

Stable isotopes reveal dietary differences and site fidelity in juvenile green turtles foraging around São Tomé Island, West Central Africa

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ABSTRACT: Green sea turtles are common in West Central Africa, but little is known about the occurrence of immatures in foraging grounds in the Gulf of Guinea islands, known for their volcanic origin and narrow coastal fringes. This study presents results of in-water surveys in foraging grounds off São Tomé Island, in the São Tomé and Príncipe archipelago, providing the first available data on the size distribution of immature green sea turtles of different life-stage groups on these islands. Two sites offering distinct types of food sources were studied, and isotopic signatures of immature turtles hand-captured at each foraging site were used to infer (1) how long they were established at the foraging sites and (2) their diet preferences. Recruitment in the region was estimated to occur at a minimum size of 34 cm curved carapace length (CCL), and resident immature turtles ranged from 53 to 87 cm CCL. Immatures sampled at each site showed clear differences in isotopic signatures, suggesting that they establish specific home ranges related to the available diet items and use them for extended periods of at least several months. Macroalgae were as important as or more important than seagrasses for the turtles' diets, and there was evidence that these individuals are not strictly herbivorous. Our study provides the first dataset for comparison with demographic data from other locations in West Africa, where current knowledge on green turtle foraging behavior is limited, and indicates that even oceanic islands that are geologically recent like São Tomé may provide important recruitment and development habitats for juvenile green turtles.

KEY WORDS: *Chelonia mydas* · Settlement · Stable isotopes · Foraging ecology · Gulf of Guinea

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INTRODUCTION

Studies on sea turtle biology have typically focused on the reproduction and post-nesting movements of females, as they are logistically more accessible. Only more recently have multidisciplinary approaches tackled the lives of cryptic life stages, such as males and immatures, and their use of neritic foraging habitats (Rees et al. 2016). Population assessments at the foraging grounds provide local size-class distributions

and may contribute information essential for establishing population abundance trends (Seminoff et al. 2003, Bjørndal et al. 2005, 2010). Furthermore, knowledge of resource use can help determine the importance of different marine habitats for the different turtle life stages and improve our understanding of migratory connectivity among breeding, foraging and developmental habitats (e.g. Bradshaw et al. 2017).

The analysis of stable isotopes of both sea turtles and their diets has been increasingly combined with

in-water population assessments to study foraging behavior and resource use due to their high versatility. Stable isotope ratios in the tissues of consumers reflect those of their diet in a predictable manner (Hobson 1999, 2007, Post 2002). The ratio of nitrogen isotopes ($\delta^{15}\text{N}$) increases along each trophic transfer and can be used to estimate the trophic position of organisms (Minagawa & Wada 1984, Peterson & Fry 1987, Post 2002b), while the ratio of carbon isotopes ($\delta^{13}\text{C}$) varies substantially among primary producers with different photosynthetic pathways, and thus can be used to determine the sources of dietary carbon (DeNiro & Epstein 1978). In addition, as both carbon and nitrogen stable isotope ratios at the base of food webs vary spatially, this is reflected in the spatial variability of isotopic composition among food webs (Jennings et al. 1997, Finlay 2001, Bearhop et al. 2004, Graham et al. 2010). The quantification of stable isotopes is thus particularly useful for studying ontogenic shifts in sea turtle foraging strategies (Arthur et al. 2008, Shimada et al. 2014, Ramirez et al. 2015, Vélez-Rubio et al. 2016, Tomaszewicz et al. 2017), identifying the geographic location of foraging habitats (Dodge et al. 2011, López-Castro et al. 2013, 2014, Ceriani et al. 2014), as well as clarifying sea turtle trophic position and resource use (Lemons et al. 2011, Goodman Hall et al. 2015, Pajuelo et al. 2016, Sampson et al. 2017).

In West Central Africa, 2 green turtle regional management units overlap (South Central and Eastern Atlantic, Wallace et al. 2010) where turtles are exposed to multiple threats, both on nesting and foraging grounds (Formia et al. 2003, Carranza et al. 2006, Fitzgerald et al. 2011, Riskas & Tiwari 2013). Foraging grounds, mostly used by immature green sea turtles, have been identified in the continental countries in West Africa, specifically in Cameroon, Republic of Congo and Gabon (Formia 2002, Formia et al. 2003, 2006, Hyacinthe et al. 2012, A. Girard pers. comm.), but not on the islands of the Gulf of Guinea. These islands, including Bioko and Annobon (Equatorial Guinea) and São Tomé and Príncipe, are of volcanic origin, dating from 15.7 million years ago (Deruelle et al. 1991), and have high relief, resulting in very narrow littoral fringes (Juste & Fa 1994). We conducted this study aiming to provide the first accounts of the spatial and temporal aspects of local aggregations of immature green sea turtles foraging on the Gulf of Guinea islands using in-water surveys, and to assess possible patterns of resource use using stable isotope analysis. We sampled individuals at 2 distinct habitats (seagrass vs. macroalgae) on São Tomé Island and investigated how the use of these habitats by different

size classes could be reflected in their isotopic niches. We sought validation of our results by (1) comparing the isotopic signatures of the immature, presumably local, individuals with those of breeding females, as female signatures should represent distant foraging grounds visited in the months preceding their migration (Stearns 1992), and (2) sampling a selection of putative diet items to obtain clues about preferred diets and resource use by potentially resident immatures. This dataset offers an insight into green turtle recruitment and settlement dynamics in the Gulf of Guinea islands and will be the first dataset available for comparison with demographic data from other locations in West Africa, where current knowledge on green turtle foraging behavior is limited or non-existent.

MATERIALS AND METHODS

Study sites

São Tomé Island is one of the 2 islands comprising the small, insular country of São Tomé and Príncipe that is located in the Gulf of Guinea, West Africa, approximately 250 km off the continental mainland. The littoral fringe surrounding the island covers approximately 450 km² above the 200 m isobar (Afonso et al. 1999).

Informal interviews were conducted with spear fishermen, turtle hunters and fish sellers in the main coastal communities of the island throughout 2014 and 2015, with the aim of identifying sea turtle aggregation areas or historical hunting grounds, as well as potential diet items that may be primarily consumed by the turtles using those areas. An island-wide survey of sites presumed to offer either suitable foraging habitat (including the existence of extensive, shallow macroalgae or seagrass banks) or availability of shelter and/or resting areas and with evidence of the all-year-round presence of sea turtles was conducted by boat over 2 d in September 2015. The survey covered the entire coastline and was carried out with the participation of local spear fishermen and turtle hunters. All sites that were visited and visually inspected by snorkeling are depicted in Fig. 1; we considered sites where we could not confirm the presence of turtles as 'potential foraging or aggregation sites'. Two areas where sea turtles were observed feeding or resting were selected for this study: Ilhéu das Cabras site (northern foraging ground, FGN, 0° 21.802' N, 6° 45.402' E); and Porto Alegre (southern foraging ground, FGS), with 2 sub-

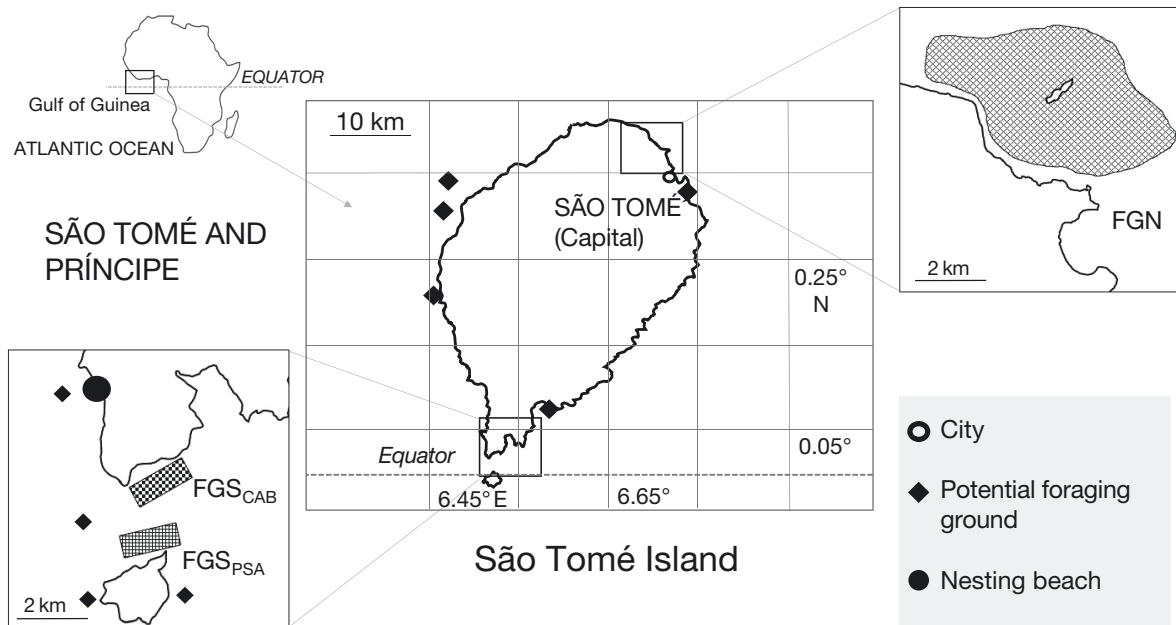


Fig. 1. Location of the 2 foraging grounds (FG) and the nesting beach (Jalé) sampled during this study, as well as potential foraging grounds (sites identified by local fishermen for which no data are available) during 2 large-scale surveys conducted in 2015. Area and location of the northern foraging ground (FGN) obtained from Alexandre et al. (2017). Sub-sites: CAB (Praia Cabana); PSA (Ponto Santo António)

sites, Praia Cabana (FGS_{CAB} , $0^{\circ} 1.310' N$, $6^{\circ} 31.407' E$) and Ponta Santo António (FGS_{PSA} , $0^{\circ} 0.408' N$, $6^{\circ} 31.622' E$) (Fig. 1).

For each site, the predominant habitat type and associated algae or seagrass species was assessed visually. Average depth was calculated by taking several readings using a depth gauge and approximate area was estimated using QGIS. A short description of the sites is provided in Table 1.

In-water visual surveys

Efforts to document sea turtle presence were carried out between November and February 2016 and

2017, and included (1) in-water visual daytime surveys either by snorkeling (underwater) or at the surface (from a boat) and (2) hand capture of live turtles during daytime (opportunistic) and night (targeted) surveys. The survey methods were adapted to the characteristics of each site, such as habitat type, depth, area and water visibility (e.g. Roos et al. 2005, Mancini et al. 2015).

The southern foraging or aggregation areas (FGS_{CAB} and FGS_{PSA}) were associated with rocky areas of spurs and groves at 8–12 m depth that offered resting and hiding areas for turtles, and dense macroalgae mats. Here, we conducted underwater visual surveys (10 and 5 transects performed at FGS_{CAB} and FGS_{PSA} , respectively), consisting of belt

Table 1. Location and habitat characterization of the study sites. FGN: northern foraging ground; FGS: southern foraging ground

| Site | Sub-site | Location | Habitat type | Distance to shore/ area surveyed | Range of depths (m) | Dominant plant or algal species |
|------|--|--|------------------|-------------------------------------|---------------------|--|
| FGN | Ilhéu das Cabras | $0^{\circ} 21.802' N$, $6^{\circ} 45.402' E$ | Seagrass patches | 2000 m 1500 ha ^a | 4–7 | Seagrass <i>Halodule wrightii</i> Macroalgae <i>Dyctiota</i> spp. and <i>Caulerpa</i> spp. |
| FGS | Praia Cabana (FGS_{CAB}) | $0^{\circ} 1.310' N$, $6^{\circ} 31.407' E$ | Rocky reef | 200 m 55 ha | 6–10 | Macroalgae <i>Dyctiota</i> spp. |
| | Ponta Santo António (FGS_{PSA}) | $0^{\circ} 0.408' N$, $6^{\circ} 31.622' E$ | Rocky platform | 500 m 40 ha | 8–15 | Macroalgae <i>Polysiphonia</i> spp. and <i>Dyctiota</i> spp. |

^aAlexandre et al. (2017)

transects following Roos et al. (2005). On these transects, 2 surface swimmers moved parallel to each other at the same speed, along one contiguous strip approximately 30 m wide (determined by the underwater visibility) and approximately 500 m long, parallel to the shore, resulting in approximately 3 ha covered in each survey. Each transect was usually covered within 30 min, depending on surface currents. In the shallow seagrass-dominated site at FGN (<7 m depth), where turtles can be easily seen from the boat, 2 surveys were conducted from the boat only under conditions of excellent water visibility, following an expanding square search pattern to maximize the area covered (e.g. Bell 1990, Christman et al. 2013, Acebes et al. 2016), covering an area of approximately 200 ha during each survey, and lasting approximately 60 min each.

At all sites, every time a turtle was sighted, the turtle's behavior (swimming, resting or feeding) and approximate size class was observed and noted, and the location was recorded using a hand-held GPS. When possible, males were identified by their external sexual characteristics (Wyneken & Witherington 2001). Sighting data were used to calculate capture per unit effort (CPUE) and to assess habitat use; size classes present at each site were only evaluated after hand capture of individual turtles (see 'Sea turtle capture and handling').

Sea turtle capture and handling

Immature and adult female turtles were sampled for this study at 2 foraging sites and 1 nesting beach, respectively. Each turtle sampled had the minimum curved carapace length (CCL_{\min} ; notch to notch, ± 0.1 cm) measured using a flexible measuring tape, and was double tagged with Inconel tags (Style 681; National Band and Tag Company)—one tag in the second large proximal scale of each front flipper. Tissue samples were collected from the trailing edge of the rear flipper of each turtle using a sterile razor scalpel and stored in 96% ethanol until processing in the lab. All seized turtles were released on-site within 30 min of capture. Turtle sampling methods are as follows.

Immatures

All immatures were hand captured. Due to the distinct characteristics of each site, we employed different approaches to capture turtles. At FGN, we used

the rodeo technique (Ehrhart & Ogren 1999), in which one person jumped into the water and attempted to capture the turtles as they were sighted at or near the surface or resting at the bottom of the sea. At FGS, we selected Cabana (FGS_{CAB}) for targeted hand captures by free-diving after dusk, as turtles were easily found resting under rocky ledges, or well camouflaged among the macroalgae beds at this time of the day (J. M. Hancock pers. obs.). Hand captures at both sites were always performed by holding the anterior and posterior medial section of the turtle's carapace, pulling it out of the water by a slow, vertical ascension, lifting its head to keep the front flippers out of the water until it could be safely hoisted onto the boat, a method that has been shown to be safe for juvenile turtles in several previous studies (e.g. van Dam & Diez 1998, Ehrhart & Ogren 1999).

Adult females

Adult female turtles were sampled at Jalé Beach ($0^{\circ} 2.496' N$, $6^{\circ} 30.734' E$), the main nesting site for this species on São Tomé Island, during night patrols conducted by the technical staff of the project Programa Tatô of São Tomé during the same period.

Sampling of putative diet items

To obtain the reference isotope ratios for different trophic levels of the foraging ground communities and investigate the variation of isotopic ratios at a local scale, we collected samples of the main algae and plant species that were referred by turtle fishermen as being either commonly consumed by green sea turtles or that were most abundant in each sampling location (see Table 1). Because incorporating too many sources would reduce the resolution of mixing models, and we were interested in assessing the differential contribution of plant or algal and animal diets for different sea turtle size groups, we selected the most common plants or algae at each site, as well as the most common primary consumer or omnivore invertebrate. These included 4 species of macroalgae of different groups (*Caulerpa* sp. among Chlorophyta, *Dictyota* sp. and *Sargassum* sp. among Phaeophyceae, and *Polysiphonia* sp. among Rhodophyta), 1 species of seagrass (*Halodule wrightii*) and the common intertidal crab *Grapsus adscensionis* (Osbeck 1765). The macroalgae *Dictyota* sp. and the crab were the only putative diet items common at both for-

aging grounds, and so were collected near FGS_{CAB} (Inhame Beach, 0° 1.464' N, 6° 31.147' E) and FGN (Gamboa Beach, 0° 22.789' N, 6° 43.173' E) to identify a possible north–south isotopic distinctiveness in $\delta^{13}\text{C}$ values. Crab samples were stored in 96% ethanol until processing. Macroalgae and seagrass samples were stored in a hypersaline solution (2:1 saltwater/salt, as suggested by Tsuda & Abbott 1985) instead of ethanol since algal material will lose pigments and become very brittle quickly if stored in ethanol; no other fixative was available, and freezing was not possible. Preserved algae samples were kept in the dark and refrigerated ($\pm 4^\circ\text{C}$) until processing.

Stable isotope analysis

From each sea turtle sample, 0.10–0.25 g of the epidermis (i.e. stratum corneum) was carefully separated from any connective tissue, rinsed with deionized water, finely diced with a scalpel blade, weighed and oven dried for at least 12 h at 60°C . Samples of putative diet items were carefully rinsed with deionized water until all salt was removed, scraped gently to remove any debris or epiphytes, finely shredded with a scalpel blade and oven dried as described above. The isotopic signature of the putative diet items was determined using 3–5 replicate samples from each item. Lipid extraction was performed on all samples, using a solvent mixture of chloroform/methanol (2:1) to a final volume 3–5 times the volume of the tissue sample (approximately 1 g of tissue in 5 ml of solvent mixture). Samples were first centrifuged in an Eppendorf centrifuge (model 5403) for 1 min at $9000 \times g$ and left to rest for 30 min. After a second centrifugation for 10 min at 4°C and at the same speed, the supernatant was entirely removed. The previous step was repeated at least 3 times until the supernatant was clear, then the remaining sample was oven dried for at least 12 h at 60°C to remove any residual solvent. Subsamples of prepared tissue (0.75–1.0 mg of animal material, 4–5 mg of plant material) were weighed with a microbalance and packed in tin capsules for mass spectrometric analysis.

The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) in the samples were determined by continuous flow isotope mass spectrometry (Preston & Owens 1983) on a Sercon Hydra 20-22 (Sercon) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector) elemental analyzer for online sample preparation by Dumas combustion. The standards used were Protein Standard OAS, Sorghum

Flour Standard OAS (Elemental Microanalysis) and IAEA-N1 (IAEA) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; $\delta^{15}\text{N}$ results were relative to air and $\delta^{13}\text{C}$ results to PeeDee Belemnite (PDB). The precision of the isotope ratio analysis, calculated using values from 6 to 9 replicates of standard laboratory material interspersed among samples in every batch analysis, was $\leq 0.2\%$.

Analytical methodology

We calculated CPUE at both sites as the sum of the number of resting or feeding turtles observed per hour of underwater survey time. For data analysis purposes, we used the estimated size at which green turtles undergo ontogenic dietary changes in the southwestern Atlantic (45 cm CCL; Vélez-Rubio et al. 2016) to separate immature turtles into 2 distinct size classes: (1) 'small immatures' (CCL < 45 cm) and (2) 'large immatures' (CCL \geq 45 cm). The minimum sizes for mature turtles were defined as CCL > 80 cm for females (minimum size observed for nesting females at São Tomé Island; S. Vieira pers. comm.) and CCL > 90 cm for males. The cut-off size for males coincides with the minimum reproductive size estimated in the Atlantic (Goshe et al. 2010); furthermore, males captured in this study with a CCL < 90 cm did not show signs of reproductive activity, such as plastron softness or mating wounds (Wibbels et al. 1991, Blanvillain et al. 2008).

Isotopic niche parameters were computed using SIBER package V.2.0 (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011) in R V.3.2.2 (R Core Team, 2013). This program fits bivariate ellipses of isotopic space using Bayesian inference to describe and compare the isotopic niche of different life stages and or/sites. Standard ellipse areas (SEA) were corrected (SEAc) for low sample size using $\text{SEAc} = \text{SEA} (n - 1)(n - 2)^{-1}$. Niche overlap was measured using the overlapping areas of the corrected standard ellipses of each life-stage group instead of the convex hulls, due to the small sample size (Jackson et al. 2011, Syväranta et al. 2013).

We used SIAR V4.2 (Stable Isotope Analysis in R; Parnell & Jackson 2013), a Bayesian-mixing model that accounts for variation in isotopic discrimination and source values (Moore & Semmens 2008), to explore the potential contributions of the most abundant groups of primary producers versus that of consumers (occupying a different trophic level) to the diets of green turtles captured at each foraging ground. Because trophic discrimination factors are not known for neritic green turtles, we used 3 different estimates of

discrimination factors that together should provide robust insights into trophic interactions of turtles (Burkholder et al. 2011), estimated for (1) juvenile green turtles fed on a carnivorous diet (Seminoff et al. 2006; skin tissue: $\delta^{15}\text{N} = 2.80 \pm 0.11\text{‰}$, $\delta^{13}\text{C} = 0.17 \pm 0.03\text{‰}$), (2) herbivorous Florida manatees *Trichechus manatus latirostris* (Alves-Stanley & Worthy 2009; skin tissue: $\delta^{15}\text{N}$ [estimated] = $5.0 \pm 0.00\text{‰}$, $\delta^{13}\text{C} = 2.80 \pm 0.09\text{‰}$), and (3) average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ discrimination factors based on meta-analysis of isotopic studies by Caut et al. (2009) ($\delta^{15}\text{N} = 2.75 \pm 0.1\text{‰}$, $\delta^{13}\text{C} = 0.75 \pm 0.11\text{‰}$). We ran the analysis per size class (small vs. large immatures) as well as per location. Adults were not considered in this analysis as they are assumed to have foraged elsewhere.

RESULTS

In-water surveys

We recorded 95 observations of *Chelonia mydas*, in a total of 17 h of combined survey time at all locations. Despite the higher number of turtles observed at the FGS sites (58 and 31 turtles observed at the Cabana and Ponta Santo Antônio sites, respectively, 6 at the FGN site [Ilhéu das Cabras]), CPUE was similar at all sites (range 5–7 individuals per hour of survey time). Rough estimates of densities (as surveys were not intensive) ranged from $0.03 \text{ ind. ha}^{-1}$ at FGN to $40\text{--}55 \text{ ind. ha}^{-1}$ at FGS_{CAB} and FGS_{PSA} , respectively. Due to the proximity of both FGS sub-sites, we consider the estimated density values representative of the FGS site as a whole. Targeted efforts resulted in the hand capture of 34 individuals, including 3 males. None showed signs of being

actively reproducing, and all were observed feeding before capture. One adult female captured at the FGN site was observed feeding on seagrass and did not show fresh mating wounds or scars, and was therefore considered as a non-breeding individual. Details and biometric parameters of turtles sampled are summarized in Table 2.

Stable isotopes

The wide range of the values of $\delta^{13}\text{C}$ (-28.3 to -10.2‰) and $\delta^{15}\text{N}$ (5.8 to 13.2‰) observed in the animals sampled is a result of the large heterogeneity of signatures observed at the different locations, although the range of $\delta^{15}\text{N}$ values are better explained by the differences observed among different size-class groups (Table 2).

The isotopic signatures of all putative diet items are presented in Fig. 2 and in Table A1 in the Appendix. Macroalgae and crab items sampled at more than one location did not vary significantly in their isotopic signatures (t -test, $p > 0.05$, $n = 5$ in both cases; Table A2 in the Appendix); therefore, the samples were pooled. As expected, all plants or algae had a very low (and similar) $\delta^{15}\text{N}$, but their $\delta^{13}\text{C}$ varied widely, mainly because of the very low values of Rhodophytes (Fig. 2), which were very abundant only at the FGS sites.

The SIBER results indicated distinctive isotopic niches for each immature size class, as well as for immatures living at each foraging ground, as the overlap among all pairs of ellipses was null (Fig. 2). Small immatures occupied an entirely different niche to the larger immatures, with their ellipse overlapping by 33% with that of the adult females sampled

Table 2. Summary of data obtained during in-water surveys at the 2 main foraging sites in São Tomé. Values are means \pm SD and range. N: number of individuals sampled; CCL_{min} : minimum curved carapace length; FGN: northern foraging ground; FGS: southern foraging ground

| Site | Size class | N | CCL_{min} (cm) (min–max) | $\delta^{15}\text{N}$ (‰) (min–max) | $\delta^{13}\text{C}$ (‰) (min–max) |
|------------|----------------------|----|---|--|--|
| FGN | Large immatures | 5 | 73.8 ± 7.1 (64.0–83.0) | 6.9 ± 1.3 (5.8–8.9) | -11.9 ± 2.3 (-15.7–-10.2) |
| | Adult (non-breeding) | 1 | 109.0 | 6.7 | -10.0 |
| FGS | Small immatures | 10 | 38.0 ± 3.7 (34.0–45.0) | 10.8 ± 1.8 (8.6–14.0) | -17.9 ± 1.2 (-19.1–-15.4) |
| | Large immatures | 8 | 73.0 ± 14.1 (53.0–87.0) | 9.0 ± 1.7 (7.5–12.9) | -24.0 ± 3.1 (-28.3–-19.3) |
| Jalé Beach | Adult females | 12 | 96.5 ± 5.3 (88.0–105.0) | 12.9 ± 1.6 (10.6–15.8) | -18.2 ± 1.3 (-20.3–-16.2) |

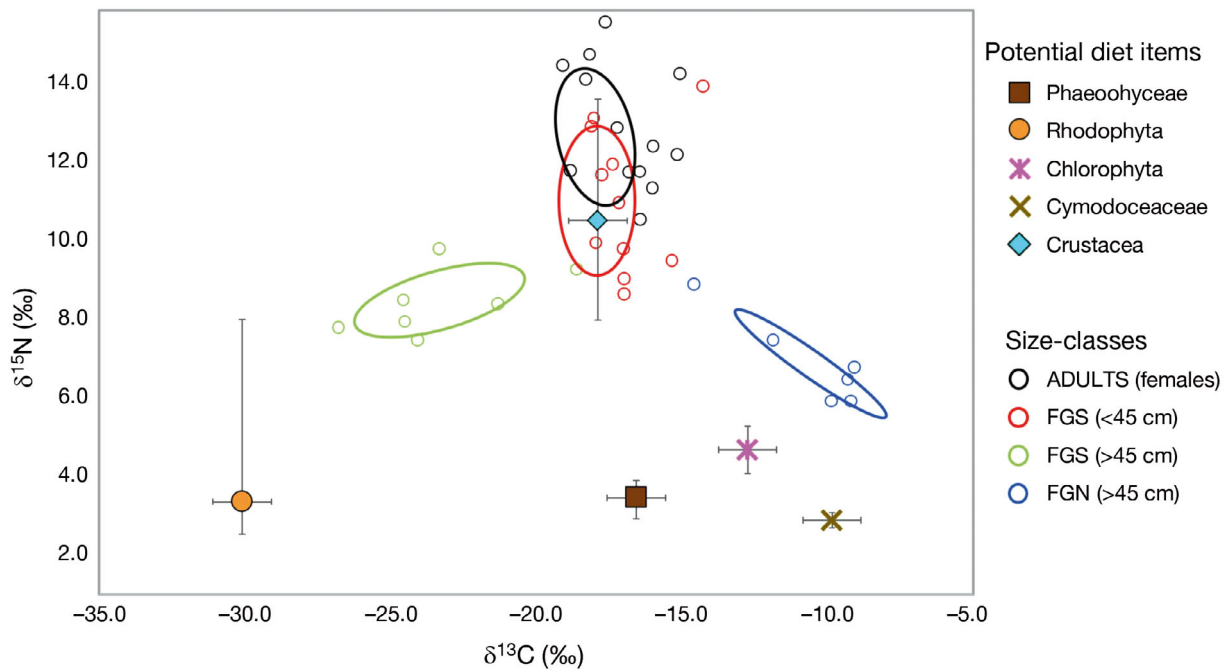


Fig. 2. Standard ellipse area corrected (SEAc) produced by SIBER indicating the trophic niches occupied by the distinct size classes. Open circles represent individual isotopic signatures. Shape symbols indicate mean isotopic signature of potential diet items and standard error values (bars)

at the nesting beach, who are not expected to forage off São Tomé Island. The distinctiveness of the larger immature’s isotopic niches and the size of their ellipses was clearly related to the 2 different foraging sites (Fig. 2). For this group, a smaller isotopic niche was calculated for those feeding at the FGN site than for those at the FGS site (Table 3). This distinction appears to be related to the relatively high contribution of Rhodophytes (*Polysiphonia* sp.) to the diet of specimens sampled in the FGS sites (Fig. 3B), while none of the algae or plants at the FGN site have a particular relevance to the turtle’s diet (Fig. 3A). The SIAR results also suggest that animal diets may be important for immatures, especially for the small size class (Fig. 3C, but see ‘Discussion’).

DISCUSSION

Foraging habitat use

Sea turtle fishermen indicated several foraging or aggregation sites (Fig. 1); however, we could not confirm this information at several sites, as no turtles were sighted during the snapshot surveys. Furthermore, the number of locations provided is likely to be limited to the fishermen’s experience and sites commonly used for fishing practices, and thus biased.

Nevertheless, the CPUEs and estimated densities recorded at the 2 selected study sites suggest that the macroalgae and seagrass patches around São Tomé Island, despite their small area, may maintain a few dozen sea turtles, at least during the months when the study was conducted (November–February). Considering that only 2 out of the several potential sites were surveyed more thoroughly and that the density of turtles in these sites was high, it is possible that future investigations will reveal more foraging grounds off the São Tomé coast.

Our results show that São Tomé hosts 2 discreet immature groups of foraging turtles: very small immatures, likely to have recruited recently to the neritic zone from their oceanic, omnivorous life stage, and larger immatures that explore the local resources for more extended periods, eventually as

Table 3. Standard ellipse area (SEA) metrics for different life-stage groups sampled at São Tomé Island. SEAc: SEA corrected

| Life-stage group | SEA | SEAc |
|---------------------|------|------|
| Adults | 6.52 | 7.03 |
| Small immatures FGS | 6.90 | 7.66 |
| Large immatures FGS | 5.93 | 7.12 |
| Large immatures FGN | 2.97 | 3.71 |

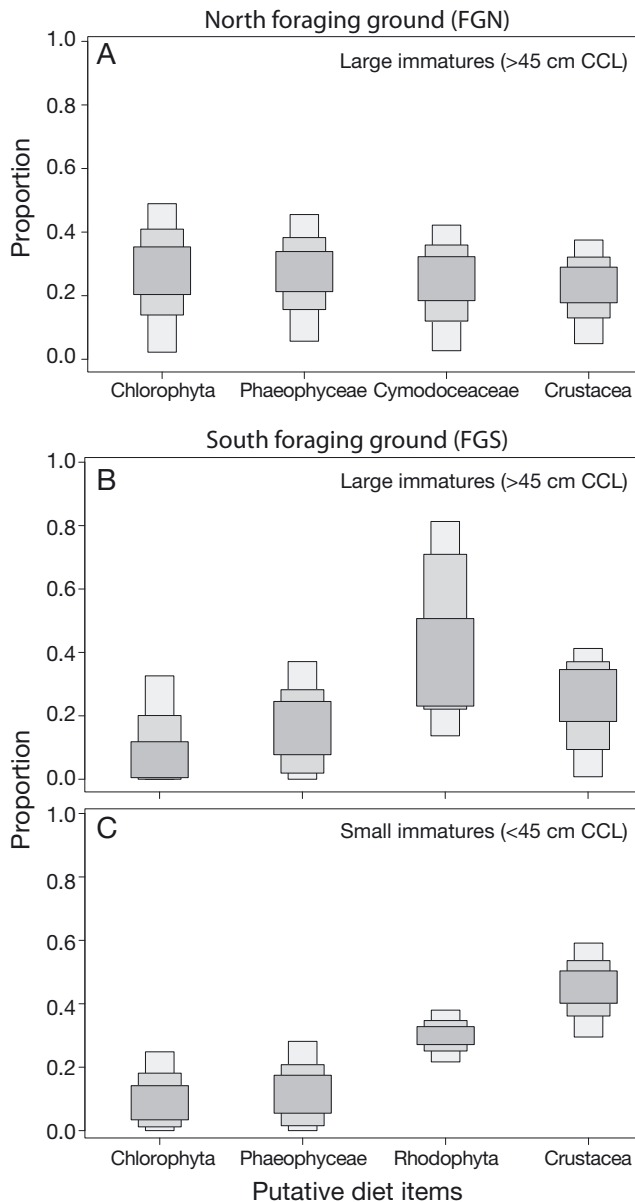


Fig. 3. Potential contribution of common diet items to the diet of immature green turtles, with credible intervals (50% inside dark grey boxes, 75% middle grey boxes, 100% outer light grey boxes, as determined by the SIAR mixing model. (A) Large immatures at FGN, (B) large immatures at FGS and (C) small immatures at FGS, as determined by the SIAR mixing model

residents. The smallest turtle captured in this study was 34 cm CCL, within the expected size at recruitment range for post-pelagic turtles of this species (Musick & Limpus 1997), and with a slightly smaller size than at other locations in the Atlantic (Reisser et al. 2013) or Pacific (Arthur et al. 2008). Small immatures were only found at the FGS sites; it is possible that the rocky substrate of the south of São Tomé is

well suited for omnivores, being rich in macroalgae and benthic invertebrates, while providing more resting or hiding sites for the smallest individuals than the exposed seagrass beds. It can also be used as a stopover area which green turtles recruit to after the pelagic phase and store resources before traveling to other developmental habitats (Bolten 2003, Reich et al. 2007). Nevertheless, it is possible that this size class was not observed at the FGN site due to the survey method used (e.g. lower detection of small individuals from the surface).

With the exception of 1 non-breeding adult female (109 cm CCL) captured at the FGN site, no adults were observed foraging at any of the sites during the breeding season, clearly indicating that São Tomé is an important recruitment and development habitat for juvenile green turtles in the region, and that after reaching maturity adults move to other foraging sites.

Recruitment and settlement

After recruiting to neritic habitats from pelagic waters, immatures of *Chelonia mydas* occupy developmental habitats, which are geographically separate from both the lost-year habitat and the adult resident habitat (Carr et al. 1978, Meylan et al. 2011). In the developmental habitats, they are expected to undergo an ontogenetic shift in foraging habits, from omnivory to feeding primarily on macroalgae or seagrass (or both) (Bjørndal 1997, Reich et al. 2007, Arthur et al. 2008) and occupy limited home ranges associated with specific grazing areas, while feeding and growing to maturity (Makowski et al. 2006, Shimada et al. 2016). As turtles settle in a foraging area, it is expected that their isotopic signatures begin to reflect those of the available diet items only after some time, since the median residence time of carbon and nitrogen stable isotopes in the epidermis of immature green turtles ranges from 27 to 35 d and from 11 to 31 d, respectively (Reich et al. 2008). There are no estimates for isotope turnover rates of large immatures, but alligator turnover rates have been shown to be up to 2 yr (Rosenblatt & Heithaus 2012). As the slower-growing tissues of larger immatures have longer turnover times (Martínez del Rio et al. 2009), the clear separation of the isotopic niches of turtles living at each foraging ground and the low variation in stable isotope values within each group is a strong indication of local settlement over time frames of at least many months (as in Bolnick et al. 2003, Bearhop et al. 2004, Cardona et al. 2009, Martínez del Rio et al. 2009). Residence periods of

immature green turtles at several foraging sites have been estimated to be as low as 744 d in Japan (Shimada et al. 2014), 11.2 yr (with a median of 2.4 yr) in Brazil (Colman et al. 2015) (interquartile range 1.2–4.2 yr), and up to 32 yr in Bermuda (Meylan et al. 2011). Moreover, as slow-maturing animals that may take from 14 to 44 yr to mature (Bjørndal et al. 2000, Bell et al. 2005, Goshe et al. 2010, Patrício et al. 2014), it is possible that these immatures remain in São Tomé waters for extended periods. Exclusive settlement to either site must, however, be interpreted with caution, as the small sample size at the FGN site may lead to an underestimation of the niche width (Syväranta et al. 2013).

Trophic status and diet preferences

Ontogenic diet shifts in green turtles from omnivory to herbivory has been thought to be abrupt and irreversible, despite growing evidence that high levels of omnivory remain among different life stages (e.g. Cardona et al. 2009, Burkholder et al. 2011, Lemons et al. 2011, González Carman et al. 2012, Burgett et al. 2018). Should immature green turtles be primarily herbivorous, their isotopic signature should be 1 trophic level above the primary producers, and reflected by tissue $\delta^{15}\text{N}$ enrichment of $\sim 2.8\%$ (Seminoff et al. 2006). The high nitrogen stable isotopic values of all the small immatures sampled suggest high levels of omnivory prior and/or soon after recruitment to neritic habitat in São Tomé, as observed elsewhere (e.g. Cardona et al. 2009, Burkholder et al. 2011, Lemons et al. 2011, González Carman et al. 2012). Even for larger immatures, the observed $\delta^{15}\text{N}$ values at both foraging grounds are higher than the expected values for strict herbivores, considering the signatures of the most common algae (Fig. 2). These animals may be supplementing their diet with animal protein (Fig. 3C), or may still be far from the isotopic equilibrium with their diets. Further evidence is obtained by the inclusion of a primary consumer in the isotope mixing models. Although it is not possible to ascertain direct consumption of these specific crustaceans or any animal matter due to the limitations of our sampling approach and of the mixing models, our results suggest that the contribution of animals to the diets of immature green turtles is not negligible. Despite the omnivory suggested for all immature stages in São Tomé waters, a clear diet ontogenic shift is suggested by the contrasting signatures of small and large immatures, reflecting adjustments to a new diet.

The differences in isotopic signatures between the 2 groups of large immatures are mainly explained by the contrasting distributions of the red algae *Polysiphonia* sp., which is the dominant species at the FGS sites, and of the seagrass *Halodule* sp. and the green algae *Caulerpa* sp., found mainly at the FGN site. Red algae such as *Polysiphonia* have more negative $\delta^{13}\text{C}$ values than other algae, which is attributed to their photosynthetic pathways (Raven et al. 2002). The importance of the red algae mats for our results is further reinforced by the lack of spatial variation found in the carbon signature of the brown algae *Dictyota* sampled at both sites. This observation is in line with other studies that show that at foraging grounds where green turtles are algal feeders, algae within the division Rhodophyta are most commonly found in the diet (e.g. Mortimer 1981, Brand-Gardner et al. 1999, López-Mendilaharsu et al. 2008). Previous studies have shown that this class of algae has a higher nutrient content (Montgomery & Gerking 1980, Brand-Gardner et al. 1999), higher protein (Fleurence 1999, McDermid & Stuercke 2003) and higher digestibility (Wong & Cheung 2001), which may be a strong factor affecting the foraging preferences of green turtles. In the north, the foraging ground is mostly limited to the existing seagrass mats, which offers a variety of prey items, yet represents a much smaller area (estimated area of 1500 ha; Alexandre et al. 2017), when compared with the southern feeding ground, and turtles appear to be less selective in their diet.

CONCLUDING REMARKS

Taking into consideration that only 2 of the available foraging areas were surveyed, and that the number of turtles at those 2 sites was high, São Tomé, as well as the similar islands in the Gulf of Guinea, may provide an important array of suitable foraging habitats for immatures of *Chelonia mydas* in the region. There is clear evidence of settlement and local exploitation of available resources, as well as of variation in foraging behavior between various size classes and life stages. These results suggest that conservation efforts should account for the possibility that subsets of the larger regional population may play different ecological roles and may be differentially vulnerable to anthropogenic impacts. Our study reveals the need for further research in neighboring islands in the Gulf of Guinea to assess the importance of these aggregations of immature turtles to each of the regional management units identified for this population in the Atlantic.

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Appendix

Table A1. Stable isotope values for the putative diet items sampled

| Diet item | Mean $\delta^{13}\text{C}$ | SD $\delta^{13}\text{C}$ | Mean $\delta^{15}\text{N}$ | SD $\delta^{15}\text{N}$ |
|---------------|----------------------------|--------------------------|----------------------------|--------------------------|
| Chlorophyta | -12.74 | 0.62 | 4.52 | 0.62 |
| Phaeophyceae | -17.41 | 0.70 | 3.01 | 2.16 |
| Rhodophyta | -30.10 | 0.84 | 3.16 | 4.76 |
| Cymodoceaceae | -9.84 | 0.21 | 2.68 | 0.21 |
| Crustacea | -17.88 | 2.60 | 10.51 | 3.18 |

Table A2. Results of statistical significance tests (*t*-test) for different isotopic signatures observed for 2 diet items (crustacean *Grapsus* sp. and brown algae *Dictyota* sp.) collected at distinct sites on São Tomé Island. FGN: northern foraging ground; FGS: southern foraging ground

| Species | Isotope | Site | N | Mean | SD | <i>t</i> -value | p-value |
|---------------------|-----------------------|------|---|--------|------|-----------------|---------|
| <i>Grapsus</i> sp. | $\delta^{15}\text{N}$ | FGS | 5 | 11.0 | 4.15 | 0.534 | 0.622 |
| | | FGN | 5 | 10.02 | 2.22 | | |
| | $\delta^{13}\text{C}$ | FGN | 5 | -17.42 | 3.63 | 0.553 | 0.609 |
| <i>Dictyota</i> sp. | $\delta^{15}\text{N}$ | FGS | 5 | 3.22 | 0.43 | 1.231 | 0.286 |
| | | FGN | 5 | 2.96 | 0.23 | | |
| | $\delta^{13}\text{C}$ | FGN | 5 | -16.62 | 0.48 | 0.775 | 0.481 |
| | | FGS | 5 | -16.76 | 0.58 | | |

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