ABSTRACT

Title of Thesis:	ON THE SHELL OF THE TURTLE: IDENTIFYING THE ISOTOPIC NICHE OF HAWKSBILL SEA TURTLES IN ANTIGUA, WEST INDIES
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The hawksbill sea turtle (*Eretmochelys imbricata*) is a critically endangered keystone species that forages in vulnerable reef habitats. Although research has focused on the hawksbill's reproductive life stage, little is known about the hawksbill's long-term foraging strategies or how their diet influences fecundity. Here, I combined stable isotope analysis of keratin tissue from 50 adult female hawksbills with long-term reproductive metrics from a hawksbill rookery beach in Antigua, West Indies to study this knowledge gap. I observed probable ontogenetic niche shifts in four individuals, and the population had high variability in isotopic composition. Niche width was not a good predictor of fecundity, but reproductive age was significantly related to niche width, with younger turtles showing higher variability. These findings provide evidence of ontogenetic dietary shifts in these hawksbills and emphasizes the necessity of protection of multiple marine habitats to aid in conservation of this critically endangered species.

ON THE SHELL OF THE TURTLE: IDENTIFYING THE ISOTOPIC NICHE OF HAWKSBILL SEA TURTLES IN ANTIGUA, WEST INDIES

by

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Table of Contents

Acknowledgements iii
Table of Contents v
List of Tables vii
List of Figures viii
List of Abbreviations ix
Chapter 1: Introduction 1
  Hawksbill biology 1
  Stable isotope analysis 2
  Applying stable isotopes to sea turtle ecology 4
  Trophic enrichment factor 6
  Conservation implications 7
  Statement of objectives and hypotheses 8
    Objective 1 8
    Objective 2 9
    Objective 3 9
  References 9
Chapter 2: Niche width and reproductive implications in the hawksbill sea turtle 15
  Introduction 15
  Methods 17
    Study site 17
    Scute sample collection 18
    Stable isotope analysis of keratin 19
    Porifera tissue collection 20
    Stable isotope analysis of Porifera 21
    Isotope data processing 21
    Assessing population level variance 22
    Individual niche width and variance calculation 22
    Fecundity and resource use models 23
    Calculating trophic enrichment factor (TEF) 25
    Visualization of repeated sampling 26
  Results 26
    Stable isotope values 26
    Fecundity and resource use models 27
    Trophic enrichment factor (TEF) 28
    Visualization of repeated sampling 29
  Discussion 29
    Ontogenetic niche shifts 30
    Population-level variability in resource use 32
    Linking resource use with reproductive output 36
    Stable isotope applications in sea turtle ecology 40
    Conclusions 43
  References 44
Tables 52

Figures.. 59
Full bibliography... 70

List of Tables

Table 1. List of all model structures for fecundity metric analysis. Listed predictors are standard ellipse area (SEA), within individual component of variance for δ^{13}C and δ^{15}N (CWIC and NWIC), reproductive age, body size (CCL), and the most recent isotopic integration of δ^{13}C and δ^{15}N (Last δ^{13}C and Last δ^{15}N). Boostrapped models are indicated with a "B".

Table 2. List of all individual hawksbill sea turtles assessed for keratin isotope composition and characteristics. Columns include: individual turtle ID, the year an individual was first tagged ($Year_{Tag}$), the year an individual was sampled for keratin ($Year_{S}$), reproductive age (Age_{R}), within individual component of variance for δ^{13}C and δ^{15}N (CWIC and NWIC), standard ellipse area (SEA), Mean δ^{13}C‰ and δ^{15}N‰ ± standard deviations, and whether an ontogenetic niche shift was apparent in the δ^{15}N record (indicated with "Yes").

Table 3. List of all bootstrapped model outputs for fecundity metric analysis. Listed predictors are standard ellipse area (SEA), within individual component of variance for δ^{13}C and δ^{15}N (CWIC and NWIC), and the most recent isotopic integration of δ^{13}C and δ^{15}N (Last δ^{13}C and Last δ^{15}N). Sample size (n) and 95% confidence intervals (CI) for models are listed.

Table 4. Normal linear regressions are listed, with p-value (*P*), slope parameter estimate, and standard error for each model. Significant results are indicated in bold and with an asterisk. Listed predictors are standard ellipse area (SEA), within individual component of variance for δ^{13}C and δ^{15}N (CWIC and NWIC), and the most recent isotopic integration of δ^{13}C and δ^{15}N (Last δ^{13}C and Last δ^{15}N).

Table 5. Minimum, maximum, mean, and standard deviation of δ^{13}C and δ^{15}N values from Porifera tissue from two locations.

Table 6. Mean δ^{15}N values ($\delta^{15}N_{SC}$), the proportion of the diet derived from dive site 1 (α), and change in δ^{15}N between Porifera and hawksbill sea turtles (Δ_n or TEF).

List of Figures

Figure 1. Long Island, Antigua in the West Indies. Pasture Beach is the primary nesting site for hawksbill sea turtles in Antigua.

Figure 2. Sampling locations for keratin tissue and examples of 100μm sections and their relative age.

Figure 3. Locations for repetitive sampling of keratin tissue. Open circle represents the first sampling locations and closed circles are secondary sampling two years later.

Figure 4. Antigua, West Indies and the two sampling locations where Porifera tissue was collected.

Figure 5. δ^{13}C and δ^{15}N values across scute tissue in individual hawksbills. Each individual is noted with a unique color.

Figure 6. Standard ellipses around individual females on a biplot of δ^{13}C and δ^{15}N values. Unique colors represent individuals.

Figure 7. Linear regression plot of last δ^{13}C and number of nests with regression line in red and confidence intervals as dashed lines.

Figure 8. Plots of individual turtles who were sampled in 2017 (black) and 2019 (red).

Figure 9. Images of sectioned keratin from the same individual in two years. A) a section of keratin from WH5766 in 2017, B) a section of keratin from an adjacent site WH5766 in 2019. Some clearly visible laminae were measured for thickness (listed in red).

Figure 10. Image of keratin regrowth in 2019 of a scute originally sampled in 2017 (circled in white). The original hole was filled in completely with new keratin, the epoxy filler was absent, but a small ring around the sample site is still visible.

Figure 11. Plot of standard ellipse area and reproductive age with regression line in red and confidence intervals as dashed lines.

List of Abbreviations

TEF	Trophic enrichment factor
JBHP	Jumby Bay Hawksbill Project
CCL	Curved carapace length
DI	Deionized water
UMCES	University of Maryland Center for Environmental Science
AL	Appalachian Laboratory
CBL	Chesapeake Biological Laboratory
MANOVA	Multivariate analysis of variance
WIC	Within individual component of variance
CWIC	Within individual component of δ^{13}C variance
NWIC	Within individual component of δ^{15}N variance
SEA	Standard ellipse area
ANOVA	Analysis of variance

Chapter 1: Introduction

Hawksbill biology

The hawksbill sea turtle (*Eretmochelys imbricata*) is a critically endangered, keystone species inhabiting coral reefs circumglobally in tropical and subtropical waters (Mortimer & Donnelly, 2008). Despite their importance within coastal ecosystems and their critically endangered status, hawksbills are understudied relative to other species of marine turtles. Similar to other marine turtles, hawksbills spend most of their adult lives in neritic and oceanic habitats (Mortimer & Donnelly, 2008), but the vast majority of research has focused on nesting beaches. Although basic life history and biological information (e.g., habitat ranges, primary diet, physiology) have been described (Hart et al., 2019; Meylan, 1988; Mortimer & Donnelly, 2008; Wyneken et al., 2013), much remains unknown about resource use and adaptability to changing environments.

Hawksbills play a unique role relative to other species of marine turtle in their ecosystems by primarily consuming a variety of Porifera (sponge) species (León & Bjorndal, 2002; Meylan, 1988). Prey composition differs among regional populations: hawksbill lavage samples from Tortuguero in Costa Rica predominantly contained *Choristida* species (Carr & Stancyk, 1975); a population in Turks and Caicos predominantly fed on *Chondrilla caribensis f. caribensis* (Stringell et al., 2016) and Caribbean studies identified *Chondrilla nucula* as the predominant prey species (León & Bjorndal, 2002; Meylan, 1988). Hawksbills appear to be primarily indiscriminate

feeders (Carr & Stancyk, 1975), however there is some evidence that selective feeding may occur (León & Bjorndal, 2002; Stringell et al., 2016). Although Caribbean hawksbills' primary diet is composed of Porifera species, elsewhere algal species can be an important component of hawksbill diets (e.g., the far northern section of the Great Barrier Reef Marine Park; Bell, 2013). By consuming sponges and macroalgae and clearing space for coral growth and recruitment, hawksbills help to maintain healthy and diverse reef structures (León & Bjorndal, 2002).

Stable isotope analysis

One of the most direct connections between an organism and its ecosystem is through the assimilation of nutrients by the organism (i.e., the diet). Understanding an organism's diet and foraging behaviors can provide information on the ecosystem, such as nutrient availability (Gannes et al., 1997) and ecosystem health (McClelland et al., 1997), as well as the trophic niche of the organism (Post, 2002). Long-term diet observations at the level of individual organisms and populations can provide information on dietary shifts in response to environmental or biological (e.g., life history) changes. Information on diet composition in conjunction with reproductive data can provide insight into which trophic resources are needed to support population growth (Bjorndal, 1985). This knowledge can be used to help inform decisions regarding conservation of vulnerable species and habitats.

Stable isotope analysis has been widely used to make inferences about the trophic ecology of consumers, including ontogenetic niche shifts, diet composition, and foraging location (Layman et al., 2012). Carbon stable isotopes are used to infer

the dietary importance of primary producers or the location of foraging, whereas nitrogen stable isotopes can serve as a proxy for the trophic level of the organism and anthropogenic nutrient inputs (Hobson, 1999). Evaluating the stable isotope composition of an organism can provide insight into their foraging patterns and resource use. Analyzing continuously accreting tissues that both record isotopic conditions and are preserved on the organism provides a unique opportunity to observe changes in resource use of an organism over time.

Isotope values are commonly expressed as a ratio of heavy to light isotopes, such as ^{13}C:^{12}C and ^{15}N:^{14}N. This 'delta notation' is derived using the below equation (Fry 2006):

$$\delta X = [(R_{sample}/R_{standard}) -1] * 1000$$

where R_{sample} is the ratio of heavy to light stable isotopes (i.e. ^{13}C:^{12}C or ^{15}N:^{14}N), and $R_{standard}$ is the international standards for each element. Therefore, a positive δ value indicates a sample has more of the heavy relative to the light stable isotope than the standard, and a negative value indicates the opposite. Animal tissues are typically more enriched in ^{15}N compared to their prey sources (DeNiro & Epstein, 1981). As consumers undergo protein deamination and transamination during metabolic processes, the lighter ^{14}N is preferentially removed from the amino acids and excreted as waste, leaving ^{15}N enriched amino acids for consumer protein synthesis (Gannes et al., 1998). Therefore, if prey stable isotope values are known, the trophic level of an organism can be determined based on the level of enrichment in their tissue. Organisms with a known trophic position can serve as a baseline upon which to compare another organism. Nitrogen enrichment and trophic position of an organism

are often calculated using the below equation with a standard (mean) enrichment factor of 3.4‰ (Minagawa & Wada, 1984; Vander Zanden & Rasmussen, 2001):

$$\text{trophic position}_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4 + \lambda$$

where λ is the trophic position of the baseline. Conversely, carbon stable isotopes of organisms are typically closer in value to those of their prey sources, as there is little to no fractionation from one trophic level to the next (DeNiro & Epstein, 1978). However, the ratio of ^{13}C:^{12}C can provide information regarding the primary producers in an ecosystem, as varying primary producers will differentially uptake carbon based on the source of available carbon to the plant (Fry, 2006). Since there is little enrichment between predatory and prey, carbon stable isotopes can be an indicator of the source of nutrients in a food web, or primary producers.

Applying stable isotopes to sea turtle ecology

Measuring an animal's stable isotope composition over long periods of time requires repeated capture and collection of metabolically active tissues (e.g., muscle, blood, skin) or collection of metabolically inert tissue that accretes over time. Examples of inert accreting tissue include keratinous structures such as nail, feather, hair, claw, or scute tissue (Rubenstein & Hobson, 2004). Sampling scute tissue on hawksbills provides a more accessible approach to studying their diet in comparison to more challenging methods such as observation, lavage, stomach and feces content analysis, or repeated sampling of metabolically active tissues.

Stable isotope analysis of sea turtle tissue has recently proliferated, however certain species and regional management units are under-represented. Loggerhead (*Caretta caretta*), leatherback (*Dermochelys coriacea*), and green sea turtles (*Chelonia mydas*) dominate the isotopic literature, while other species are relatively understudied. Given that hawksbills fulfill a unique ecological niche that is dissimilar to other species of marine turtle, understanding their resource requirements is critical. In a recent review of sea turtle isotope studies, Pearson et al. (2017) noted the lack of hawksbill analysis and highlighted the importance of using this method to better understand these critically endangered populations. One-time observations of $\delta^{13}C$ and $\delta^{15}N$ values have been completed for hawksbills using skin tissue to make predictions about their diet (Bjorndal & Bolten, 2010; Ferreira et al., 2018). Enriched $\delta^{15}N$ values have confirmed that hawksbills, even when living in close proximity to green sea turtles, forage at a different trophic level than the herbivorous green sea turtles (Bjorndal & Bolten, 2010). Bomb radiocarbon incorporation of Hawaiian hawksbill keratin indicated a significantly different age-at-maturity from the Caribbean hawksbill (Van Houtan et al., 2016), making the application of findings across distinct regional populations problematic.

Past stable isotope studies have used keratin tissue from loggerhead and green sea turtles. Vander Zanden et al. (2010) used loggerhead keratin to evaluate both individual and population level variation to better understand resource use at multiple scales. Green sea turtle keratin informed our understanding of an ontogenetic shift from an early-life oceanic habitat and carnivorous diet to a neritic habitat and herbivorous diet (Reich et al., 2007). These methods for evaluating keratin can be

applied to understudied hawksbills to better understand their resource use over time as well as changes in their threatened reef ecosystems.

Trophic enrichment factor

Stable isotope values from consumer tissue alone can supply information about an individual's relative trophic position over time or in comparison to the population. However, to make inferences regarding absolute position within an ecosystem, the enrichment in ^{15}N from one trophic level to the next, or the trophic enrichment factor (TEF) is necessary. TEFs are derived from the below equation (Fry, 2006):

$$\Delta X = \delta X_{tissue} - \delta X_{diet}$$

where ΔX is the change in $\delta^{15}N$ value (of TEF), δX_{tissue} is the $\delta^{15}N$ value of the consumer tissue and δX_{diet} is the $\delta^{15}N$ value of the prey tissue. TEFs have been determined for Atlantic/Caribbean green sea turtles (Vander Zanden et al., 2013) and Atlantic loggerheads (Reich et al., 2008) using the difference between values from prey sources and marine turtle keratin tissue. At present, a trophic enrichment factor has not yet been determined for hawksbill sea turtles.

After four years of successfully using satellite telemetry to track hawksbill movement patterns to foraging grounds, an area off the western coast of Antigua has been identified that serves as a foraging area for at least three hawksbills that nest on Long Island, Antigua (Maurer & Stapleton, *unpublished data*). Sampling potential prey sources from an identified foraging ground would allow for determination of a preliminary TEF for these hawksbills.

Conservation implications

Identifying female sea turtles on their nesting beaches is a common method of studying sea turtle reproduction and life history. On Long Island, Antigua, West Indies, the Jumby Bay Hawksbill Project (JBHP) has monitored a nesting hawksbill population since 1987 with saturation-tagging protocols, facilitating the identification of nearly every individual that has nested there during the last 32 years. Every year from June 1st to November 16th, field staff conduct hourly patrols of the main nesting beach from sunset to sunrise. All encountered turtles are tagged for identification and recaptured upon future nesting events. Nest metrics are also recorded post-hatch for at least 100 nests each year. While the nesting beach is only approximately 650m long, the JBHP has identified hundreds of individuals nesting over the 33 years of monitoring. Most hawksbill nesting sites in Antigua have seen dwindling numbers of nesting hawksbills. The Jumby Bay site on the smaller, privately-owned Long Island has been protected from threats such as poaching and invasive mammalian predators (Richardson et al., 1999), but nesting numbers appear to be on the decline recently after a sustained period of increase and stability. In recent years, the JBHP has deployed 21 platform terminal transmitters to track in-water hawksbill movements in the inter-nesting intervals and to identify regional foraging grounds of individuals. Preliminary data from these tags indicate that these turtles forage throughout the Caribbean (Maurer & Stapleton, *unpublished data*). The JBHP's high-resolution dataset provides a unique foundation for relating individual hawksbill age, growth, and fecundity to questions regarding in-water behavior. Assessing linkages between fecundity and foraging strategies can inform

conservation management decisions about habitat in both home ranges and nesting grounds.

Statement of objectives and hypotheses

There is an identified knowledge gap in how hawksbills use trophic resources through ontogeny and during their inter-nesting interval, how their resource use affects their reproduction, and how hawksbills are affected by environmental perturbations in their foraging grounds. Understanding resource use strategies will allow us to better understand the availability of resources within coral reef ecosystems. In this study, I completed one of the first trophic analyses of the hawksbill sea turtle using stable isotope composition of keratin tissue. I evaluated hawksbill trophic ecology and two hypotheses: (1) Hawksbills, at the individual and population levels, are displaying one of two resource use strategies: generalist or specialist. (2) Individual turtle foraging history is a good predictor of reproductive output. I identified three primary objectives to help me address these questions:

Objective 1

Assign individual hawksbills and the overall population a defined foraging strategy based on stable isotope chronologies. Sampling transects across the layered sequential growth structures in carapace keratin allows for assessment of patterns in δ^{13}C and δ^{15}N. The level of variation in diet of hawksbills over time provides information on both individual and population level foraging strategies, illuminating the trophic niche that hawksbills occupy in their reef ecosystems.

Objective 2

Use statistical models to evaluate relationships between foraging strategy and individual fecundity. The comprehensive data set from the JBHP will be used to examine individual foraging strategy as a predictor of reproductive success. Response variables will include remigration interval, number of nests, clutch size, and hatch success.

Objective 3

Determine a carapace keratin TEF and estimate keratin growth rate for the hawksbill sea turtle using δ^{13}C and δ^{15}N analysis of hawksbill and prey tissue (Porifera) from a known foraging area and repetitive sampling of keratin tissue across multiple years.

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Chapter 2: Niche width and reproductive implications in the hawksbill sea turtle

Introduction

The Critically Endangered hawksbill sea turtle is a keystone coral reef species (Mortimer & Donnelly, 2008). Hawksbills contribute to biodiversity in coral reef ecosystems by primarily foraging on sponges (Porifera), a common coral competitor (León & Bjorndal, 2002). Whereas studies of hawksbill diet have broadly indicated a spongivorous diet (Meylan, 1988), little is known about long-term hawksbill resource use, or how different resource use strategies may contribute to population dynamics.

Past studies of hawksbill diet have relied upon intensive and invasive techniques such as gastric lavage (León & Bjorndal, 2002; Meylan, 1988), post-mortem stomach content analysis (Stringell et al., 2015), or visual observation of foraging (Fernandes et al., 2017; Stampar et al., 2007). Minimally invasive techniques such as stable isotope analysis of body tissue provide an alternative to direct diet measurements and have been used as a method to understand resource use in a wide range of organisms (Layman et al., 2012). Tissue carbon stable isotope ratios ($\delta^{13}C$) can provide spatial information, such as insight into the geographic location of foraging, or serve as a tracer of trophic resources by highlighting the dietary importance of different primary producers (i.e., horizontal trophic niche) (Thomas & Cahoon, 1993). Tissue nitrogen stable isotope ratios ($\delta^{15}N$) often become enriched in consumers relative to their prey. Nitrogen stable isotopes therefore are useful in determining the trophic position of a consumer (i.e., vertical trophic niche) and also

can provide insight into nutrient inputs in an environment (Hobson, 1999). When $\delta^{13}C$ and $\delta^{15}N$ values are assessed together, they can be evaluated as a proxy for trophic niche width (Bearhop et al., 2004; Jackson et al., 2011).

Stable isotope analysis of accreting, diet-derived tissues such as hair, claw, feather, or scutes (Rubenstein & Hobson, 2004) provide an opportunity to assess stable isotope values over long periods of time. Hard tissues such as keratin contain a long-term record of assimilated diet that aren't readily available in metabolically active tissues such as blood or skin. The hawksbill's distinct tortoiseshell is entirely made of continuously accreting hard keratin tissue. Transect sampling of sequential keratin layers in other sea turtle species such as loggerheads (Vander Zanden et al., 2010, 2013) and green sea turtles (Reich et al., 2008) has provided insight into resource use strategies at both the individual and population level. A review of sea turtle stable isotope literature recently notes that hawksbills are underrepresented in the stable isotope literature (Pearson et al., 2017). Given their global status as Critically Endangered, their ecological importance in coral reef ecosystems, and the vulnerability of reefs to coastal development and changing climates (Hughes et al., 2003), understanding hawksbill resource use is critical for developing best management practices for the conservation of both hawksbills and their habitats.

The relationship between resource use and fecundity has been explored in taxa such as birds (Golet et al., 2000; Resano-Mayor et al., 2014), fish (Holbrook & Schmitt, 1992), arthropods (Griffen, 2014), and marine turtles (Hatase et al., 2013). Using similar techniques to examine these linkages in hawksbills could indicate

whether observed (actual) isotope values or derived isotopic niche metrics effectively capture modalities in resource use and their potential effect on fecundity.

A well-studied hawksbill rookery on Long Island, Antigua, West Indies (also known as Jumby Bay) provides a unique setting for studying hawksbill diet and fecundity. Since 1987, the Jumby Bay Hawksbill Project (JBHP) has implemented saturation tagging protocols on Long Island to study the life history of this long-lived species. High nesting site fidelity through natal homing allows for consistent recapture of nesting females and identification of all newcomers to the Jumby Bay nesting site (Levasseur et al., 2019; Richardson et al., 1999). This high-resolution fecundity data on individual turtles provides an ideal foundation to study the link between diet and reproduction.

My research investigates the use of stable isotope biomarkers to identify resource use patterns in the life history of the hawksbill sea turtle. Broadly, I used stable isotope analysis of nesting hawksbill sea turtles paired with long-term reproductive data to evaluate trophic niche, foraging strategy, and effects on fecundity. More specifically, I assessed keratin isotopic records for distinct niche shifts, analyzed among and within individual variability, and tested isotopic-based metrics as predictors of four fecundity metrics.

Methods

Study site

Long Island is a 120-ha barrier island located to the northeast of Antigua. Multiple man-made beaches on the northern and northwestern sides of the island are

known hawksbill nesting sites, with the natural Pasture Beach (~650 m) as the primary nesting site (17.158567°N, 61.755545°W) (**Figure 1**). The primary hawksbill nesting season in Antigua is June through November (Richardson et al., 1999).

Scute sample collection

Field staff patrolled Pasture Beach from June 15 – November 16th 1987-2006 and June 1st to November 16th, 2007–2019 following an apparent shift in the nesting season. The field staff used foot patrols of the nesting beach every hour from one hour post-sunset (19:00–20:00) to the appearance of first light (5:00–6:00) to identify nesting activity. The JBHP implements saturation tagging protocols: outfitting all hawksbills successfully ovipositing with flipper tags on the trailing edge of the foreflippers and secondarily marking individuals with a unique pattern in the supracaudal scutes using a battery-powered drill. These methods facilitate identification during future nesting events. Biometric data included curved carapace length (CCL). I used the CCL measurement procured closest to or on the date of keratin sampling in this analysis. Staff also conducted post-hatch excavations of > 100 nests each year starting in 2003 to evaluate clutch size and hatching success. From 2017-2019, I also sampled individuals for keratin tissue. I opportunistically sampled keratin from all individuals where possible. During oviposition, I collected scute tissue from the right second costal scute (**Figure 2**) in two locations (anterior and posterior) to collect the maximum range of keratin growth. I collected the keratin using a battery powered hand drill and a ¼" plug cutter attachment. The plug cutter was used to cut a piece of keratin until the section could be removed with forceps. I

filled in the resulting holes in the scute with epoxy. If individuals returned to the nesting beach having been sampled in a previous year, I sampled them a second time at an adjacent site to the previous plugs (**Figure 3**). All samples were stored in non-iodized NaCl at room temperature for transport to the Chesapeake Biological Laboratory.

Stable isotope analysis of keratin

For this analysis, I selected 50 individual hawksbills to account for the full range of ages of individuals. In the laboratory, I soaked scute samples in deionized water (DI) for a minimum of 24 hours, then triple-rinsed the samples in DI to remove potential contaminants from the surface. I conducted lipid extraction via soaks in 2:1 chloroform:methanol solution, a modified version of the Bligh and Dyer method (Bligh & Dyer, 1959; Wedemeyer-Strombel, 2019). I soaked keratin in the chloroform:methanol solution for 24 hours, then triple-rinsed the samples with DI water. I repeated this process three times, then dried the samples at 60°C in a drying oven for ≥ 48 hours. I temporarily removed 0.2-mm sections from 20 samples using a Buehler IsoMet™ Low Speed cutting machine. I took digital images of these sections using an Olympus SZX16 stereo-microscope with a 17 megapixel Olympus DP73 color camera. Sections were then placed back with the original sample, and all pieces were affixed to glass slides using Crystalbond™ 509 mounting adhesive.

I determined that 100 µm of ground keratin tissue was the minimum amount necessary per layer for stable isotope analysis. I ground scutes at 100-µm intervals using a micromill equipped with a carbide steel dental drill bit. One individual turtle's

scute (WS1189) was ground initially at 100-μm intervals until a depth of 2300 μm, then 200-μm intervals from 2300 to 3100 μm to account for uneven and chipped surfaces. All keratin plugs were ground until 200–300 μm of tissue remained on the glass slide so as to not drill into the mounting adhesive underneath the sample. I weighed samples into tin foil capsules and analyzed them for $\delta^{13}C$ and $\delta^{15}N$ on either a Carlo Erba NC2500 elemental analyzer interfaced with a Thermo Delta V+ isotope ratio mass spectrometer at the University of Maryland Center for Environmental Science (UMCES) Appalachian Laboratory (AL) or a Thermo Scientific Delta V Plus continuous flow isotope ratio mass spectrometer coupled with a Costech ECS 4010 elemental analyzer at the UMCES Chesapeake Biological Laboratory (CBL). In both labs, Vienna Pee Dee Belemnite and atmospheric N_2 standards were used for $\delta^{13}C$ and $\delta^{15}N$, respectively. Delta notation is derived using the below equation (Fry, 2006):

$$\delta X = [(R_{sample}/R_{standard}) -1] * 1000$$

where R_{sample} is the ratio of heavy to light stable isotopes (i.e. ^{13}C:^{12}C or ^{15}N:^{14}N) in the sample material, and $R_{standard}$ is ratio of heavy to light stable isotopes of the same element measured in the international standard.

Porifera tissue collection

I collected sponge samples on August 12th, 2019. Based on known hawksbill foraging ground information (Maurer & Stapleton, *unpublished data*), two dive sites were selected off the western coast of Antigua (17.018°N, 61.93°W [depth ~ 15 m]

and 17.069°N, 61.927°W [depth ~ 8.5 m]; **Figure 4**). At each site, I collected a sample of approximately 3 cm^3 of sponge tissue from a total of 26 individuals (12 at the first site, and 14 at the second) using a diver's knife. I attempted to sample a representative range of sponge taxa present at each site. I stored sponges in non-iodized NaCl at room temperature for transport to the CBL.

Stable isotope analysis of Porifera

Sponge samples were soaked in DI for a minimum of 24 hours, triple-rinsed in DI, then dried in a 60°C drying oven for ≥ 48 hours. I halved the samples, and acidified one half via direct application of 1N HCl to remove carbonates which have isotopically heavier δ^{13}C values than organic tissue (DeNiro & Epstein, 1978). I triple rinsed the acidified samples with DI then dried them in a 60°C drying oven for at least 48 hours. I homogenized all samples using a mortar and pestle then weighed them into tin foil capsules. Acidified tissue was analyzed for δ^{13}C and un-acidified tissue was analyzed for δ^{15}N.

Isotope data processing

I calculated an offset from n = 10 replicate keratin samples analyzed at CBL and AL to account for variation between the two mass spectrometers used. The offsets (-0.55‰ for δ^{13}C and -3.36‰ for δ^{15}N) were calculated by averaging the differences between the same samples analyzed at the two laboratories and subsequently applied to the samples analyzed at CBL to allow for comparison of

tissue assessed at both labs. If individuals contained a difference between subsequent δ^{15}N values of $> 5‰$, the I excluded the less enriched values from statistical analyses to avoid incorporating information from earlier life stages.

Assessing population level variance

To assess within population-level differences, I used multivariate analysis of variance (MANOVA) with individual treated as the predictor variable and δ^{13}C and δ^{15}N considered response variables. I used the non-parametric function "Adonis" to account for non-Gaussian data (Anderson, 2001) and included the keratin layer as a blocking variable to account for individual variation (Jari Oksanen et al., 2020). I also performed univariate ANOVA tests on δ^{13}C and δ^{15}N values separately. All analyses were conducted in the statistical program R (R Core Team, 2020).

Individual niche width and variance calculation

To assess individual variance, I used standard variance (s^2) for each individual and standard variance of the population for both δ^{13}C and δ^{15}N to calculate the within individual component of variance (WIC) values for carbon (CWIC) and nitrogen (NWIC) (Matich et al., 2011) using:

$$WIC = s^2_{individual} / s^2_{population}$$

To assess individual- and population-level niche width across δ^{13}C and δ^{15}N values, I used the SIBER package to calculate standard bivariate ellipses (encompassing 95% of the data) on a biplot of δ^{13}C and δ^{15}N for each individual

(Jackson et al., 2011). Standard ellipse area (SEA) in C-N isotope space is used as a proxy for individual trophic niche width. Both the SEA and WIC account for differing types of individual variation: SEA encompasses both δ^{13}C and δ^{15}N, whereas WIC incorporates the niche width in the context of the population.

Fecundity and resource use models

I compiled nesting data from 1987–2019 and post-hatch data between 2003–2019 to calculate four individual-based fecundity metrics: remigration interval (the number of years elapsing between successive nesting seasons); number of nests (the number of nests laid in each nesting season); clutch size (the number of eggs in each clutch; and hatching success (the percentage of eggs that successfully hatched). Number of nest calculations for individuals who nested in the first or last month of the monitored nesting season were excluded due to the possibility of missed nests (i.e., nesting that occurred outside the monitoring period). Neophyte (first time nesting) turtles were excluded from remigration interval analysis given they had no interval between multiple nesting years. I also calculated the reproductive age of each individual as the year the turtle was sampled for keratin (2017–2019) minus the year the turtle was first tagged.

I used 26 individual linear regression models to test three groups of models. In the first, I tested the three niche width proxies (SEA, CWIC, and NWIC) as a predictor of the four fecundity metrics. In the second, I independently tested reproductive age and body size as predictors of the three niche width proxies. Lastly, in order to focus on the most recent nesting period, I used the δ^{13}C and δ^{15}N values of

the most recently formed keratin layer as predictors of the four fecundity metrics from the same year that the turtle was sampled for keratin. Box-cox transformations were used to account for non-normal residuals (tested via Shapiro Wilk test) (Box & Cox, 1964; Venables & Ripley, 2002). This method selects the power transformation that transforms the data most closely to normal distribution. In determining model significance, I set $\alpha = 0.05$.

All model structures are listed in **Table 1.** Given that each individual had data spanning multiple years and multiple nests, but nest and year counts are not constant for each turtle (i.e. older individuals often had more observed remigration intervals, turtles that nested in the middle of the season had higher observations for number of nests, and individuals that nested earlier in the season had higher number of observations for clutch size and hatching success), I bootstrapped each of the fecundity metrics to account for inconsistencies in the number of data points per individual. I replicated each fecundity metric 500 times, and evaluated 95% confidence intervals around each individual model for statistical significance. I identified several statistical outliers for SEA, CWIC, NWIC, and the last δ^{13}C and δ^{15}N. Outliers were defined based on Quartiles (Q) as below Q_1-1.5*(Q_3-Q_1) or above Q_3-1.5*(Q_3-Q_1). Further analysis showed no difference in statistical significance between models with and without outliers. Therefore, model results that include all data are presented below.

Calculating trophic enrichment factor (TEF)

Three individual turtles were identified as foraging in western Antiguan reefs between nesting seasons (Maurer & Stapleton, *unpublished data*). I averaged all within-turtle isotope values for these individuals to derive a single, mean $\delta^{13}C$ and $\delta^{15}N$ value for each turtle. I used a student's t-test to compare $\delta^{13}C$ and $\delta^{15}N$ values of sponges collected from each dive site ($\delta^{13}C$: t-test, $df = 23.83$, $t = -4.05$, $P = 0.0005$; $\delta^{15}N$: t-test, $df = 21.50$, $F = 0.59$, $P = 0.56$). Given the significant difference in sponge $\delta^{13}C$ values between dive sites, I treated each site as a separate resource pool. I averaged sponge $\delta^{13}C$ and $\delta^{15}N$ values for each dive site to get a representative $\delta^{13}C$ and $\delta^{15}N$ value for that site. These turtle and sponge data were treated as a pilot dataset from which to calculate enrichment in ^{15}N from prey to hawksbills. I calculated a TEF using the below equation for each individual turtle (modified from Post, 2002):

$$\Delta_n = (\delta^{15}N_{SC} - [\delta^{15}N_{base1} * \alpha + \delta^{15}N_{base2} * (1 - \alpha)])$$

where $\delta^{15}N_{SC}$ is the average $\delta^{15}N$ value of each Antiguan hawksbill, $\delta^{15}N_{base1}$ is the mean $\delta^{15}N$ value of Porifera from dive site 1, $\delta^{15}N_{base2}$ is the mean $\delta^{15}N$ value of Porifera from dive site 2, and α is the proportion of the diet derived from dive site 1 and $\alpha - 1$ is the proportion of the diet derived from dive site 2. The α variable is calculated using the two-endmember mixing model equation below:

$$\alpha = (\delta^{13}C_{SC} - \delta^{13}C_{base2})/(\delta^{13}C_{base1} - \delta^{13}C_{base2})$$

where $\delta^{13}C_{SC}$ is the average $\delta^{13}C$ value of each Antiguan hawksbill, $\delta^{13}C_{base2}$ is the $\delta^{13}C$ value of Porifera from dive site 1, and $\delta^{13}C_{base1}$ is the $\delta^{13}C$ value of Porifera from dive site 2.

Visualization of repeated sampling

In order to understand adult hawksbill keratin growth rates between nesting years, I used two methods to evaluate typical keratin growth rates in adults: visual assessment of optical properties of keratin sections, and sequential stable isotope analysis. I visually evaluated section images of keratin from individuals sampled in both 2017 and 2019 for corresponding patterns in coloration and growth lines. Isotopic values from individuals sampled in both 2017 and 2019 were plotted adjacently. I then visually assessed plots for evidence of shared or lagged patterns.

Results

Stable isotope values

I analyzed samples from a total of 50 individual female hawksbills nesting on Long Island, Antigua for keratin isotope composition (**Figure 5, Table 2**). These individuals ranged from 80.5 cm to 98.6 cm CCL, with reproductive age ranging from 0 years to 30 years. The number of samples per individual ranged from 6 to 33, and

averaged 16.52 ± 6.8 (SD). Four individuals contained a difference of greater than 5‰ in δ^{15}N values between sequential scute layers. Of these four, each individual's oldest keratin layer had a δ^{15}N value ≤ 3.1‰ and a newest layer ≥ 7.5‰. These individuals ranged in reproductive age from 0 to 21 yrs. Across the population, patterns in δ^{13}C (s^2 = 2.9) were less pronounced than δ^{15}N (s^2 = 4.5). However, the average standard variances for each individual's CWIC and NWIC indicated that δ^{13}C (mean s^2 = 0.9± 1.3) had similar variability within individuals than δ^{15}N (mean s^2 = 0.7 ± 1.2).

Population-level analysis of δ^{13}C and δ^{15}N values of keratin demonstrated significant differences among individuals (MANOVA, *df* = 52, *F*= 61.35, *P* = 0.0001). ANOVA tests indicated significant variance in both δ^{13}C and δ^{15}N individually, with among-individual variance (δ^{13}C sum of squares = 1813.3, δ^{15}N sum of squares = 2001.3) accounting for more variance than within-individual variance (δ^{13}C sum of squares = 38.5, δ^{15}N sum of squares = 35.2). SEAs calculated for all individuals ranged from 0.06‰^{2} to 18.61‰^{2}, with a high level of overlap in ellipses in C-N for all individuals (**Figure 6**). CWIC ranged from 0.006 to 2.075 and NWIC ranged from 0.002 to 1.38.

Fecundity and resource use models

After Box-Cox transformation was performed (λ= 0.18), SEA and NWIC were linearly related to reproductive age (SEA regression: estimate = -0.014, R^2 = 0.16, *df* = 48, *P* = 0.003; NWIC regression: estimate = -0.008, R^2 = 0.14, *df* = 48, *P* = 0.008). Body size (CCL) was not linearly related to SEA (SEA regression: estimate =

0.008, R^2 = 0.01, df = 48, P = 0.42), CWIC (CWIC regression: estimate = 0, R^2 <0.001, df = 48, P = 0.84), or NWIC (NWIC regression: estimate = -0.005R^2 = 0.01, df = 48, P = 0.45).

Regression analysis indicated that no fecundity metrics tested had significant relationships with SEA, CWIC, or NWIC (**Table 3** and **4**). Among the models tested using the δ^{13}C and δ^{15}N values from the most recently formed keratin, only one model was significant given an alpha value of 0.05. I found that the last δ^{13}C value is a significant predictor of the number of nests laid (linear regression, n = 34, F= 4.37, P = 0.045, R^2 = 0.12), with depleted δ^{13}C values associated with fewer nests in the year sampled for keratin (**Figure 7)**. However, the p-value (0.045) was very close to my selected alpha value (0.05), indicating a potentially weak relationship between the two variables.

Trophic enrichment factor (TEF)

Sponge tissue stable isotope composition averaged -20.3 ± 2.1‰ for δ^{13}C and 5.6 ± 1.4‰ for δ^{15}N at site 1, and -16.6 ± 2.6‰ for δ^{13}C and 5.2 ± 2.3‰ for δ^{15}N at site 2 (**Table 5**). Among those three individual hawksbills identified as Antiguan foragers, WH10133 showed a niche shift in her δ^{15}N record; therefore, δ^{15}N average values were assessed excluding the less enriched trophic phase. Calculated trophic enrichment factor values for individuals range from 2.87 to 4.15 (**Table 6**).

Visualization of repeated sampling

All three individuals who were sampled in two different years displayed different δ^{15}N values between years, with the three 2019 patterns all displaying more enriched δ^{15}N values (**Figure 8**). The high variation between years made analysis of vertical keratin growth rate difficult to interpret. Despite evidence of vertical layering in the keratin, there was no obvious pattern in isotope values or optical layer structure (i.e., width, color) at similar or lagged increment intervals that would allow alignment of keratin from different sampling years **(Figure 9)**. Outwardly, two out of the three individuals showed complete regrowth of the scute tissue at the sample site (**Figure 10**). The epoxy filler was absent, and the only indication of previous sampling was a smooth, lighter colored circle at the sample site. The third individual suffered damage to the shell, making assessment of the previous sample collection site difficult. Visual assessment of sectioned images did not produce significant observations of patterns of similarity in coloration or growth lines.

Discussion

The goal of my study was to evaluate long-term patterns in stable isotope composition of Caribbean hawksbill sea turtle keratin to better understand the foraging ecology of this species. I assessed 50 individual female turtles for a full keratin stable isotope record (including three individuals with repeated sampling). I observed trophic niche shifts in the δ^{15}N record for multiple individuals, and overall,

the population appears to display a generalist pattern of resource use based on high variance of isotope composition. Niche width, evaluated as SEA, was negatively related to reproductive age. Overall, I documented few strong patterns in stable isotope composition and reproductive output. Repeated hawksbill sampling and assessment of Porifera tissue provide a foundation for further analysis to aid in assessment of keratin growth rate and stable isotope-tissue fractionation for the hawksbill sea turtle.

Ontogenetic niche shifts

Four of the 50 individual hawksbills assessed in this study demonstrated a distinct resource use shift from less enriched δ^{15}N values to more enriched values. These shifts all indicated one-way trajectories of δ^{15}N values from less to more enriched. These isotopic changes support previous evidence of an ontogenetic shift from a juvenile diet dominated by primary producers (represented by less enriched δ^{15}N) (Meylan, 1988) to a sponge-dominated diet (represented by more enriched δ^{15}N) associated with the transition to adulthood (Carr & Stancyk, 1975). Further, this finding of less enriched δ^{15}N in keratin tissue provides evidence that juveniles are not only consuming primary producers, but also assimilating them into growth and production of bodily tissue.

All of the isotopic niche shifts show a consistent enrichment trend in these individuals, but the timing of these shifts within the keratin record is inconsistent across individuals. Assuming that vertical hawksbill keratin growth rate is similar

among individual turtles, only the youngest turtles should display a shift, because older turtles will have shed older layers of tissue. Similarly, if keratin growth rate is consistent across the lifespan of an individual, the shifts would be within the same approximate layer of keratin in similarly aged turtles. Neither of these assumptions are met within this study, suggesting that keratin growth rate in hawksbills can vary widely both among and within individuals. In fact, one of the oldest individuals sampled (reproductive age = 21 years) displayed the largest keratin tissue record prior to a niche shift (depleted in $\delta^{15}N$), with the shift to more enriched $\delta^{15}N$ occurring in the third to last layer of keratin. In comparison, one neophyte (first time nester, reproductive age = 0) displayed a niche shift in the first ~20 % of the keratin transect (layer 3 out of 14), and another neophyte had a shift at ~50 % of the keratin transect distance (layer 12 out of 23). Of note, 10 additional neophytes were assessed that did not contain a niche shift in the $\delta^{15}N$ record. Levasseur et al. (2020) indicated a range of ages for hawksbill sexual maturity of 14 – 24 years based on genetic assessment of the Jumby Bay population. This range in ages of sexual maturity supports the theory of variable growth rates among individuals. Neophytes with a trophic niche shift in the keratin record could have matured at faster rates than neophytes without the shift, leading to less time represented within the keratin record and earlier life stages represented.

These discrepancies make evaluating the keratin in absolute time difficult. Past studies have calculated keratin growth rates for adult green sea turtles (*Chelonia mydas*) and adult loggerhead (*Caretta caretta*) to be 121.95 µm/year (50µm /0.41 years) (Vander Zanden et al., 2013) and 83.38 µm/year (50 µm / 0.6 years) (Vander

Zanden et al., 2010), respectively. However, given differences in diet, taxonomy, and sea turtle body size, these calculations cannot be broadly applied to different sea turtle species. Because I was not able to directly convert hawksbill keratin increments to absolute time, my inferences are restricted to relative patterns in isotopic signatures. Suggestions for further work seeking to develop a method for aging keratin in hawksbills are discussed below.

The varying timing of dietary shifts in the keratin record could also be a result of a dietary shift during the adult life stage. The relatively later shift in the individual at a reproductive age of 21 years seems unlikely to reflect a shift during a juvenile life stage. However, there is little to no evidence that hawksbills would undergo a large and sudden dietary shift during their adult life stages. Marine turtles display high fidelity to geographic foraging locations (Broderick et al., 2007), and there is little to no evidence of trophic or prey item shifts for hawksbills within the adult life stage. If this shift in δ^{15}N values did occur in the adult life stage, possible causes could include environmental disturbance such as a destructive storm or change in nutrient inputs into the foraging habitat. In the future, inclusion of geographic foraging location and historical context of the site could provide more insight into these δ^{15}N shifts.

Population-level variability in resource use

Bearhop et al. (2004) proposed using isotopic variance as a proxy for niche width in consumers and using stable isotope patterns to explore foraging strategy (i.e., specialists vs generalists). High variance in isotopic values across the sampled hawksbill population supports the classification of a population with a generalist

resource use strategy. Vander Zanden et al. (2010) described three possible population patterns in resource use over time, a specialist population, a generalist population, or a generalist population with individual specialists. Broadly, this population of hawksbills fits well into the generalist population pattern, with statistically significant variance in isotope composition. The observed high among individual variability could indicate a generalist resource use strategy at the population level, or differences in trophic baselines in the nitrogen pools available at varying foraging locations within this population of hawksbills. However, this population doesn't appear to fit solely within an overall generalist population, or a generalist population with individual specialists, as within individual variance differed a great deal among individuals. Bearhop et al. (2004) predicts that tissues with longer isotope integration rates (i.e., bone, feathers, scute, etc.) are more likely to display specialist patterns, given the opportunity for mixing of multiple prey sources. Our strategy of repeated sampling in scute tissue appears, to some extent, to have avoided this bias towards specialization.

Despite all of the females sampled for this study belonging to the same reproductive population (Levasseur et al., 2019), initial satellite tracking of in-water movements has suggested this population uses numerous geographic foraging habitats across the Caribbean (Maurer & Stapleton, *unpublished data*). The variance in among-individual $\delta^{13}C$ values likely reflects differences in stable isotope composition of local carbon pools supporting food webs used by individuals. Patterns in local $\delta^{13}C$ baselines have been previously reported across coastal environments, with inshore habitats having relatively enriched carbon pools as compared to offshore

environments (France, 1995; Hobson et al., 1994). Future analysis that integrates spatial foraging data alongside stable isotope data could provide insight into the geographic or local environmental drivers of hawksbill trophic niche or keratin $\delta^{13}C$ stable isotope composition.

Nitrogen stable isotope values often differ with trophic position or between trophic baselines in different foraging locations. Although I was unable to differentiate between these two sources of variability in this study, the inclusion of $\delta^{15}N$ values in my analysis provides a mechanism for evaluating resource use variability outside of the spatial information typically represented by carbon stable isotope data. In this population of hawksbills, diversity in trophic position appears to be another important driver of trophic niche variability. Observations of hawksbill omnivory are well documented and incidents of herbivory have been observed in hawksbill populations in regions such as the eastern Pacific (Méndez-Salgado et al., 2020), the northern Great Barrier Reef (Bell, 2013), and the Caribbean (Stringell et al., 2016). Given the variation observed in these adult hawksbills, even for those individuals with no isotopic evidence of an ontogenetic trophic niche shift, fluctuating $\delta^{15}N$ values could indicate herbivory continues to occur among adults. Alternatively, spatial patterns in nitrogen isotope values of basal resources also occur (Schell et al. 1998), and the observed variability in $\delta^{15}N$ values during the adult life-stage could reflect feeding over a broad range of habitats with unique baseline conditions.

Sea turtle reproductive age was observed to be a significant predictor of niche width (**Figure 11**). SEA calculations were based on isotope composition after a probable niche shift; therefore, this pattern of narrowing niche width should be

independent of a sudden ontogenetic trophic niche shift. This narrowing of niche width could be linked to changes in adult foraging behavior, such as selection for certain prey items or the homogenization of overall diet composition (Vander Zanden et al., 2013). For this study, I was unable to determine if the narrowing niche width stems from diet specialization of only a small number of prey items or a diet consisting of many prey items and constant ratios. Both scenarios would lead to smaller variability in isotopic composition and a narrower niche width. While I cannot distinguish between distinct prey sources, this relationship between age and niche width provides insight into the factors that relate to resource use strategy. Individual turtle age is a factor that is largely disconnected from environmental factors such as reef diversity, foraging location, or baseline nutrients and primary producers. It could then follow that niche width might have more to do with variability in preferences as individuals age, rather than environmental effects such as reef health and the stability of local food webs.

This finding, paired with the evidence of a trophic niche shift in hawksbills, suggests some level of difference of δ^{15}N values across different life stages. It appears that younger individuals have both lighter δ^{15}N isotopic composition and increased variability across both δ^{13}C and δ^{15}N than adults in the population. This provides isotopic support for hawksbills of different ages using different habitats and/or prey sources. To my knowledge, this is the first time biomarkers have been used to confirm this ecological differentiation in resource use patterns between older and younger hawksbill turtles.

Linking resource use with reproductive output

A significant relationship between the most recent δ^{13}C value and the number of nests laid was identified. The positive relationship suggests that depleted δ^{13}C values were associated with fewer nests in the year the keratin was sampled. This result could indicate a relationship between carbon resource use and yearly clutch production, but the lack of relationship between the most recent δ^{13}C value and any of the other three fecundity metrics makes characterization of a definitive relationship difficult. Discrimination of δ^{13}C is often associated with inshore vs. offshore feeding (France, 1995; Hobson et al., 1994; Wyatt et al., 2012) and Wedemeyer-Strombel (2019) documented a shift in juvenile Eastern Pacific hawksbills from more enriched to more depleted δ^{13}C values in a gradient from pelagic to estuarine and mangrove habitats. Given the significant differences in baseline Porifera tissue from two nearby hawksbill foraging habitats, differences in the δ^{13}C composition of local resource pools among individuals seems likely.

It is important to note that while this relationship did have a *p*-value less than the selected α ($P = 0.045$, $\alpha = 0.05$), the *p*-value was close to α. Similarly, the large number of models tested in this analysis could indicate that a lower α value might be more appropriate in evaluating true significance of the models. Therefore, while these results suggest a relationship may exist, further study is necessary to understand the potentially complex relationship between carbon stable isotope value and sea turtle fecundity. Future research that integrates stable isotope data from metabolically active tissues with faster turnover times (e.g., skin, blood, muscle) (Rubenstein & Hobson, 2004) and spatial foraging data could reveal additional insight into the

relationships between recent diet, spatial foraging patterns, and fecundity. To my knowledge, this is the first study examining linkages between stable isotope metrics and reproductive output in hawksbill sea turtles.

None of the tested fecundity metrics showed a significant relationship with niche width (either as SEA or WIC). This lack of relationship between reproduction and stable isotope variance could indicate that 1) untested variables play a more significant role in predicting reproductive success in hawksbills, 2) our sampling and analytical methods are too coarse to distinguish a relationship, and/or 3) isotopic variance doesn't properly capture the relationship between environmental resource use and fecundity. Concurrently, relatively low variability in the derived fecundity metrics could also account for weak relationships outside of stable isotope analysis.

This study focused solely on isotopic variance of δ^{13}C and δ^{15}N values to predict fecundity metrics, however the complex behaviors, long migrations, and oceanic habitats of marine species include many factors that could influence reproductive success. Important factors include nest site selection (e.g., vegetative cover, clutch size, date of deposition, the individual's chronological nest of the season) (Ditmer & Stapleton, 2012), sea surface temperature (Solow et al., 2002), and possibly factors such as genetics, length of migration, and a suite of environmental factors between the migratory route and nesting grounds (though see Price et al., 2004). It is also important to note that only female sea turtle metrics were evaluated in this study. Female behavior (Ditmer & Stapleton, 2012), trophic ecology (Hatase et al., 2013), and physiology (Leblanc et al., 2014) should account for most of the variability in reproductive output (e.g., energy acquisition and expenditure, nest site

selection), but any contribution to realized fecundity by male genetics or sperm quality would be missed in this analysis.

The high among-individual variance observed suggests a generalist feeding strategy at the population-level. The overall ranges of δ^{13}C (-25 – -13.15‰, total = 11.85‰) and δ^{15}N (-0.83 –13.87‰, total = 14.7‰) values for these hawksbills are comparable or larger ranges than what are reported in other species of marine turtle. Loggerheads had δ^{13}C ranges from ~ -20 – -9‰ (total = 11‰) and δ^{15}N ranges from ~3 – 13‰ (total = 10‰) (Vander Zanden et al. 2010) and green sea turtles had δ^{13}C ranges from ~ -13 – -6.5‰ (total = 6.5‰) and δ^{15}N ranges from ~2.5 – 9.9‰ (7.4‰) (Vander Zanden et al. 2013). SEA and WIC had wide ranges across the sampled turtles, indicating varying levels of specialization and generalism among individuals. Given that keratin growth rate is unknown for hawksbills but appears to vary among and within individuals, it is possible that consistent sampling at 100 μm intervals does not account for all or enough variation in hawksbill diet. If fluctuations in resource use are integrated quickly (faster than can be assessed in 100 μm layers), this variability would be missed and absent from the SEA evaluation for individuals. Based on previously determined keratin growth rates for other species of sea turtle (Vander Zanden et al., 2010, 2013) the range of sizes in keratin samples sampled in this study should include sufficient data to elucidate fine-scale temporal changes in diet during the adult life stage (i.e., 0.82 – 1.2 years/100 μm layer).

Finally, stable isotope analysis is a powerful tool in trophic ecology, but relies on a suite of assumptions. In this study, if the assumption is made that all foraging habitat variability (i.e., changes in diet, primary producers, location, reef stability,

etc.) are captured in the $\delta^{13}C$ and $\delta^{15}N$ stable isotope record, based on the lack of significant models, I might infer that foraging strategy plays no role in reproductive success. However, this most likely is not the case. Given the current lack of spatial and environmental data corresponding to the observed stable isotope composition of individual hawksbills, the specific relationship between foraging conditions and fecundity remains unclear. With the high complexity of reef systems, varying strategies in different locations may benefit individuals. For example, a specialist diet in a stable environment and generalist diet in an unstable environment may yield similar energetic input and reproductive output. In a stable environment, individuals can select the most nutritious prey items year-round. In an unstable environment, individuals who adapt to select an array of nutritious prey items in a changing landscape may increase energetic inputs. These two strategies would appear different within the isotopic record, but could provide similar energetic input and reproductive output to individuals. Further, stable isotope approaches often require standardization to a specific baseline (Schell et al. 1998), even for niche-based metrics such as SEA (e.g. Warry et al., 2016). It is possible that baseline-correction of stable isotope niche-metrics would explain some of the variability in reproductive success, but the spatially explicit data needed to correct stable isotope values for each individual turtle do not exist. Overall, the relationship between foraging grounds and reproductive success is likely highly complex, and further work that leverages additional sources of data to better understand the patterns between diet and fecundity will aid in conservation of this species.

Multiple sampling of individuals

I tested two methods of evaluation, stable isotope analysis and visual observation of growth laminae and coloration, for utility in developing a method for aging vertical keratin growth in hawksbills. While neither method produced compelling results, these methods (and others) should continue to be examined with a larger sample size. Information based on larger sample sizes should be available in coming years as more previously sampled individuals return to the nesting beach and can be resampled for analysis.

Identifying growth lines under the microscope proved difficult. While some patterns in broad colorations and putative growth laminae were distinguishable in individual keratin samples, identifying similar patterns across individuals in multiple years was not initially feasible. Measuring the growth between distinguishable lines could provide some insight into this method with more turtles, but for those individuals examined here, no patterns were discernible.

Stable isotope analysis of tissue from individuals two years apart, while not necessarily providing growth rate information, did provide interesting results. Given the regrowth of new keratin tissue in the overlapped sample, I was unable to use this method as a reliable adult stage keratin growth rate. In at least two individuals (the third suffered shell damage, making distinct evaluation difficult), the 2017 sample site was completely regrown with keratin. Despite this regrowth, it is very unlikely that the adult life stage keratin growth rate for hawksbills is actually 1500–1800 µm per 2 years. Growth rates in other species of marine turtle are ~100 µm/year (Vander

Zanden et al., 2010, 2013), which is less than 10 times the perceived rate of the hawksbill keratin growing the amount required to completely fill the previous sample site. The fastest maturing individuals within this Caribbean hawksbill population reach sexual maturity at a maximum of 14 years based on genetic analysis (Levasseur et al., 2020), but a turtle from this study with a reproductive age of 21 years showed evidence of a clear niche shift (**Figure 5**). Assuming the niche shift reflected an ontogenetic shift, the 1200 μm of keratin for that female would span a minimum of 21 years, but likely at least 35 years. These inconsistencies in growth could indicate that the regrowth of tissue at the sample site was caused by an increased growth rate due to injury recovery to fill the hole in the shell, rather than a true standard keratin growth rate.

All three of the resampled turtles had low initial δ^{15}N values, with two individuals showing a niche shift to more enriched values, and one maintaining the lower δ^{15}N values through the keratin plug. In 2019, all three individuals had exclusively enriched δ^{15}N values. Combined with the visual observation of complete regrowth of keratin tissue in the two years between sampling, this could indicate that the 2019 sampling locations were composed primarily of tissue exclusively grown between 2017–2019. The higher δ^{15}N values are then consistent with an ontogenetic niche shift to adult life stage resource use. Individual WH5664 did not contain an apparent niche shift in the tissue record (**Figure 8**). However, it is possible that the sampling or sectioning methods missed tissue after the niche shift if it was in a deeper section of keratin. Given constraints in sampling and drilling layers (i.e., consideration to not drill past the keratin layers into bone when sampling and leaving

~200–300 µm of tissue on the glass slide so as to not contaminate the samples with Crystalbond™ 509), it is possible that the niche shift in WH5664 did occur but is not represented in the keratin record sampled here. The 2019 sample of enriched δ^{15}N values supports this possibility.

Trophic Enrichment Factor (TEF) calculation

A range of TEFs was calculated from three individual turtles using Porifera tissue from a known foraging location (two sites within the larger range). While this information provides an initial TEF estimate for hawksbills, further assessment with a larger sample size is necessary prior to application of these TEFs to other hawksbill individuals. Still, despite the small sample size and range in TEF values that spans 2.87 to 4.15‰, the mean value of 3.67‰ (standard deviation = 0.7‰) reported here is comparable to values reported in the literature for other sea turtles (Vander Zanden et al., 2010, 2013). Collaborative efforts to sample and assess Porifera tissue from other identified foraging grounds would strengthen this relationship between diet and consumer isotopic composition, particularly if coupled with satellite-based tracking of individuals to pinpoint their local foraging areas. Caribbean hawksbills have been tracked to foraging ground locations near at least 14 Caribbean countries (Hart et al. 2019). Future work that would inform this analysis includes collecting prey tissue from multiple foraging grounds, a better resolution of the available range of potential prey items, and matching prey collections with foraging areas of known individuals. Calculation of a robust TEF for hawksbill keratin will allow for evaluation of absolute trophic position and the application of more highly resolved multi-

endmember mixing model approaches for studying diet within ecosystems (McClellan et al., 2010) in addition to the relative patterns described above.

Conclusions

Hawksbill sea turtles are of conservation concern, and understanding their life histories can aid in prioritizing management strategies. This study is one of the first to pair stable isotope analysis with reproductive metrics for the hawksbill and to use biomarkers to differentiate resource use in different life stages for Caribbean hawksbills. The results of this assessment highlight the complexity of using stable isotopes as a proxy for resource use, but provides insight into how hawksbills may be changing over their lives. The results of this analysis show differences in how older and younger individuals are integrating carbon and nitrogen. These differences will be crucial in identifying conservation priorities for hawksbills. Conservation strategies for younger life stages will need to be distinct from older individuals, and both strategies should consider inclusion of primary producers, not only sponges, as potential prey sources for hawksbills of varying ages. Future work to expand on the growth rate and TEF calculation will further clarify these relationships. TEF calculation of individuals will provide insights into hawksbill trophic position in varying geographic regions and can highlight important prey sources within ecosystems. Identifying patterns in keratin growth will allow for further identification of relationships between fecundity and resource use, in particular on finer time scales such as annual differences. Understanding these relationships between prey items,

geographic location, differences in varying life stages, and reproductive output can inform prioritization of conservation efforts for this critically endangered species.

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Tables

Table 1. List of all model structures for fecundity metric analysis. Listed predictors are standard ellipse area (SEA), within individual component of variance for δ^{13}C and δ^{15}N (CWIC and NWIC), reproductive age, body size (CCL), and the most recent isotopic integration of δ^{13}C and δ^{15}N (Last δ^{13}C and Last δ^{15}N). Boostrapped models are indicated with a "B".

Predictor	Response	n	Bootstrapped?
SEA	Remigration interval	36	B
	Number of Nests	34	B
	Clutch size	50	B
	Hatch success	50	B
CWIC	Remigration interval	36	B
	Number of Nests	34	B
	Clutch size	50	B
	Hatch success	50	B
NWIC	Remigration interval	36	B
	Number of Nests	34	B
	Clutch size	50	B
	Hatch success	50	B
Reproductive age	SEA	50	-
	CWIC	50	-
	NWIC	50	-
Body size (CCL)	SEA	50	-
	CWIC	50	-
	NWIC	50	-
Last C13	Sampled year Remigration interval	50	-
	Sampled year Number of Nests	50	-
	Sampled year Clutch size	15	B
	Sampled year Hatch success	15	B
Last N13	Sampled year Remigration interval	50	-
	Sampled year Number of Nests	50	-
	Sampled year Clutch size	15	B
	Sampled year Hatch success	15	B

Table 2. List of all individual hawksbill sea turtles assessed for keratin isotope composition and characteristics. Columns include: individual turtle ID, the year an individual was first tagged ($Year_{Tag}$), the year an individual was sampled for keratin ($Year_S$), reproductive age (Age_R), within individual component of variance for $\delta^{13}C$ and $\delta^{15}N$ (CWIC and NWIC), standard ellipse area (SEA), Mean $\delta^{13}C$‰ and $\delta^{15}N$‰ ± standard deviations, and whether an ontogenetic niche shift was apparent in the $\delta^{15}N$ record (indicated with "Yes").

Turtle ID	$Year_{Tag}$	$Year_S$	Age_R	CWIC	NWIC	SEA	Mean $\delta^{13}C$‰	Mean $\delta^{15}N$‰	Niche shift?
WH10133	2018	2018	0	0.20	0.04	0.67	-20.36 ± 1.34	5.4 ± 4.56	Yes
WH10048	2018	2018	0	0.08	0.20	1.05	-23.16 ± 1.05	10.21 ± 0.71	-
WH10147	2018	2018	0	0.29	0.28	1.56	-18.56 ± 0.86	8.86 ± 0.84	-
WH10035	2017	2017	0	0.09	0.14	1.02	-18.12 ± 0.54	8.61 ± 0.56	-
WH10005	2017	2017	0	0.18	0.15	1.44	-19.78 ± 0.78	8.2 ± 2.08	Yes
WH10047	2017	2017	0	0.15	0.15	1.32	-18.52 ± 1.05	10.7 ± 0.66	-
WH10144	2018	2018	0	1.09	0.50	2.27	-14.96 ± 1.97	7.7 ± 1.23	-
WH10078	2019	2019	0	0.01	0.01	0.07	-17.75 ± 0.22	10.26 ± 0.3	-
WH10080	2019	2019	0	0.94	0.03	1.56	-18.3 ± 1.63	9.79 ± 2.53	-
WH10040	2017	2017	0	0.16	0.01	0.36	-17.71 ± 0.72	9.22 ± 0.48	-
WH10068	2018	2018	0	0.02	0.04	0.23	-18.08 ± 0.26	10.78 ± 0.35	-
WH10141	2018	2018	0	0.03	0.02	0.22	-17.97 ± 0.92	9.8 ± 0.67	-
WS8801	2014	2017	3	0.18	0.23	1.39	-16.85 ± 0.59	7.25 ± 0.89	-
WS1163	2014	2017	3	0.22	0.06	0.99	-18.02 ± 0.45	10.17 ± 0.36	-
WS8949	2016	2019	3	0.01	0.03	0.08	-18.09 ± 0.19	10.32 ± 0.35	-
WS8824	2015	2019	4	0.30	0.17	1.65	-18.25 ± 0.97	9.6 ± 0.71	-
WS1189	2014	2018	4	0.11	0.05	0.57	-18.21 ± 0.4	8.69 ± 0.37	-
WS8864	2015	2019	4	0.01	0.07	0.20	-17.25 ± 0.26	8.62 ± 0.53	-
WS1175	2014	2018	4	0.74	0.30	3.10	-18.18 ± 1.64	8.83 ± 0.92	-
WS8858	2015	2019	4	0.05	0.02	0.26	-17.44 ± 0.38	9.08 ± 0.31	-
WS1164	2014	2019	5	0.11	0.39	1.89	-18.35 ± 0.55	9.43 ± 1.03	-
WS1020	2012	2017	5	0.49	1.38	7.35	-21.53 ± 1.41	1.45 ± 2.81	-
WS1151	2013	2018	5	0.55	0.67	5.25	-20.83 ± 1.27	8.75 ± 1.37	-

WS1142	2013	2018	5	0.88	0.02	1.00	-18.79 ± 1.66	9.96 ± 0.33	-
WS1070	2012	2018	6	0.15	0.14	0.80	-17.69 ± 0.66	8.16 ± 0.78	-
WH5762	2010	2017	7	1.86	0.11	2.78	-20.65 ± 2.68	10.57 ± 3.34	-
WH5766	2010	2017	7	0.77	0.16	1.36	-18.35 ± 1.77	4.87 ± 3.15	Yes
WH5742	2010	2017	7	0.16	0.00	0.16	-18.1 ± 0.65	8.94 ± 0.11	-
WH5664	2009	2017	8	2.08	1.17	12.44	-20.56 ± 2.25	1.89 ± 3.73	-
WH5690	2009	2017	8	0.02	0.02	0.12	-17.59 ± 0.28	9.93 ± 0.35	-
WH5692	2009	2018	9	0.45	0.01	0.64	-17.47 ± 1.15	8.54 ± 0.21	-
WH5746	2010	2019	9	0.24	0.01	0.49	-18.24 ± 1.35	8.99 ± 0.47	-
WH5658	2008	2017	9	0.01	0.02	0.09	-17.57 ± 0.14	9.27 ± 1.79	-
WE5294	2008	2018	10	0.80	0.33	3.12	-19.08 ± 1.38	9.6 ± 2.21	-
WH5616	2008	2018	10	0.46	0.36	2.34	-18.69 ± 1.2	9.83 ± 1	-
WE5180	2006	2017	11	0.08	0.01	0.25	-17.49 ± 0.48	8.98 ± 0.34	-
WE5068	2005	2017	12	0.79	0.08	1.83	-18.63 ± 1.53	8.54 ± 0.46	-
WE5211	2007	2019	12	0.01	0.04	0.17	-17.88 ± 1.3	10.64 ± 0.49	-
WE5148	2006	2018	12	0.02	0.03	0.20	-18.06 ± 0.32	9.09 ± 0.26	-
WE5025	2004	2017	13	0.01	0.07	0.25	-17.41 ± 0.21	9.31 ± 0.44	-
WE5032	2004	2017	13	0.05	0.01	0.24	-17.76 ± 0.4	9.77 ± 0.22	-
WE5055	2005	2019	14	0.51	0.03	1.05	-17.78 ± 1.21	9.43 ± 0.34	-
WE363	2003	2018	15	0.03	0.02	0.19	-17.6 ± 0.3	9.72 ± 0.21	-
XXA282	2002	2018	16	0.01	0.03	0.17	-17.57 ± 0.2	8.75 ± 0.28	-
XXA202	2000	2017	17	0.07	0.00	0.11	-18.5 ± 0.55	11.25 ± 0.84	-
QQZ195	1996	2017	21	0.05	0.00	0.00	-19.25 ± 1.81	5.35 ± 4.19	Yes
PPC903	1996	2017	21	0.02	0.01	0.11	-17.38 ± 0.24	9.34 ± 0.28	-
QQB996	1992	2018	26	0.24	0.01	0.26	-18.4 ± 0.84	9.82 ± 0.15	-
QQZ108	1993	2019	26	0.01	0.03	0.15	-17.94 ± 0.73	10.37 ± 2.13	-
PPN051	1988	2018	30	0.01	0.03	0.17	-17.32 ± 0.27	10.1 ± 0.3	-

Table 3. List of all bootstrapped model outputs for fecundity metric analysis. Listed predictors are standard ellipse area (SEA), within individual component of variance for $\delta^{13}C$ and $\delta^{15}N$ (CWIC and NWIC), and the most recent isotopic integration of $\delta^{13}C$ and $\delta^{15}N$ (Last $\delta^{13}C$ and Last $\delta^{15}N$). Sample size (n) and 95% confidence intervals (CI) for models are listed.

Predictor	Response	n	CI	
			Upper	Lower
SEA	Remigration interval	36	-1.7594	0.1974
	Number of Nests	34	-0.095	1.1927
	Clutch size	50	-0.0074	0.0522
	Hatch success	50	-2.606	5.569
CWIC	Remigration interval	36	-0.3462	0.1117
	Number of Nests	34	-0.0807	0.2205
	Clutch size	50	-0.0024	0.011
	Hatch success	50	-0.7871	0.9539
NWIC	Remigration interval	36	-0.2119	0.0344
	Number of Nests	34	-0.0629	0.1076
	Clutch size	50	-0.0015	0.0055
	Hatch success	50	-0.1526	1.0172
Last C13	Sampled year Clutch size	15	-0.0382	0.1029
	Sampled year Hatch success	15	-14.643	13.703
Last N13	Sampled year Clutch size	15	-0.0444	0.0241
	Sampled year Hatch success	15	-6.114	3.3652

Table 4. Normal linear regressions are listed, with p-value (*P*), slope parameter estimate, and standard error for each model. Significant results are indicated in bold and with an asterisk. Listed predictors are standard ellipse area (SEA), within individual component of variance for $\delta^{13}C$ and $\delta^{15}N$ (CWIC and NWIC), and the most recent isotopic integration of $\delta^{13}C$ and $\delta^{15}N$ (Last $\delta^{13}C$ and Last $\delta^{15}N$).

Predictor	Response	n	Boxcox?	*P*	Estimate	Standard error
Reproductive age	SEA	50	Yes	**0.003 ***	**-0.014**	**0.005**
	CWIC	50	Yes	0.053	-0.003	0.001
	NWIC	50	Yes	**0.003 ***	**-0.008**	**0.003**
Body size (CCL)	SEA	50	Yes	0.434	0.008	0.011
	CWIC	50	Yes	0.841	0	0.003
	NWIC	50	Yes	0.454	-0.005	0.007
Last C13	Sampled year Remigration interval	50	-	0.563	-0.202	0.346
	Sampled year Number of Nests	50	-	**0.045 ***	**0.697**	**0.333**
Last N15	Sampled year Remigration interval	50	Yes	0.92	-0.513	5.062
	Sampled year Number of Nests	50	-	0.406	0.178	0.211

Table 5. Minimum, maximum, mean, and standard deviation of δ^{13}C and δ^{15}N values from Porifera tissue from two locations.

	δ^{13}C‰			δ^{15}N‰		
	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD
Site 1	-22.79	-16.57	-20.23 ± 2.03	5.08	13.14	10.09 ± 2.92
Site 2	-19.66	-8.54	-16.56 ± 2.59	2.77	12.27	8.49 ± 3.52

Table 6. Mean δ^{15}N values ($\delta^{15}N_{SC}$), the proportion of the diet derived from dive site 1 (α), and change in δ^{15}N between Porifera and hawksbill sea turtles (Δ_n or TEF).

	$\delta^{15}N_{SC}$‰	α	Δ_n‰
WH10133	9.7	1.28	3.99
WE5294	9.6	0.69	4.15
WS1070	8.16	0.31	2.87

Figures

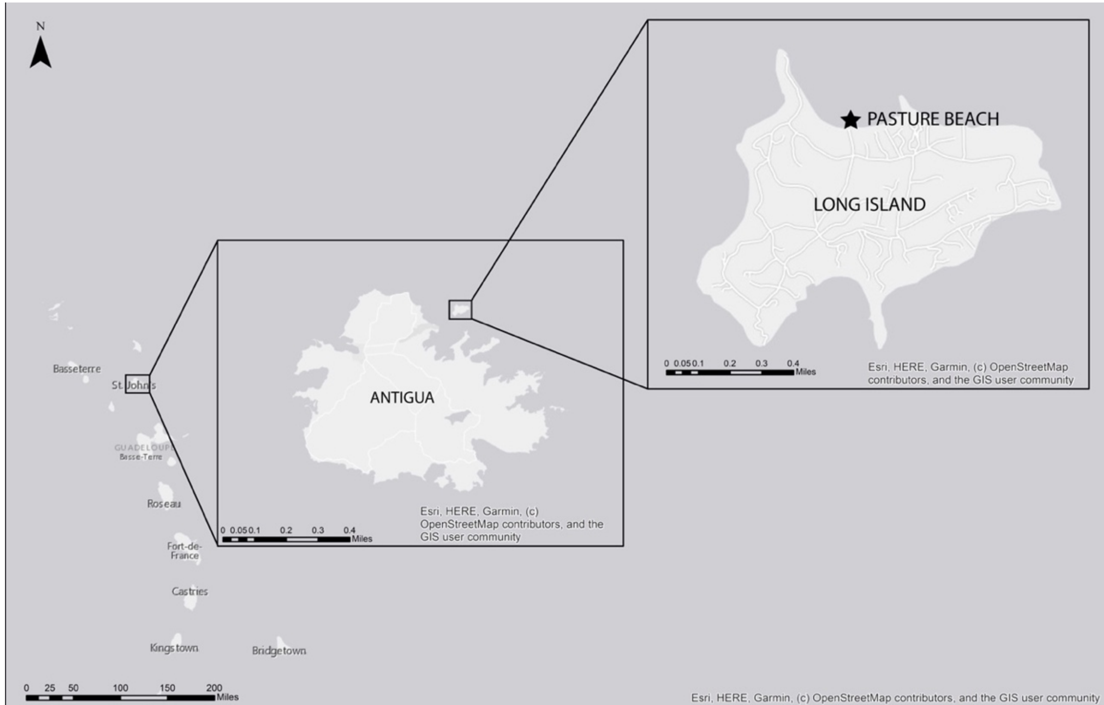


Figure 1. Long Island, Antigua in the West Indies. Pasture Beach is the primary nesting site for hawksbill sea turtles in Antigua.

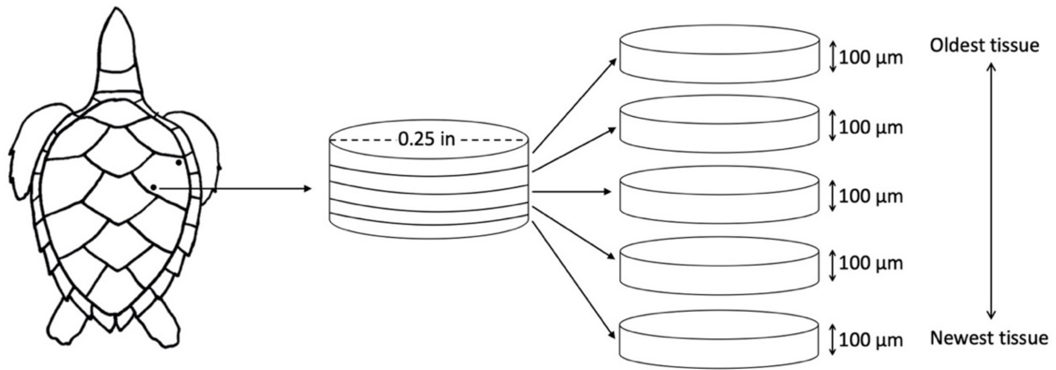


Figure 2. Sampling locations for keratin tissue and examples of 100µm sections and their relative age.

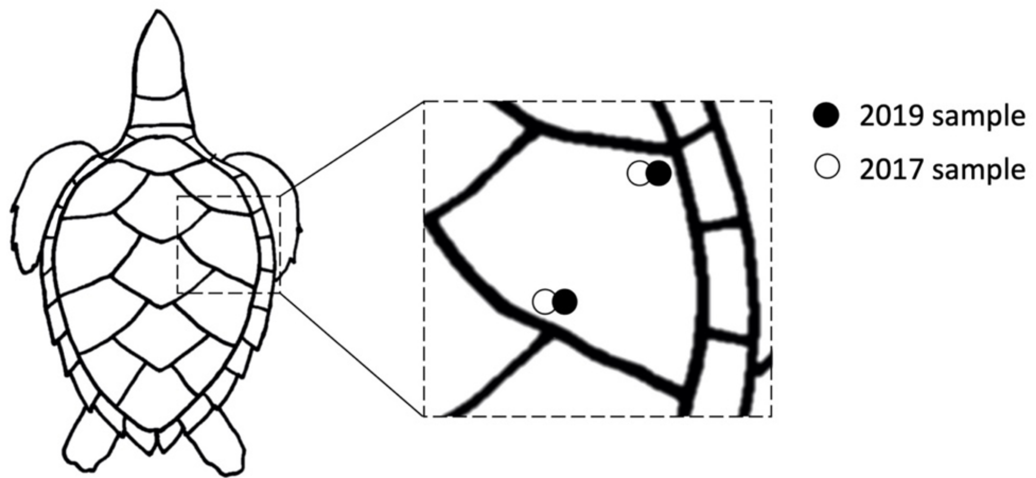


Figure 3. Locations for repetitive sampling of keratin tissue. Open circle represents the first sampling locations and closed circles are secondary sampling two years later.

Figure 4. Antigua, West Indies and the two sampling locations where Porifera tissue was collected.

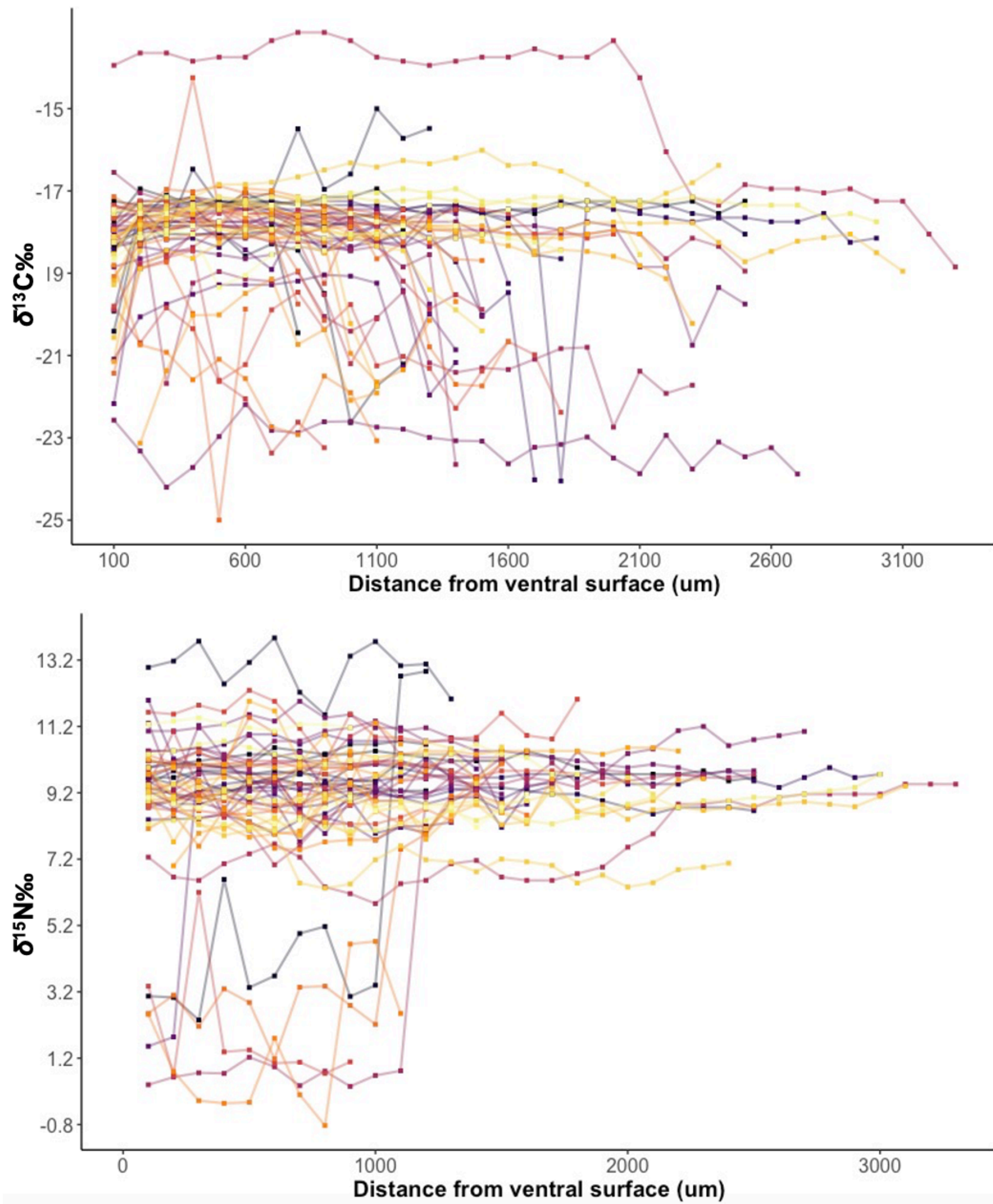


Figure 5. δ^{13}C and δ^{15}N values across scute tissue in individual hawksbills. Each individual is noted with a unique color.

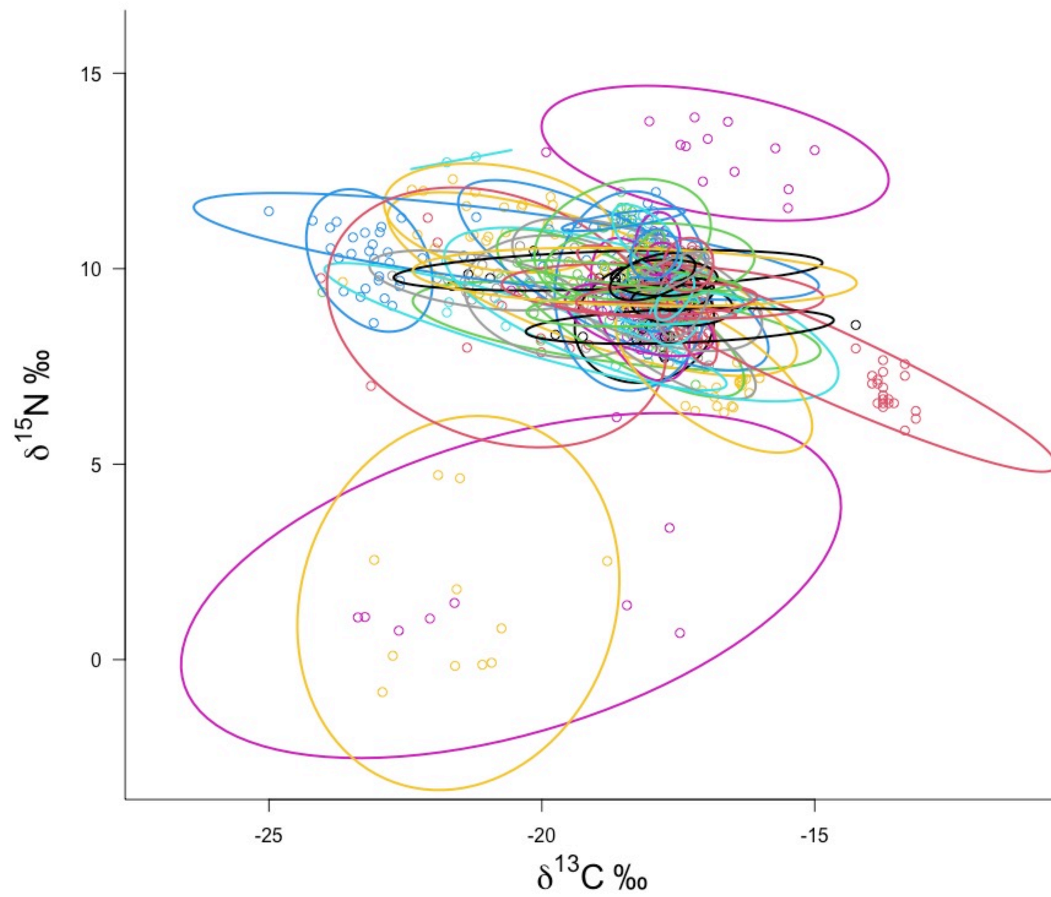


Figure 6. Standard ellipses around individual females on a biplot of δ^{13}C and δ^{15}N values. Unique colors represent individuals.

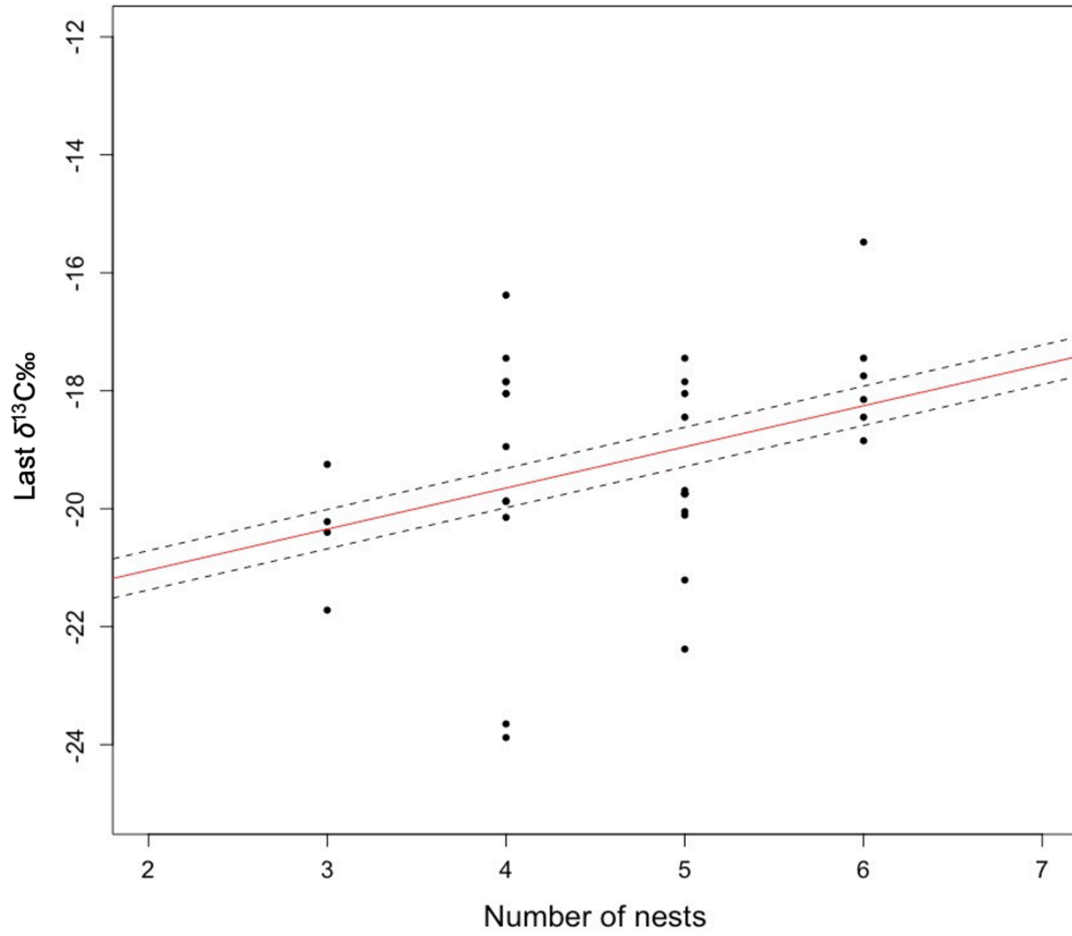


Figure 7. Linear regression plot of last δ^{13}C and number of nests with regression line in red and confidence intervals as dashed lines.

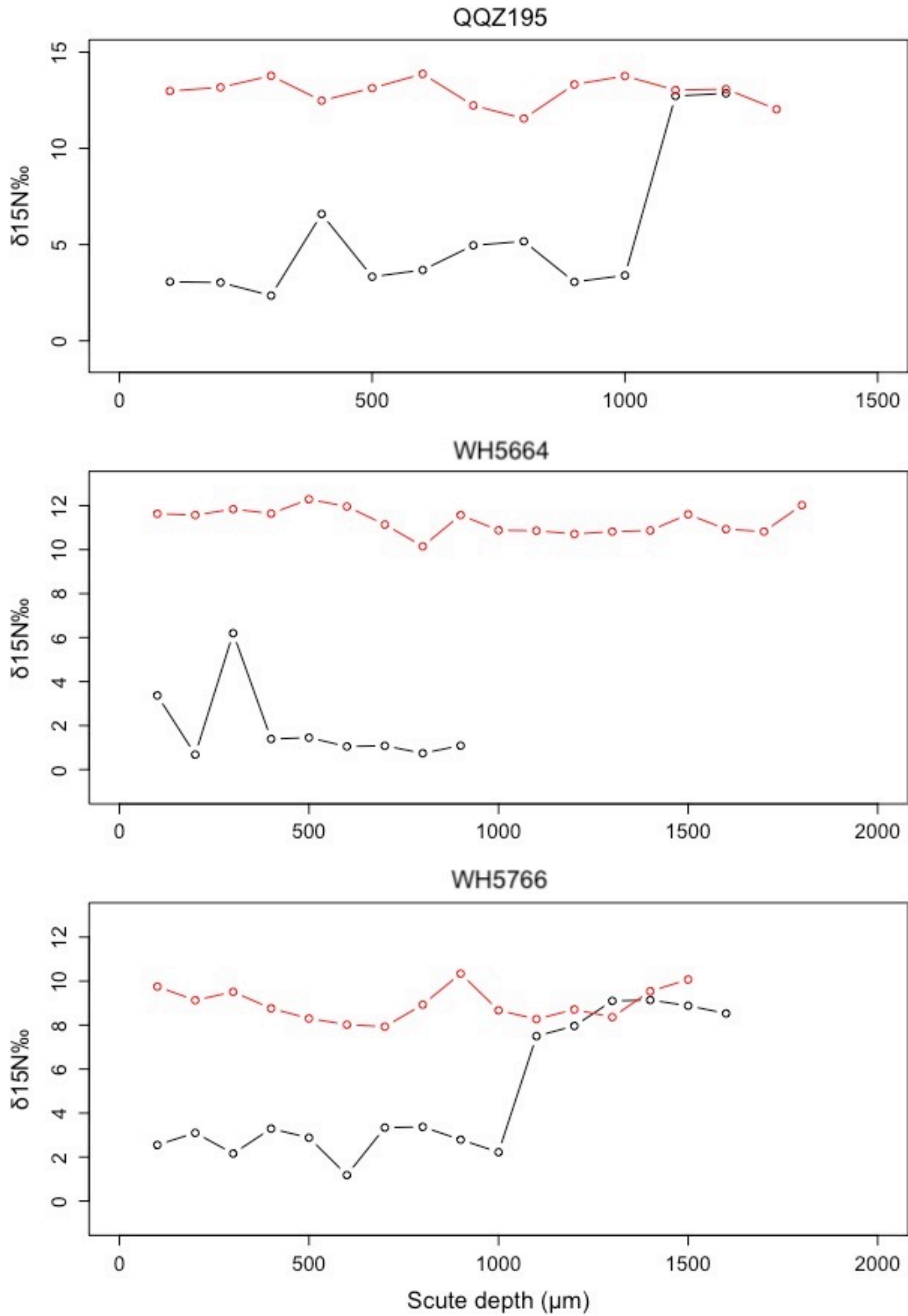
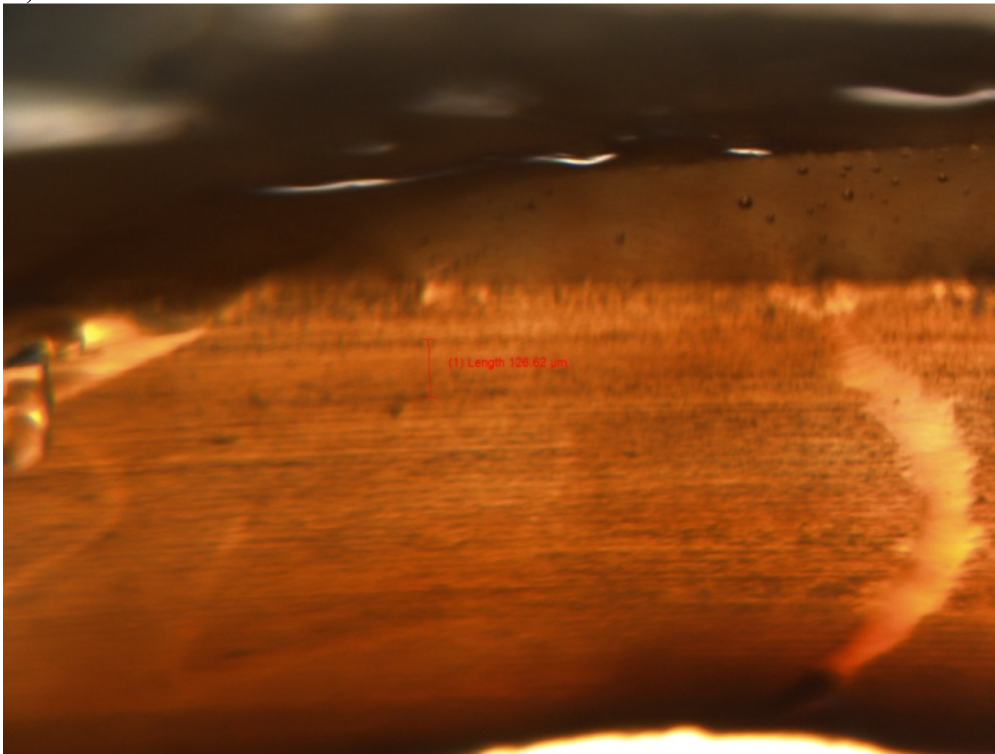


Figure 8. Plots of individual turtles who were sampled in 2017 (black) and 2019 (red).

A)



B)

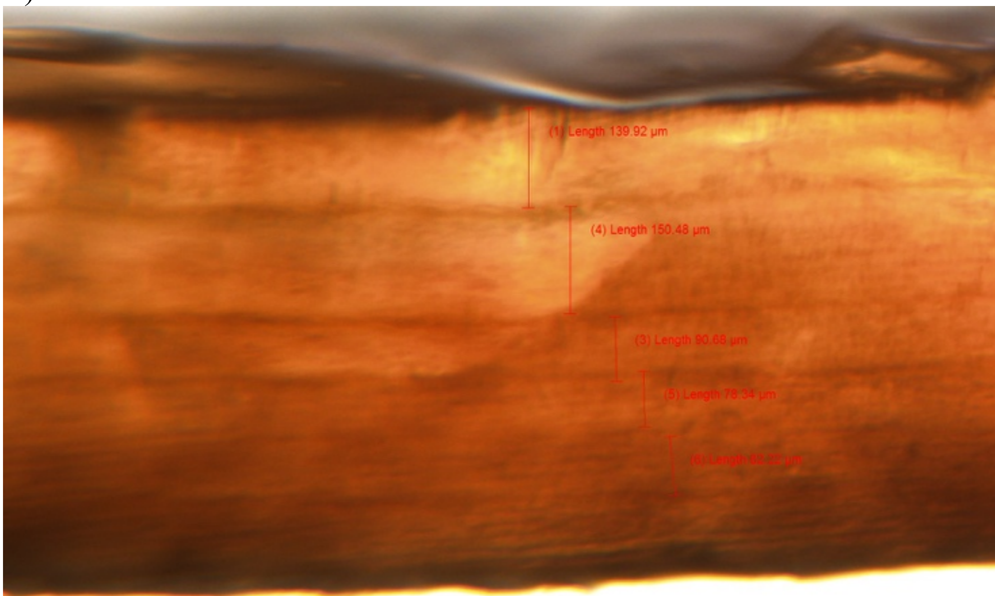


Figure 9. Images of sectioned keratin from the same individual in two years. A) a section of keratin from WH5766 in 2017, B) a section of keratin from an adjacent site WH5766 in 2019. Some clearly visible laminae were measured for thickness (listed in red).

Figure 10. Image of keratin regrowth in 2019 of a scute originally sampled in 2017 (circled in white). The original hole was filled in completely with new keratin, the epoxy filler was absent, but a small ring around the sample site is still visible.

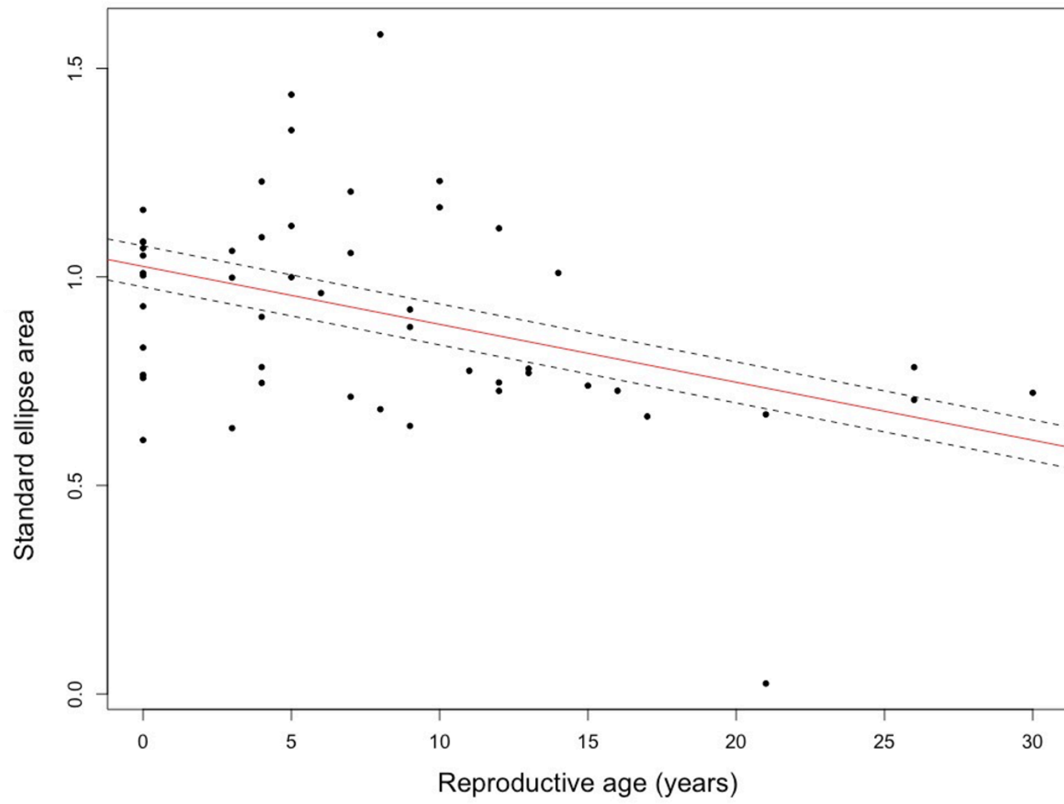


Figure 11. Plot of standard ellipse area and reproductive age with regression line in red and confidence intervals as dashed lines.

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