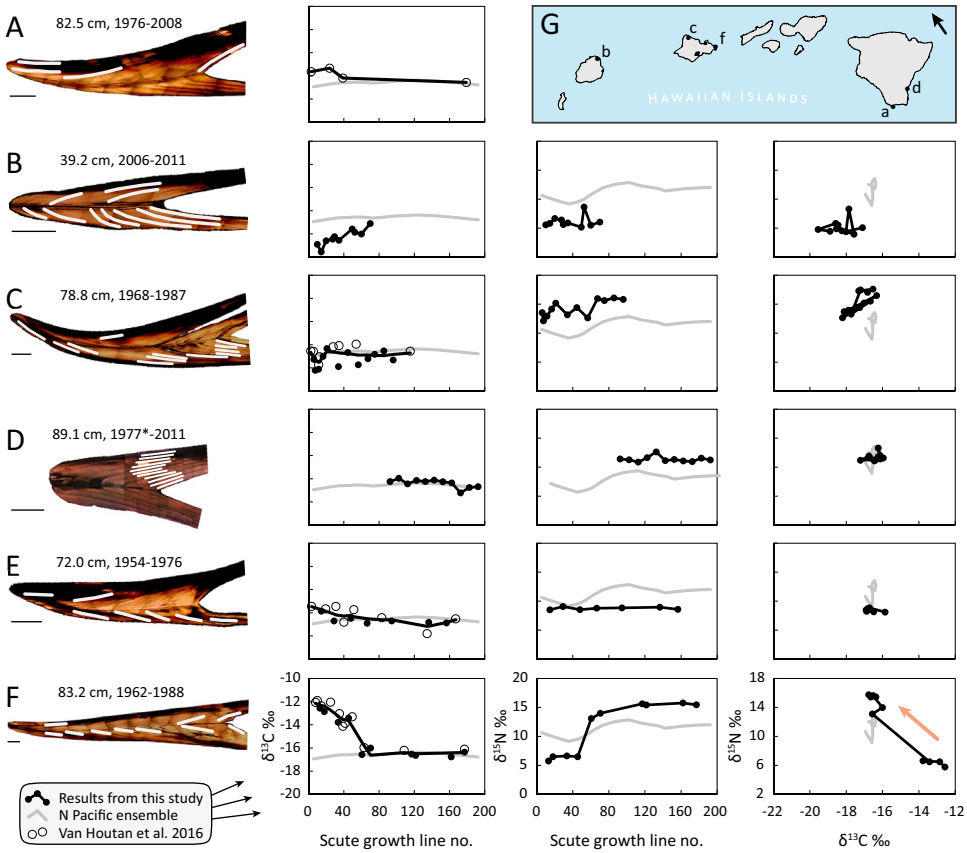

Fig. 3 Stable isotope values from scute growth contours shows several developmental patterns in Hawaii hawksbills. **A–F** Imaged cross sections, drilled transects, and stable isotope results for six hawksbill turtles. Hollow circle $\delta^{13}C$ values and age estimates are from a previous study (Van Houtan et al. 2016a), and filled circles are from the current study. Black lines interpolate values, are a LOESS when multiple data sources are available, and gray line is the ensemble average for all individuals (Figs. 3–4). From left to right **A–C**, **E–F** or top to bottom **D**, drill line paths follow the chronology of maturation. Juvenile tissue in one turtle **D** is missing from abrasion. **G** Map of the Hawaiian archipelago locates each turtle's stranding site (E is unknown). There is no single pattern in either C or N isotopes across development envisioned in either single or dual variable plots. **F** Demonstrates a discrete habitat shift (orange arrow), reflected in $\delta^{13}C$ and $\delta^{15}N$ values, perhaps due to nearshore settlement. Gradual shifts in $\delta^{13}C$ values **A–B**, **E** and **E** also indicate potential habitat shifts. Scale bars near each PM cross section are 5 mm. Asterisk (*) indicates turtle age estimated with a Von Bertalanffy growth function (see Methods)

five turtles reveal more subtle patterns and suggest no distinct developmental biogeography. Figure 3A, E shows a slight decrease in $\delta^{13}C$ values in growth lines 0–40, but the accompanying $\delta^{15}N$ values are either absent or constant. Two turtles (Fig. 3B, C) show a gradual enrichment in $\delta^{13}C$ in growth lines 0–80 but reveal no significant $\delta^{15}N$ patterns. The last turtle (Fig. 3D) abraded its early life history tissue, so this record is lost, but has remarkably constant $\delta^{13}C$ and $\delta^{15}N$ values throughout. Figure 3G shows the geographic origins of the samples in the Main Hawaiian Islands, unless unknown (Fig. 3E). Though the ^{13}C Suess effect is seemingly strongest in the surface waters of the North Pacific (Eide et al. 2017), it seems an unlikely influence to these patterns as we observe no consistent $\delta^{13}C$ trend, and its magnitude is weak (< 0.02 ‰ yr^{-1}) to our observed changes (Fig. 3).

Continuous PM microsampling of stable isotope values for three Western Pacific hawksbills suggests ontogenetic shifts across isotopically distinct areas might be more common in this region (Fig. 4). Despite a lack of adult tissues (these juveniles measured 42–50 cm SCL, estimated at 5–7 years old), each turtle shows some evidence of a distinct developmental shift. Here, individual isotope biplots of $\delta^{13}C$ against $\delta^{15}N$ are particularly revealing with each showing two clusters of data points. Though these isotope data suggest developmental changes to diet and ecosystem through development, they do not record the same pattern across turtles. The isotope data clusters reveal dramatic $\delta^{13}C$ increases with gradual $\delta^{15}N$ increases (Fig. 4A), gradual $\delta^{13}C$ declines with dramatic $\delta^{15}N$ declines (Fig. 4B), and notable $\delta^{13}C$ increases with similar $\delta^{15}N$ declines (Fig. 4C). Like the Hawaii hawksbills in Fig. 3, there is no clear agreement in

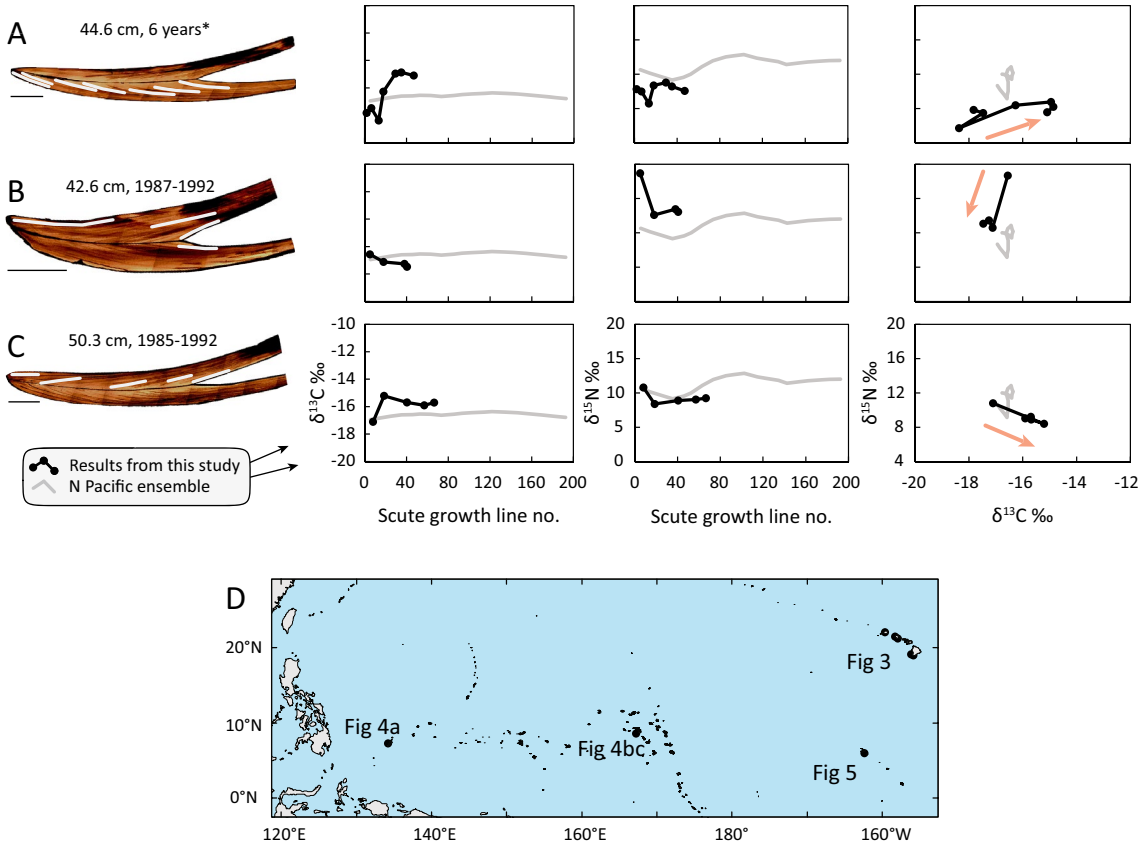

Fig. 4 Stable isotope values from scute growth contours of Western Pacific hawksbills. Though the turtles from **A** Palau and **B, C** Kwajalein Atoll are all relatively immature, stable isotopes document potential shifts in both habitat and forage. Symbology retained from Fig. 3, and scale bars near each PM cross section remain 5 mm. Asterisk. Asterisk (*) indicates age estimated using measured length and previously derived VBGF parameters (Van Houtan et al. 2016a) as we had no date metadata for this specimen. Stranding dates of **B**, **C** allow us to estimate birth year; **A** has no stranding date. Orange arrows indicate an apparent habitat and dietary shift. **D** Map of the central and Western Pacific with all sample collection regions noted

overall pattern. Figure 4D shows the geographic origin of these turtles in Palau and the Marshall Islands, and origin of some of the forage samples in Fig. 5.

Though non-exhaustive, Fig. 5 summarizes available bulk stable isotope values of typical hawksbill forage items from four Pacific Ocean regions. The data comprise 89 samples from 36 morphospecies representing five major forage groups: sponges, other macroinvertebrates, red algae, green algae, and brown algae. Hawksbills are omnivores, and while this dataset is not exhaustive, it represents all known forage groups for hawksbills in this region (Graham 2009). Based on the limited data from these samples, the isotope biplot reveals some apparent structure of hawksbill forage items between Pacific regions. This is particularly true for $\delta^{15}N$ values. The macroinvertebrates and sponges of Palmyra Atoll ($\delta^{15}N > 9$ ‰), for example, have mean $\delta^{15}N$ values almost twice that of the same groups in the Main Hawaiian Islands ($\delta^{15}N < 5$ ‰). The macroinvertebrates and sponges sampled from Kwajalein Atoll are between the two extremes with mean $\delta^{15}N$ values near 7 ‰. Across locations and forage groups, $\delta^{13}C$ values are highly variable by comparison with $\delta^{15}N$ values. The limited representation of only green algae from Rose Atoll shows high variability in both $\delta^{13}C$ and $\delta^{15}N$ values. Tables S2 provides more details on these forage items, including species and samples sizes.

Discussion

Given their critical conservation status and ongoing exploitation (Mortimer and Donnelly 2008; Miller et al. 2019), understanding the spatial population structure of hawksbill turtles is important for developing effective management strategies (Monzón-Argüello et al. 2010; Wallace et al. 2010; Seminoff et al. 2015). This may require a dedicated endeavor for hawksbills; however, as their omnivorous and variable life history traits defy simple characterization both within and among geographic regions. For example, unlike other sea turtle species, satellite telemetry (Hawkes et al. 2012; Marcovaldi et al. 2012; Walcott et al. 2012) and fishery

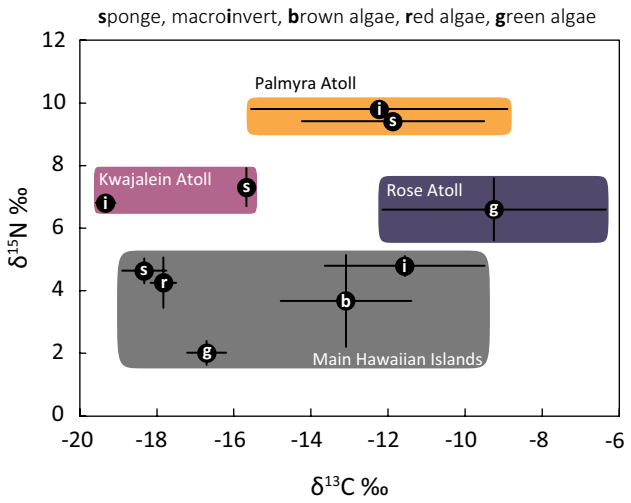

Fig. 5 Stable isotope values of typical hawksbill forage item groups from 4 Pacific Island regions. Hawksbills are omnivores that forage on sponges, other macroinvertebrates, and macroalgae. Above stable isotope values are crowdsourced from the literature (Palmyra Atoll, Pacific Remote Island Areas USA), our own collection efforts (Kwajalein Atoll, Marshall Islands; Rose Atoll, American Samoa), or a combination of both (Main Hawaiian Islands). Filled circles represent the group mean, lines are standard error. Though there is significant variability in $\delta^{13}C$ values within each region (and sometimes within a single region's forage item groups), there is some structure of $\delta^{15}N$ values between regions. These data are meant to report what currently exists, but not represent an exhaustive list of all potential hawksbill forage items in all population regions

bycatch data (Van Houtan et al. 2016b) reveal no clear spatial population structure for hawksbills. Furthermore, existing population sampling data indicate that much remains cryptic about hawksbill life history and habitat use (Gaos et al. 2012; Liles et al. 2015). Stable isotope analysis of preserved tissues is an established technique that has demonstrated promise to help resolve the developmental biogeography of hawksbill populations.

Progress here has been limited as the current knowledge of hawksbill tissue stable isotope values is largely restricted to the Neotropics. Early, limited analysis of homogenized outer scute layers from two Florida and two Bahamas hawksbills had mean $\delta^{15}N$ of ~ 5.5 ‰ and $\delta^{13}C$ of ~ − 17 ‰ (Reich et al. 2007), providing the first insights into hawksbill scute stable isotopes in those ocean regions. Comparing distinct developmental stages of scute growth layers in nesting females from the Lesser Antilles, Fireman (2021) found "high variability" in stable isotope values (mean: $\delta^{15}N = 8.9 \pm 2.0$ ‰, $\delta^{13}C = -18.4 \pm 1.3$ ‰) of all individuals sampled, but clear evidence of ontogenetic shifts in just four of 50 individuals sampled (8%). Whole blood and skin biopsy analysis from juvenile hawksbills in the eastern tropical Pacific of Costa Rica showed a broad $\delta^{13}C$ niche (range: − 19 to − 13 ‰) by comparison to $\delta^{15}N$ (range: 12 to ~ 14 ‰) (Clyde-Brockway et al. 2022). Biopsy samples of four scute growth layers in juvenile and sub-adult hawksbills in the eastern tropical Pacific of Nicaragua and El Salvador found a general $\delta^{13}C$ depletion as turtles matured (range: -27 to -17 ‰) (Wedemeyer-Strombel et al. 2021). Longitudinal skeletal sampling of hawksbills in Pacific of El Salvador showed a consistent decline through development of $\delta^{13}C$ (from − 15 to − 24 ‰) and $\delta^{15}N$ (from ~ 14 to ~ 11 ‰), which researchers attributed to an oceanic to nearshore habitat shift (Turner Tomaszewicz et al. 2022).

A primary aim of this study was to provide novel sampling methods to describe the contiguous isotope life history of hawksbills. We first demonstrated that sampling the ventral surface of scutes detects no clear isotope patterns across development from hatchlings to breeding females (Fig. 1). Like skin or blood samples, surface scute samples summarize a life history record that is both recent and brief. Analogous to tree rings (Schweingruber 2012) and fish otoliths (Pannella 1971), cross-sectioned and polished PM scutes reveal a near-complete life-history record (Fig. 2) with potential to yield new insights into age, diet, and movements (Van Houtan et al. 2016a). For Hawaiian hawksbills, one of six turtles (17%) has clear evidence of an ontogenetic shift (Fig. 3). This result corroborates a previous analysis of bycatch, strandings, and opportunistic observation data that Hawaiian hawksbills aged 0–4 years likely remain in the region's coastal waters (Van Houtan et al. 2016b). The proportion of ontogenetic shifts in Hawaii is also consistent with what has been inferred from isotopes from hawksbills from the Lesser Antilles (Fireman 2021), but is significantly less than the eastern tropical Pacific populations (Wedemeyer-Strombel et al. 2021; Turner Tomaszewicz et al. 2022). The longitudinal trends in stable carbon values of Hawaiian hawksbills are inconsistent, containing both patterns of ^{13}C enrichment and depletion through development (Fig. 3). By contrast to the Hawaii specimens, all three Western Pacific turtles show a clear ontogenetic shift. However, their isotope biplots reveal three different patterns and no single habitat-use type (see orange arrows in Fig. 4A–C). By comparison to Hawaiian hawksbills, together this suggests that the early development phase of Western Pacific hawksbills may have less association with nearshore waters. While such sequential and repeated sampling within individual tissues holds promise, especially as a complement to other sampling techniques, the present analysis represents a small sample and should be expanded.

A second goal of the present analysis was providing new stable isotope information for Central, Western, and South Pacific hawksbills. In this respect, our results add to the growing body of evidence that hawksbill tissue isotopes vary regionally between populations. Figure 1 presents novel data that demonstrate broad $\delta^{15}N$ structure in scutes from Caribbean, Central Pacific, and South Pacific populations.

These $\delta^{15}N$ values are consistent with the isotope values of the regional forage items we report here (Fig. 5), with previously published isotopes of Caribbean hawksbill scutes (Reich et al. 2007; Fireman 2021) and published values of other reef taxa in these regions (CocheretdelaMorinière et al. 2003; Fiore et al. 2013). While this alignment is encouraging for developing applied isoscapes, there remains a substantial need to document site-specific dietary composition and forage characteristics across hawksbill populations. Though recent studies present important additions to this literature (Méndez-Salgado et al. 2020; Clyde-Brockway et al. 2022; Turner Tomaszewicz et al. 2022), most geographic regions are persistently data poor, limiting ecological knowledge and conservation planning. Future studies may therefore expand ecological monitoring efforts to increase data collection on the habitat use and foraging ecology of hawksbills as well as the diagnostic analysis of their forage items. Of note, the regional $\delta^{15}N$ patterns that we describe here (Fig. 1C, D) from hawksbill scutes parallel the differences in seabird trophic position from the same marine regions (see Fig. S1) which were correlated with anthropogenic impacts (Gagné et al. 2018a). As $\delta^{15}N$ patterns of consumers are derived from ^{15}N values at the food web base, future work may investigate whether regional values are fixed in time or whether they are impacted by anthropogenic pressures such as overfishing and climatic change.

To help fill existing data gaps, here we summarized available stable isotope values for common hawksbill forage items in the Central, South, and Western Pacific (Fig. 5). As these forage data are not exhaustive, we are prevented from running a formal mixing model (Lemons et al. 2011; Stock and Semmens 2016; Gagné et al. 2018b; Stock et al. 2018), and cannot infer diets or dietary shifts through development for individuals in the present study. From the data we possess, however, one thing may be clear. The sampled forage items from Hawaii are considered relatively exhaustive (41 samples from 21 species across 5 forage groups, see Table S2), have $\delta^{15}N$ values ranging from 2–5 ‰, yet are somehow lacking the full complement of hawksbill prey species. While the mean $\delta^{15}N$ values across development for Hawaiian hawksbills in Fig. 3 is ≥ 9 ‰, two adult turtles (Fig. 3C, F) have $\delta^{15}N$ values that exceed 15 ‰. Given that published sea turtle tissue $\delta^{15}N$ discrimination values are ≤ 4.0 ‰ (Seminoff et al. 2006; Vander Zanden et al. 2012), these two adults likely consumed forage items not displayed in Fig. 5. A possible explanation is that these individuals recently migrated from another region where they consumed forage more enriched in $\delta^{15}N$. However, this is unlikely given the isolation of the Hawaiian archipelago, and that none of the foraging items for any Pacific regions in Fig. 5 can support such high tissue $\delta^{15}N$ values. Since these turtles both stranded in the 1980s, these turtles may have foraged on high-trophic level species that no longer occur in such abundance, or this may be reflecting that food web compression has occurred in recent decades in Hawaii's reef ecosystems. Another explanation is that these individuals foraged in impaired watersheds with high N footprints (e.g., Van Houtan et al. 2010). This explanation may be unlikely, however, as eutrophication has increased over time, yet elevated $\delta^{15}N$ tissue values were observed in hawksbills decades ago and not recently.

Resolving individual life histories though the longitudinal analysis of hawksbill scutes is promising, but substantial work remains. In this study, we expand on earlier pioneering research that first demonstrated successional layering in hawksbill scutes (Tucker et al. 2001; Palaniappan 2007), and later documented a near-complete chronology in the ultimate PM scutes (Van Houtan et al. 2016a). Using the same tissues and preparations, here we sequentially sampled along scute growth line contours and performed SIA to understand individual life histories and regional population structure. As we have shown, especially when combined with other traditional and diagnostic tools, such methods can reveal previously unknown information with important conservation applications. Moving forward, future progress can be made in several distinct ways.

The novel sclerochronology methods we developed here can be applied universally to reconstruct the long-term habitat use of individual hawksbill turtles in any geographic region. We recommend expanding the approach to increase both the samples and populations analyzed here. This might prioritize data poor regions of the South Atlantic, Indian, West Pacific and South Pacific basins as well as the Eastern Pacific and the Northwest Atlantic. We also recommend refining our techniques with ultimate PM scutes, comparing it with other scute tissues, and further aligning it with growth line and ageing studies (e.g., Van Houtan et al. 2016a). As it was here, partnerships with museums, natural history repositories, law enforcement agencies, and stranding programs may be important to obtain specimens as well as to demonstrate additional applied contexts for such isotopic research (Espinoza et al. 2007). In addition to replicating and refining this work, we recommend supplementing the existing mass spectrometry diagnostics of carbon and nitrogen to additional elements. As Fig. 1E, F demonstrates, 36% of scute tissues are composed of H, O, S, and other trace elements. Although H and O can display low variability between regions, δD, $\delta^{18}O$, and $\delta^{34}S$ have demonstrated use in marine systems (Cardona et al. 2009; Clark and Fritz 2013; Tucker et al. 2014; Duarte et al. 2018; Miller et al. 2019) and may be useful for sea turtle populations. Together, these programs will allow for the development of robust mixing models, advance our understanding of individual life histories, and increase the effectiveness of conservation management for critically endangered hawksbill turtles.

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Author Contributions KV and JAS designed the study and wrote the manuscript. KV, TJ, MH, and GP provided and prepared specimens. JAS and JS performed the diagnostic analyses, while KV analyzed the data and generated the figures. All authors made contributions to and reviewed the manuscript.

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Data Availability Raw data are available in the Supplementary Information. High-resolution figures and data are also available at the third-party Open Science Framework repository: https://osf.io/2fh39/.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethical approval All research followed the NOAA Institutional Animal Care and Use Committee criteria.

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