

*Research Article*

## Trophic ecology of hawksbill turtles (*Eretmochelys imbricata*) in Golfo Dulce, Costa Rica: integrating esophageal lavage and stable isotope ( $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ ) analysis

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**ABSTRACT.** Hawksbill turtles (*Eretmochelys imbricata*), considered Critically Endangered, have several small populations in the Eastern Pacific (EP). Knowledge about their diet and habitat use can aid in developing conservation strategies and promoting population recovery in the region. Although considered a spongivore in the Caribbean, data from the EP region indicate that hawksbills consume a wide array of prey species, including angiosperms. We used two approaches to study the diet of hawksbills at Golfo Dulce, Costa Rica: esophageal lavage and stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) analysis of bulk skin tissue and blood plasma. Lavage samples collected from 41 turtles revealed macroalgae as the predominant diet item ( $R_w = 20.22$ ), followed by sea snails and excavating worms. Stable isotope values for blood plasma from 44 turtles ranged from  $-23.0\text{‰}$  to  $-15.7\text{‰}$  for  $\delta^{13}\text{C}$  and  $6.9\text{‰}$  to  $10.4\text{‰}$  for  $\delta^{15}\text{N}$ , whereas values for skin tissue were  $-20.4\text{‰}$  to  $-13.9\text{‰}$  and  $9.3\text{‰}$  to  $11.0\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. We compared these isotope values with those of five potential prey groups (sponge, sea snail, excavating worm, mangrove, macroalgae) using a multisource stable isotope mixing model analysis in R (SIAR). Our results indicated that multiple prey resources are important for hawksbills in Golfo Dulce, where sea snails, sponges and excavating worms contributed up to 63% of the assimilated diet per individual, and mangrove and macroalgae up to 50%. These data show that hawksbills in Golfo Dulce, and perhaps the wider EP region, are omnivorous, underscoring the importance for considering alternative habitats, aside of coral reefs, for its management and restoration.

**Keywords:** *Eretmochelys imbricata*; marine turtles; diet; resource requirements; eastern Pacific

### INTRODUCTION

Within coastal anthropogenically impacted habitats understanding the biology of threatened species is fundamental for their conservation (Wallace *et al.*, 2010, 2011). Knowledge about the species' feeding behaviour, diet and trophic ecology can be useful for identifying the most important resources within foraging areas and can help pinpoint priority sites for habitat protection (Blumenthal *et al.*, 2009; Gaos *et al.*, 2012b; Seminoff *et al.*, 2012; Revuelta *et al.*, 2015). Sea turtles represent a valuable and enigmatic taxonomic group, for which this information is appropriate

for conservation practices (Hamann *et al.*, 2010; Rees *et al.*, 2016).

A variety of methods have been developed to study sea turtle feeding behavior and diet composition, including direct observation, esophageal lavage, faecal examination and stable isotopes analysis (SIA) (Forbes, 1999; Gaos *et al.*, 2011; Jones & Seminoff, 2013). Each of these methods has its pros and cons: for example, esophageal lavage is a relatively simple, low-cost technique that provides information about recently ingested items, but the recovered diet items are often biased toward softer and smaller items, many of which are too small to identify to species. Lavage may also

reveal items that are consumed but not assimilated, which thus hinders the interpretation of the most important foods for nutrition (Forbes, 1999; Seminoff *et al.*, 2002; Lemons *et al.*, 2011). In contrast, direct observation may be a less invasive way to observe diet intake by sea turtles in the wild, but this approach is often logistically difficult for cryptic species that respond negatively to human observers in their surroundings. Furthermore, this technique can be time-consuming and susceptible to inconsistent results due to environmental variability (*e.g.*, water visibility, Blumenthal *et al.*, 2009; Mancini *et al.*, 2015).

A relatively new approach for determining the diet and trophic ecology of consumer species, such as sea turtles, is bulk tissue SIA (Godley *et al.*, 1998). This technique yields information about the trophic status and dietary history of an individual via collection of small tissue samples and analysis of their stable isotope compositions. This is based on the fact that the isotopic composition of a consumer's body tissues is derived from its prey sources and the ecosystem within which it lives (Reich *et al.*, 2008; Jones & Seminoff, 2013; Carpentier *et al.*, 2015). Stable nitrogen isotope values ( $\delta^{15}\text{N}$ ) are used for estimating the trophic position of a consumer within a food web due to the step-wise enrichment of  $\delta^{15}\text{N}$  with each trophic step (Jones & Seminoff, 2013; Carpentier *et al.*, 2015), whereas stable carbon isotope ( $\delta^{13}\text{C}$ ) values of consumer tissues are generally used to determine the source of nutrients, with a continuum of progressive  $\delta^{13}\text{C}$  depletion in tissue of animals as they forage farther from the coast (Gannes *et al.*, 1998; Jones & Seminoff, 2013). In omnivores or herbivores,  $\delta^{13}\text{C}$  values in tissue may also be influenced by the photosynthetic reactions and glucose synthesis of plant species, as well as the carbon sources for these consumed foods (Hobbie & Werner, 2004). For example, in coastal estuarine habitats, mangroves (*e.g.*, *Rhizophora mangle*), which are marine angiosperms that derive carbon from terrestrial-based systems, will have substantially depleted  $\delta^{13}\text{C}$  values relative to seagrass and, to a lesser extent, marine macroalgae located only a few meters away (Lepoint *et al.*, 2004). As a result, SIA is complementary to other techniques such as esophageal lavage, and their concurrent application can provide a more holistic perspective about what prey items are most important for nutrient assimilation in wildlife species (Williams *et al.*, 2014; Espinoza *et al.*, 2015).

The hawksbill turtle (*Eretmochelys imbricata*) is listed as Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2017) because of population declines around the world, due largely to the commercialization of its shell and eggs (IUCN, 2016). In the Caribbean, the

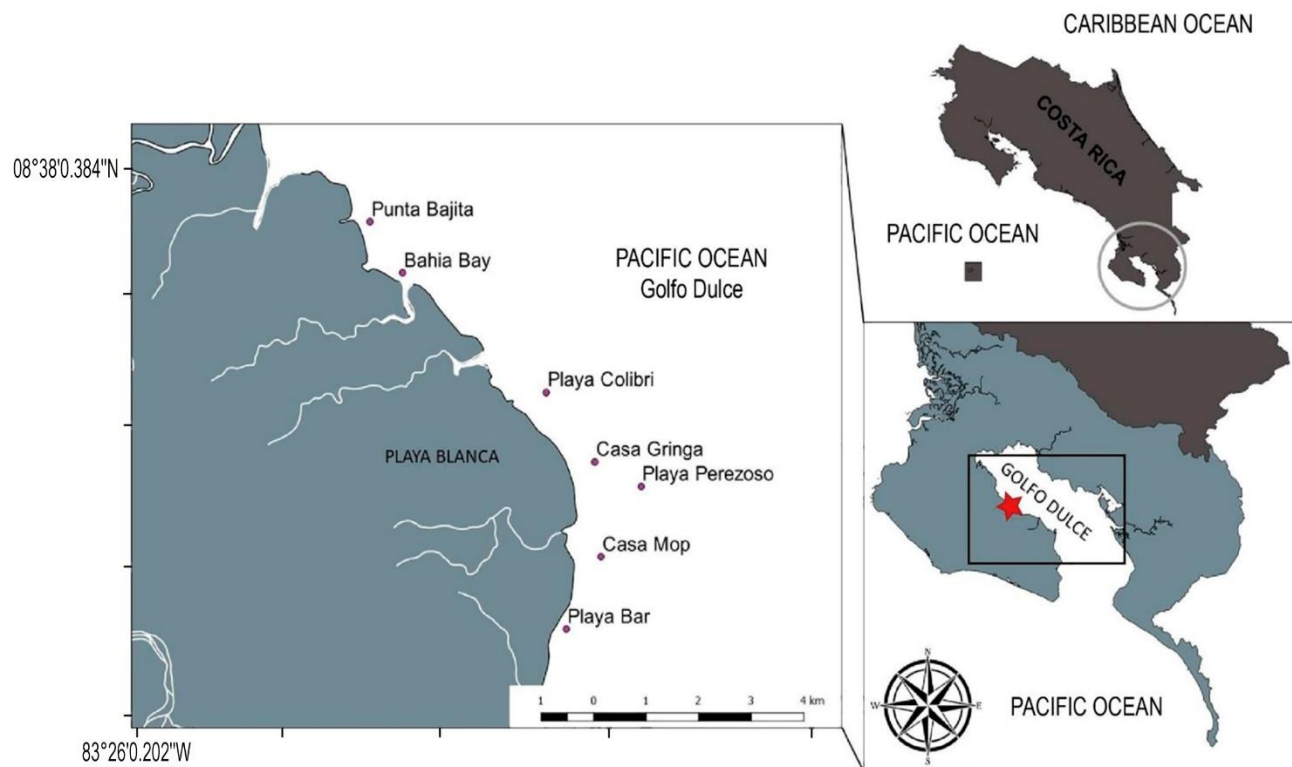
species is considered spongivorous (Meylan, 1988; León & Bjørndal, 2002; Parker *et al.*, 2009; Bell, 2012; Wood *et al.*, 2017); however, the feeding ecology of hawksbills in the eastern Pacific (EP) is unclear, and may vary spatially, depending on the habitat occupied. Seminoff *et al.* (2003) reported that the stomachs of two dead hawksbills in the Gulf of California, at the extreme northern edge of their range, consisted largely of the sponge *Haliclona* sp., whereas Carrión *et al.* (2013) described diet for 13 individuals along the Nicoya Peninsula, Costa Rica, reporting that their diet consisted of the sponge *Geodia* sp. and the ascidian *Rhopalaea birkelandi*. Considering the small sample sizes of previous studies, additional information is needed to better understand hawksbill foraging ecology in the EP region.

One area that hosts relatively large numbers of hawksbills, but at which diet has not yet been studied, is Golfo Dulce, a tropical estuarine fjord along the Pacific coast of Costa Rica (Fig. 1). Here, we examined the diet and trophic status of hawksbills using multiple approaches. We used esophageal lavage to evaluate ingested diet items, and we applied stable-carbon ( $\delta^{13}\text{C}$ ) and -nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis of bulk skin tissue and blood plasma to infer the most important assimilated foods for hawksbills. Our stable isotope data were also used to infer individual fidelity to this ecosystem by analyzing the information obtained from recaptured individuals and inspecting for aberrant isotopic values that were not indicative of the local stable isotope regime. These findings will contribute greater information to support the conservation strategies for this protected coastal ecosystem and its resources, indirectly ensuring the conservation of this emblematic species. Our data will also serve as a baseline for which to compare with future studies of hawksbill foraging ecology in Golfo Dulce and other areas within the EP.

## MATERIALS AND METHODS

### Study area

We studied hawksbill turtles along the coast of Playa Blanca in the western portion of Golfo Dulce, Costa Rica (N08°38'0.384" W83°26'0.202"; Fig. 1). This region is characterized by the presence of seagrasses, macroalgae, and mangroves with small patches of degraded coral reefs (Cortés, 1991; León & Vargas, 1998; Morales, 2010; Samper *et al.*, 2014). Golfo Dulce is a broad, fjord-like embayment approximately 50 km long and 10-15 km wide. A sill area 20 km from the bay's entrance separates an inner, flat bottom basin with depths up to 215 m from a shallow outer basin. Annually, the air temperature typically varies from



**Figure 1.** Sampling areas of hawksbill turtles (*Eretmochelys imbricata*) foraging in Golfo Dulce, Costa Rica.

23°C to 33°C, and rarely gets below 21°C or above 35°C. The hot season lasts from January to April, with an average daily maximum air temperature above 32°C. The cool season occurs from August to December, with an average daily maximum air temperature below 29°C (Svendsen *et al.*, 2006).

### Turtle capture

Seven hawksbill capture sites were established (Fig. 1), which cover the variety of habitat types within Playa Blanca (Table 1). Two entanglement nets (mesh size 50 cm), one measuring 100×8 m and the other 80×8 m, were joined together and used for hawksbill captures. The attached nets were set for a minimum of six hours, starting at 08:00, and were constantly monitored during the entire soak time. Net orientation and distance from shore (100 to 500 m) varied depending on the tides and precipitation (Chacón *et al.*, 2015). Once the nets were positioned, they were georeferenced with a global positioning system (Model 62S, Garmin®). We calculated catch per unit effort (CPUE) as the total number of hawksbill turtles caught in each sampling event divided by the number of capture effort units, defined as the deployment of 180 m of net for 6 h (Chacón *et al.*, 2015).

Upon capture, turtles were transported to the shore where they were processed (Chacón *et al.*, 2015). We measured curved carapace length (CCL) from the nuchal notch to the posterior-most tip of the carapace. We conducted each measurement three times with a flexible tape measure and recorded the mean of these three measurements ( $\pm 1.0$  mm) to reduce error (Labrada, 2011; Carrión *et al.*, 2013; Chacón *et al.*, 2015). Turtles were classified as putative adults if their  $CCL \geq 62.2$  cm (Gaos *et al.*, 2017). Each turtle was tagged with an Inconel® flipper tag (Style 681, National Band and Tag Co., Newport, KY, USA) in the second axillary scale of each front flipper (Chacón *et al.*, 2015) if it was not previously tagged. All recaptured turtles were re-measured, but tissue samples were collected only from turtles recaptured  $\geq 1$  month after the previous capture (Seminoff *et al.*, 2007). All personnel involved in the handling process used latex gloves as per biosecurity regulations and all tissue collection and flipper tagging sites were disinfected with alcohol (Forbes, 1999; Chacón *et al.*, 2015). After measurement and sampling, all turtles were released at the site of initial capture (Labrada, 2011; Chacón *et al.*, 2015), usually within 30 min.

**Table 1.** Description of areas where hawksbill turtle *Eretmochelys imbricata* sampling took place between March and August 2015.

Location	Coordinates (CR-05)	Description
Playa Bar	08°38'0.87"N 83°26'0.325"W	Predominantly rocky and sandy bottom, with patches of seagrass. Coastal vegetation mainly of palms with patches of mangrove.
Casa Mop	08°38'0.280"N 83°26'0.276"W	A substrate of rocky, muddy, and sandy patches, with seagrass and presence of degraded coral formations. The coast of mixed vegetation with mangrove and palm. River mouth.
Playa Perezoso	08°38'0.504"N 83°25'0.513"W	Mixed rocky, sandy, and muddy substrate. Coast with mangroves and palms.
Casa Gringa	08°39'0.830"N 83°25'0.537"W	Muddy and sandy bottom, with seagrass. Coast with mangrove and palms.
Playa Colibrí	08°39'0.352"N 83°25'0.548"W	Muddy and sandy bottom, with seagrass. Coast mainly of palms. River mouth.
Bahía Bay	08°40'0.999"N 83°26'0.301"W	Muddy and sandy soil with seagrass. Coastal mangrove vegetation. River mouth.
Punta Bajita	08°40'0.324"N 83°26'0.462"W	Muddy and sandy bottom with seagrass. Coast with mangroves. River mouth.

### Esophageal lavage

We conducted oesophageal lavage on each captured turtle following previously established protocols (Forbes, 1999; Stokes & Epperly, 2008; Bell, 2012; Carrión *et al.*, 2013). Briefly, each turtle was placed on its plastron at a 45° angle and a tube was inserted into the turtle's mouth no deeper than the intersection between the neck and anterior plastron. The diameter of the wash tube ranged from 0.5 to 1 cm outside diameter and was changed according to the size of each turtle so as to diminish the possibility of injuring its oesophagus (Bell, 2012; Carrión *et al.*, 2013). For each turtle, lavage efforts lasted 5 min or less and were conducted with three litres of clean seawater collected from the same area where the turtle was captured. Lavage samples were preserved in a saturated salt (NaCl) solution and refrigerated until analysis. After transfer to the laboratory of the Regional Institute of Toxic Substances Studies of the National University of Costa Rica (UNA, for its acronym in Spanish), the net weight of each sample was determined and all recovered diet items were examined by microscope and identified to the lowest taxonomic unit (Forbes, 1999; Stokes & Epperly, 2008; Carrión *et al.*, 2013).

### Skin tissue collection and preparation

We collected epidermis (*i.e.*, skin) tissue (stratum corneum) from the dorsal surface of the neck of each captured turtle using a razor blade. We were careful to collect only epidermal tissue, avoiding the connective tissue layer. Skin samples were preserved in saturated salt solution and placed on ice for transport to the laboratory, where they were stored at -20°C until preparation and analysis (McClellan *et al.*, 2010; Reich

& Seminoff, 2010; Lemons *et al.*, 2011). Prior to SIA, skin samples were thawed, rinsed with distilled water, frozen at -80°C and lyophilized for 24 h in the Laboratory of Bacteriology at UNA. Dried samples were then stored in labelled vials until preparation for SIA.

### Blood sample collection and preparation

Blood samples were obtained via the dorsal cervical sinus (Owens & Ruiz, 1980). Puncture sites were cleansed with alcohol and betadine and a 23 or 25 gauge needle was used to obtain the sample (Strik *et al.*, 2007). From each individual, 4 mL of blood were collected, stored in Vacuette® lithium heparin tubes, and centrifuged for five minutes to separate the plasma from cellular blood constituents (Owens & Ruiz, 1980; Seminoff *et al.*, 2006; Lemons *et al.*, 2012). Plasma was then transferred to labelled tubes and stored in liquid nitrogen until preparation for SIA. Prior to analysis, each plasma sample was lyophilized for 24 h then stored in a microtube at room temperature until  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses (Reich & Seminoff, 2010; Seminoff *et al.*, 2012).

### Prey sample collection and preparation

Tissue and whole bodies of putative prey species were collected during opportunistic SCUBA efforts along the coast of Playa Blanca, mostly gathered from rocky-bottom areas such as Playa Bar, Casa Mop and Playa Perezoso (Fig. 1). We collected three 'indicator' species in as many areas as possible to determine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for putative hawksbill prey within the study area. These included the sponge (*Mycale* sp.), an excavating worm (*Amphicteis* sp.) and a snail (*Cerithium* sp.). These species were abundant within Golfo Dulce and were

considered possible dietary items based on previous reports (Gaos *et al.*, 2011, 2012b; Carrión *et al.*, 2013). In total, we collected eight snail samples, six worm samples, and four sponge samples. Diet samples were preserved in saturated salt solution and stored at  $-20^{\circ}\text{C}$  until preparation and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses. We were unable to collect mangrove (*Rhizophora mangle*) and macroalgae within our study area due to logistic constraints; we therefore used values published in the literature for Gulf of Chiriquí, Panamá (Viana *et al.*, 2015), which is relatively close to Golfo Dulce and within the same marine isotopic regime (Table 2; Seminoff *et al.*, 2012, *unpubl. data*).

### Stable isotope measurement

Prepared samples were sent to the Department of Geosciences Stable Isotope Laboratory at University of Florida, Gainesville, Florida USA, where they were loaded into sterilized tin capsules and analysed by a continuous-flow isotope-ratio mass spectrometer (Lemons *et al.*, 2011). We used a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT, Bremen, Germany) to a Deltaplus gas isotope-ratio mass spectrometer (Finnigan MAT, Bremen, Germany) (Lemons *et al.*, 2011). Sample SI ratios relative to the isotope standard are expressed in the following conventional delta ( $\delta$ ) notation in parts per thousand (‰):  $\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the corresponding ratios of heavy to light isotopes ( $^{13}\text{C}/^{12}\text{C}$ :  $\delta^{13}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ :  $\delta^{15}\text{N}$ ) in the sample and standard, respectively (Lemons *et al.*, 2011).  $R_{\text{standard}}$  for  $^{13}\text{C}$  was Baker Acetanilide ( $\text{C}_8\text{H}_9\text{NO}$ ;  $\delta^{13}\text{C} = -10.4\text{‰}$ ) calibrated monthly against the Peedee Belemnite (PDB) limestone formation international standard;  $R_{\text{standard}}$  for  $^{15}\text{N}$  was IAEA N1 Ammonium Sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ;  $\delta^{15}\text{N} = +0.4\text{‰}$ ) calibrated against atmospheric  $\text{N}_2$  and USGS Nitrogen standards. All analytical runs included samples of standard materials inserted every 6 to 7 samples to calibrate the system and compensate for any drift over time. Replicate assays of standard materials indicated measurement errors of 0.05‰ and 0.095‰ for carbon and nitrogen, respectively. In addition to SI ratios, we measured %C and %N for each diet and tissue sample (Lemons *et al.*, 2011). Samples were combusted in pure oxygen in the elemental analyser. Resultant  $\text{CO}_2$  and  $\text{N}_2$  gasses were passed through a series of thermal conductivity detectors and element traps to determine percent compositions. Acetanilide standards (10.36% N, 71.09% C) were used for calibration (Lemons *et al.*, 2011).

### Statistical analysis

We determined the percent volume (%VO) for each of the items recovered in oesophageal lavages, we

determined the percent volume for each one (%VO) where  $\% \text{VO} = \frac{\text{item weight in diet}}{\text{total sample weight}} \times 100$ . Any element with a %VO greater than 5% in at least one sample was considered to be major diet item and not incidentally consumed (Arthur & Balazs, 2008; Carrión *et al.*, 2013). We calculated the frequency of occurrence (%FO) for all diet constituents (Mohan & Sankara, 1988; Carrión *et al.*, 2013), where:

$$\% \text{FO} = \frac{\text{number of samples in which the item was observed}}{\text{total number of samples}} \times 100$$

The results from these two calculations were combined to create a weighted index (Rw) that allowed us to estimate the order of importance of each element for the suite of items consumed (Mohan & Sankaran, 1988; Carrión *et al.*, 2013). This index was plotted as a function of the angle ( $\theta$ ), allowing us to interpret the importance of each element based on the consistency of %VO and %FO (Mohan & Sankaran, 1988).

$$\text{Rw} = \frac{Q(\text{V}^2 + \text{FO}^2)^{\frac{1}{2}}}{\sum Q(\text{V}^2 + \text{FO}^2)} \times 100, \text{ where}$$

$$Q = \frac{45 - |\theta - 45|}{45}, \text{ where } \theta = \tan^{-1} \frac{\text{FO}}{\text{V}}$$

Rw values varied between 0 and 100, where the elements closer to 100 represented the most important dietary components, whereas those items closer to 0 were of lesser dietary importance (Carrión *et al.*, 2013).

We used the stable isotope mixing model Stable Isotope Analysis in R (SIAR) to establish the possible dietary constituents and their contributions to hawksbill diet. The model was run using the diet-tissue discrimination factors ( $\Delta_{\text{dt}}$ ) from small juvenile loggerhead turtles (*Caretta caretta*; Reich *et al.*, 2008), as this is the most closely-related and ecologically-similar sea turtle species to hawksbills for which  $\Delta_{\text{dt}}$  values are available. For skin,  $\Delta_{\text{dt}}^{13}\text{C} = 2.62 \pm 0.34$  and  $\Delta_{\text{dt}}^{15}\text{N} = 1.65 \pm 0.12$ , whereas for plasma,  $\Delta_{\text{dt}}^{13}\text{C} = 0.29 \pm 0.20$  and  $\Delta_{\text{dt}}^{15}\text{N} = 0.32 \pm 0.09$ . Monthly distributions of prey contributions were generated with SIAR (Lemons *et al.*, 2011) to determine if prey contributions changed during the study duration.

## RESULTS

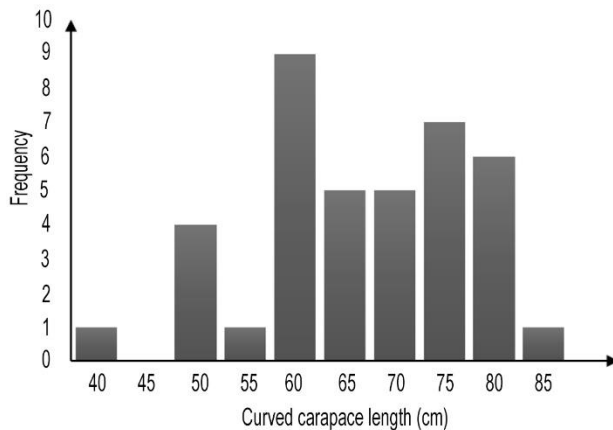
We conducted 42 field capture efforts and successfully captured at least one hawksbill turtle during 36 of these campaigns. There were 51 captures of 41 individuals, with four turtles captured two times, and three turtles captured three times. The mean CPUE during our study was 0.2 captured turtles per hour of effort, although the capture rate varied among the monitoring sites (Fig. 3). A total of 25 turtles were classified as putative adults and 16 as juveniles. The average CCL of putative adults

**Table 2.** Stable isotope values for the putative hawksbill turtles *Eretmochelys imbricata* prey, which were used in mixing model analyses. All values are shown with standard deviation (SD).

Element	n	Mean $\delta^{15}\text{N} \pm \text{SD}(\text{‰})$	Mean $\delta^{13}\text{C} \pm \text{SD}(\text{‰})$
<i>Cerithium</i> sp.	5	5.381 $\pm$ 1.173	-10.046 $\pm$ 1.425
<i>Amphicteis</i> sp.	3	2.966 $\pm$ 0.265	-15.224 $\pm$ 0.615
<i>Mycale</i> sp.	4	6.079 $\pm$ 1.727	-9.319 $\pm$ 5.172
Mangrove ( <i>R. mangle</i> , <i>P. rhizophorae</i> and <i>A. germinans</i> ) (Viana <i>et al.</i> , 2015)	-	3.700 $\pm$ 1.000	-29.200 $\pm$ 1.200
Macroalgae (Viana <i>et al.</i> , 2015)	-	3.300 $\pm$ 1.200	-30.700 $\pm$ 1.200

**Table 3.** Summary of hawksbill turtles *Eretmochelys imbricata* captured in their feeding area in Golfo Dulce, Costa Rica, between March and August 2015. CCL: curved carapace length. SD: standard deviation.

Month	Number of captures		Size (cm)	
	Juveniles	Putative adults	CCL $\pm$ SD	Range
March	1	9	70.7 $\pm$ 6	59.8 - 81.0
April	0	3	69.3 $\pm$ 4.4	63.0 - 72.7
May	5	5	62.1 $\pm$ 10.6	47.1 - 79.4
June	3	3	65.8 $\pm$ 8.2	52.1 - 77.1
July	6	6	60.6 $\pm$ 11.1	39.5 - 77.7

**Figure 2.** Summary of curved carapace lengths of hawksbill turtles *Eretmochelys imbricata* (n = 40) captured near Playa Blanca in Golfo Dulce, Costa Rica.

was 70.3  $\pm$  4.9 cm (range = 62.1 to 81 cm), whereas that for juveniles was 53.2  $\pm$  5.68 cm (range = 39.5 to 59.77 cm; Table 3, Fig. 2).

### Oesophageal lavage

Esophageal lavage was performed on all 41 captured hawksbills with a mean wet weight per lavage sample of 0.05  $\pm$  0.07 g (range = 0.0005 to 0.35 g). From one to seven dietary elements were obtained per sample, and a total of 12 species were distinguished (Table 4).

The predominant diet items for hawksbills examined during this study were the macroalgae *Bostrychia moriziana* (%VO = 44.73  $\pm$  33.99, %FO = 64) and

*Laurencia obtusa* (%VO = 30.86  $\pm$  27.13, %FO = 62), and the mollusk *Cerithium* sp. (%VO = 29.37  $\pm$  23.71, %FO = 50) (Fig. 3).

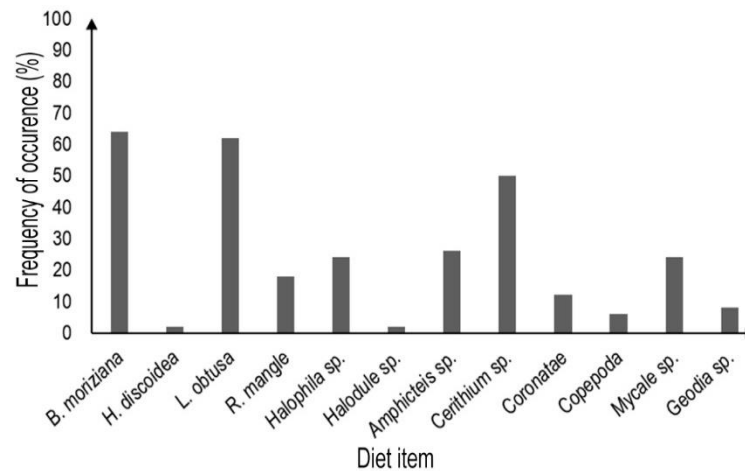
According to the *R<sub>w</sub>* values, the order of importance of diet elements for hawksbills was *B. moriziana* > *L. obtusa* > *Cerithium* sp. > *Amphicteis* sp. > *Halophila* sp. > *Mycale* sp. > *R. mangle* > *Coronatae* > *Geodia* sp. > *Copepoda* > *Halodule* sp. > *Halimeda discoidea* (Fig. 4). *B. moriziana* was the diet element of greatest importance with a high *R<sub>w</sub>* value (20.22). The angles of consumed items that are close to 45° are considered as abundant and frequent in consumption, and, based on this approach, the annelid *Amphicteis* sp. and the sponge *Mycale* sp. were both frequent and abundant in hawksbill diet as indicated by their angles (41.09 and 48.95°, respectively).

### Stable carbon and nitrogen isotope values

Elemental concentrations (%C, %N) and isotopic values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) for skin tissue and blood plasma collected from hawksbills from March to August 2015 are shown in Table 5. The %C range for plasma was 36.0 to 45.6‰, whereas for %N ranged from 9.2 to 11.7‰. The %C and %N ranges in the skin were 34.7 to 44.5‰ and of 12.4 to 15.7‰, respectively. Plasma  $\delta^{13}\text{C}$  values ranged from -23.0 to -15.7‰ (mean = -18.2  $\pm$  1.7‰) and for  $\delta^{15}\text{N}$  from 6.9 to 10.4‰ (mean = 7.8  $\pm$  0.5‰). For skin,  $\delta^{13}\text{C}$  ranged from -20.4 to -13.9‰ (mean = -15.9  $\pm$  1.5‰) and  $\delta^{15}\text{N}$  ranged from 9.3 to 11.0‰ (mean = 10.0  $\pm$  0.6‰).

**Table 4.** Items recovered in the esophageal lavage samples of hawksbill turtles *Eretmochelys imbricata* caught in Golfo Dulce. Values are presented as mean volume percentage (%VO) and standard deviation (SD), frequency of occurrence (%FO), angle, angle deviation measure = 45° (Q) and the resulting weighted index (Rw).

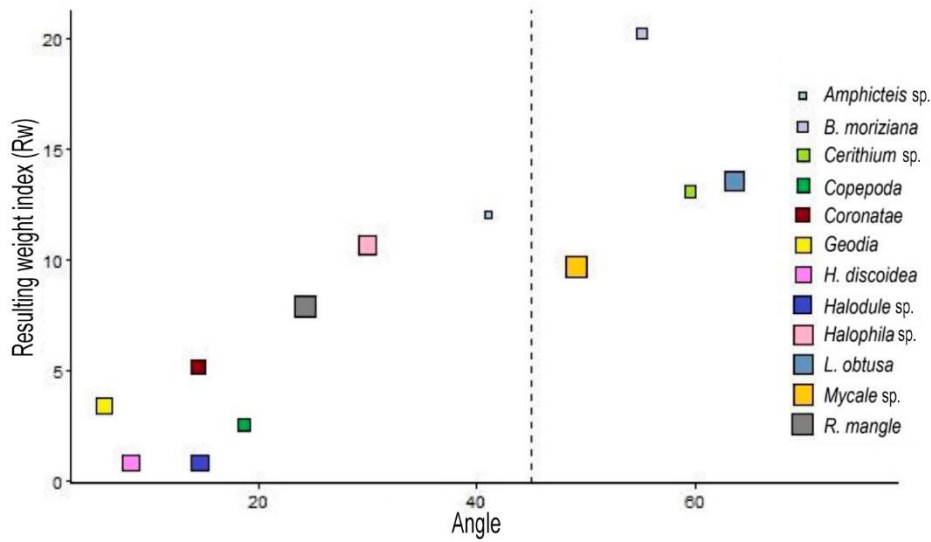
Species	Mean % VO (n) ± SD	%FO (n)	Angle	Q	Rw
Macroalgae					
Phylum Chlorophyta					
<i>Halimeda discoidea</i>	13.28 (n = 1) ± 0.00	2 (n = 1)	8.56	0.19	0.85
Phylum Rhodophyta					
<i>Laurencia obtusa</i>	30.86 (n = 31) ± 27.13	62 (n = 31)	63.54	0.59	13.58
<i>Bostrychia moriziana</i>	44.73 (n = 32) ± 33.99	64 (n = 32)	55.05	0.78	20.22
Mangrove					
Phylum Tracheophyta					
<i>Rhizophora mangle</i>	39.81 (n = 9) ± 33.66	18 (n = 9)	24.33	0.54	7.88
Seagrass		24 (n = 12)			
Phylum Tracheophyta					
<i>Halophila</i> sp.	41.84 (n = 12) ± 33.73		29.84	0.66	10.67
<i>Halodule</i> sp.	7.57 (n = 1) ± 0.00	2 (n = 1)	14.80	0.33	0.86
Annelids					
Phylum Annelida					
<i>Amphiteis</i> sp.	29.82 (n = 13) ± 29.40	26 (n = 13)	41.09	0.91	12.04
Artropodes					
Phylum Arthropoda					
Copepods	17.79 (n = 3) ± 20.37	6 (n = 3)	18.64	0.41	2.59
Mollusks					
Phylum Mollusca					
<i>Cerithium</i> sp.	29.37 (n = 25) ± 23.71	50 (n = 25)	59.57	0.68	13.08
Jellyfish					
Phylum Cnidaria					
<i>Laurencia obtusa</i>	46.83 (n = 6) ± 41.96	12 (n = 6)	14.37	0.32	5.15
Sponges					
Phylum Porifera					
<i>Mycale</i> sp.	20.90 (n = 1) ± 0.00	24 (n = 12)	48.95	0.91	9.68
<i>Geodia</i> sp.	77.49 (n = 1) ± 0.00	8 (n = 8)	5.89	0.13	3.40



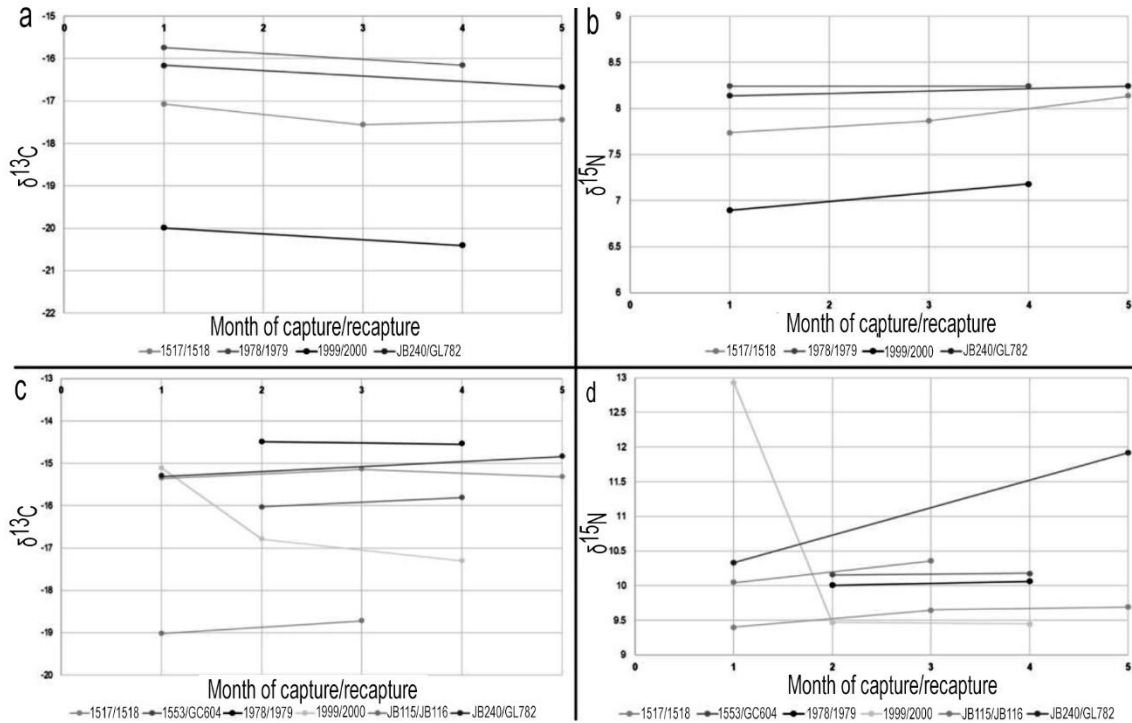
**Figure 3.** Frequency of occurrence of items found in esophageal lavages.

We were able to monitor temporal changes in SI values for turtles that were recaptured on more than one occasion during this study (Fig. 5). We found that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were consistent in plasma for recaptured

turtles, were the mean  $\delta^{13}\text{C}$  was  $-18.1593 \pm 0.1\text{‰}$ , and mean  $\delta^{15}\text{N}$  was  $7.9 \pm 0.06\text{‰}$ . However, SI values of skin varied among capture events for two individuals.



**Figure 4.** Importance (Rw) of the primary dietary components (*i.e.*, mean % VO > 5) for hawksbills turtles *Eretmochelys imbricata* in Golfo Dulce, Costa Rica. Order of importance (from most-to-least important) is *Bostrychia moriziana* > *Laurencia obtusa* > *Cerithium* sp. > *Amphicteis* sp. > *Halophila* sp. > *Mycale* sp. > *Rhizophora mangle* > *Coronatae* > *Geodia* sp. > *Copepoda* > *Halodule* sp. > *Halimeda discoidea*.



**Figure 5.** Stable isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , in ‰) in hawksbill turtles (*Eretmochelys imbricata*) plasma (a,b; n = 4) and skin (c,d; n = 6) corresponding to first capture and recaptures of hawksbill turtles in Golfo Dulce, Costa Rica. Stable isotope values are given according to the month of capture: 1) March, 2) April, 3) May, 4) June, 5) July.

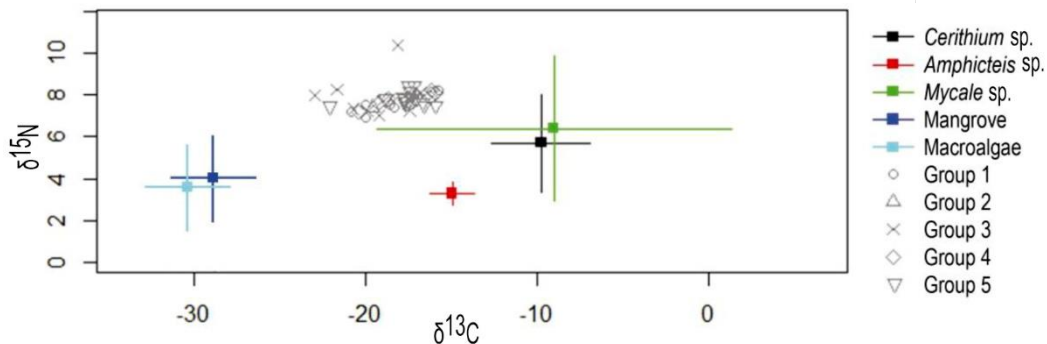
For turtle 1999/2000, changes occurred between the first (March 27, 2015) and second (July 13, 2015) captures, with a  $\delta^{13}\text{C}$  changing from -15.1 to -16.8‰ and  $\delta^{15}\text{N}$  from 12.9 to 9.5‰. Turtle JB240/GL782 also

showed variation between its first (March 3, 2015) to its last capture (July 23, 2015), with  $\delta^{13}\text{C}$  changing from -15.3 to -14.8‰, and  $\delta^{15}\text{N}$  changing from 10.3 to 11.9‰.



**Table 5.** Mean elemental concentrations (%C, %N, in ‰) and stable isotope values ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ) for plasma and skin samples of hawksbill turtles *Eretmochelys imbricata* collected from March to August 2015 in Golfo Dulce, Costa Rica. SD: standard deviation. n: number of individuals.

Month	n	%C		%N		$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Plasma									
March	9	42.5 $\pm$ 1.7	40.6 to 45.6	11.1 $\pm$ 0.4	10.4 to 11.6	-18.2 $\pm$ 1.7	-20.8 to -15.7	7.6 $\pm$ 0.4	6.9 to 8.2
April	2	42.6 $\pm$ 0.3	42.3 to 42.9	10.6 $\pm$ 0.1	10.5 to 10.7	-18.3 $\pm$ 1.2	-19.6 to -17.1	7.7 $\pm$ 0.3	7.4 to 8.0
May	11	41.7 $\pm$ 2.3	36.0 to 45.1	10.6 $\pm$ 0.8	9.2 to 11.7	-19.3 $\pm$ 1.8	-23.0 to -16.8	7.9 $\pm$ 0.9	7.0 to 10.4
June	7	42.7 $\pm$ 0.9	41.2 to 43.9	10.9 $\pm$ 0.3	10.3 to 11.3	-17.7 $\pm$ 1.6	-20.4 to -15.9	7.8 $\pm$ 0.3	7.2 to 8.2
July	14	42.6 $\pm$ 1.6	40.1 to 45.4	10.8 $\pm$ 0.5	9.8 to 11.4	-17.7 $\pm$ 1.4	-22.1 to -15.9	7.8 $\pm$ 0.3	7.5 to 8.5
Skin									
March	6	38.4 $\pm$ 1.2	37.4 to 40.8	13.5 $\pm$ 0.6	12.8 to 14.7	-16.6 $\pm$ 1.5	-19.0 to -15.1	10.1 $\pm$ 1.0	9.3 to 12.2
April	3	38.8 $\pm$ 2.4	35.6 to 41.2	13.7 $\pm$ 0.8	12.5 to 14.4	-15.8 $\pm$ 1.0	-16.8 to -14.5	9.9 $\pm$ 0.3	9.5 to 10.2
May	9	40.0 $\pm$ 1.5	37.9 to 42.9	14.4 $\pm$ 0.7	13.1 to 15.3	-16.4 $\pm$ 1.9	-20.4 to -14.6	10.0 $\pm$ 0.5	9.4 to 10.8
June	8	39.3 $\pm$ 2.3	37.2 to 44.5	13.9 $\pm$ 0.8	13.2 to 15.7	-15.4 $\pm$ 1.0	-16.8 to -13.9	10.0 $\pm$ 0.5	9.3 to 11.0
July	4	39.3 $\pm$ 2.8	34.7 to 41.9	13.5 $\pm$ 0.7	12.4 to 14.3	-15.1 $\pm$ 0.4	-15.6 to -14.4	9.9 $\pm$ 0.2	9.7 to 10.3



**Figure 6.** Plasma isotopic values (‰) of hawksbill turtles *Eretmochelys imbricata* in Golfo Dulce, Costa Rica, plotted with isotope values of possible food sources. Open symbols represent turtle values; each group reflects one month of study (Group 1: March, Group 2: April, Group 3: May, Group 4: June, Group 5: July). Filled squares represent prey item mean values and variance.

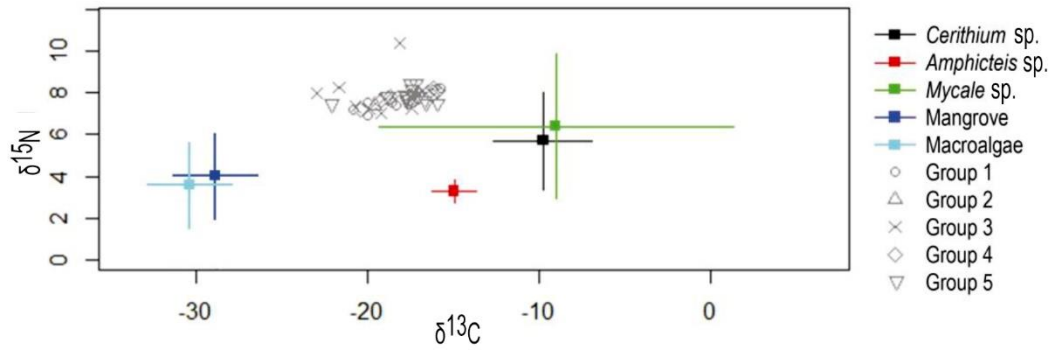
Among the potential foods,  $\delta^{13}\text{C}$  ranged from -30.7 to -9.3‰ and  $\delta^{15}\text{N}$  ranged from 3.0 to 6.1‰. Lower values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were observed for marine algae, whereas invertebrates, except for *Amphicteis* sp., had higher values; *Mycale* sp. was the most enriched in both  $^{13}\text{C}$  and  $^{15}\text{N}$  (Table 5; Figs. 6-7).

The proportional contributions of all possible prey species were modeled with SIAR (Figs. 8, 9), and the contribution of each item ranged from 0 to 62% (Table 6). For models based on skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, snail (*Cerithium* sp.) was the invertebrate with the highest dietary contribution, with a proportional contribution range of 1 to 63% (mean = 38%). Mangrove (*R. mangle*, *P. rhizophorae* and *A. germinans*) and sponge (*Mycale* sp.) had the largest contributions based on their contribution percentages for both plasma and skin. Mangrove contributions based on plasma and skin model outputs were from 1 to 50% (mean = 30%) and

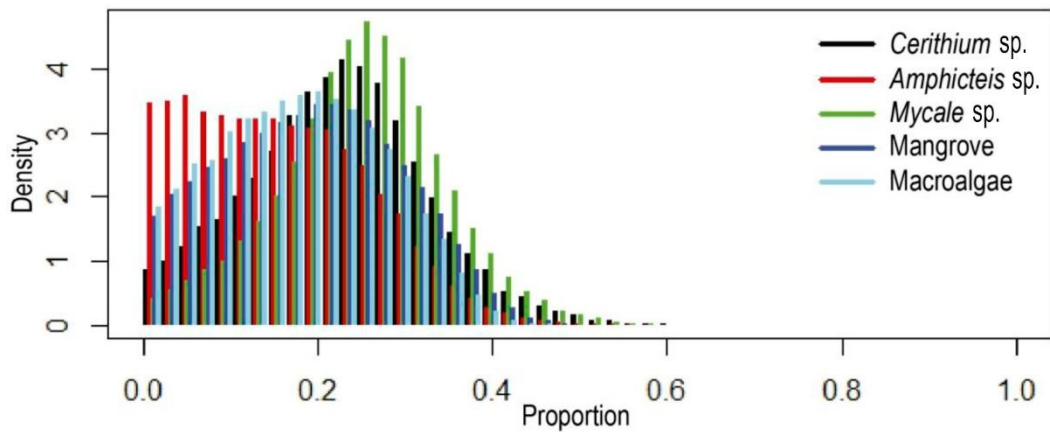
from 0 to 42% (mean = 30%), respectively. The dietary contribution values for *Mycale* sp. were 0 to 59% (mean = 25%) based on plasma SI values, and from 2 to 40% (mean = 23%) based on skin SI values.

When evaluated by month, proportional contributions based on plasma SI values showed that *Cerithium* sp., *Mycale* sp., mangroves and macroalgae were consistent in the diet throughout the study (from 0 to 63%). The excavating worm (*Amphicteis* sp.) had relatively smaller contributions throughout the study (0 to 38%) and was similar in its importance to these other prey items only during April (Fig. 10).

For skin tissue-derived dietary proportional calculations, the contribution of *Cerithium* sp. was generally constant, with a peak observed in June. In May, a decrease in the contribution by *Amphicteis* sp. was observed, whereas the biggest contributor was *Mycale* sp. As our study progressed from March to July,



**Figure 7.** Skin isotope values (‰) of hawksbills *Eretmochelys imbricata* in Golfo Dulce, Costa Rica, plotted with isotope values of possible food sources. Open symbols represent turtle values; each group reflects one month of study (Group 1: March, Group 2: April, Group 3: May, Group 4: June, Group 5: July). Filled squares represent prey item mean values and variance.



**Figure 8.** SIAR histogram projection incorporating the discrimination factors from Reich *et al.* (2008) in plasma of sampled hawksbill turtles *Eretmochelys imbricata* and the distribution of the contribution of the five possible diet sources for the period of March to August 2015 in Golfo Dulce, Costa Rica.

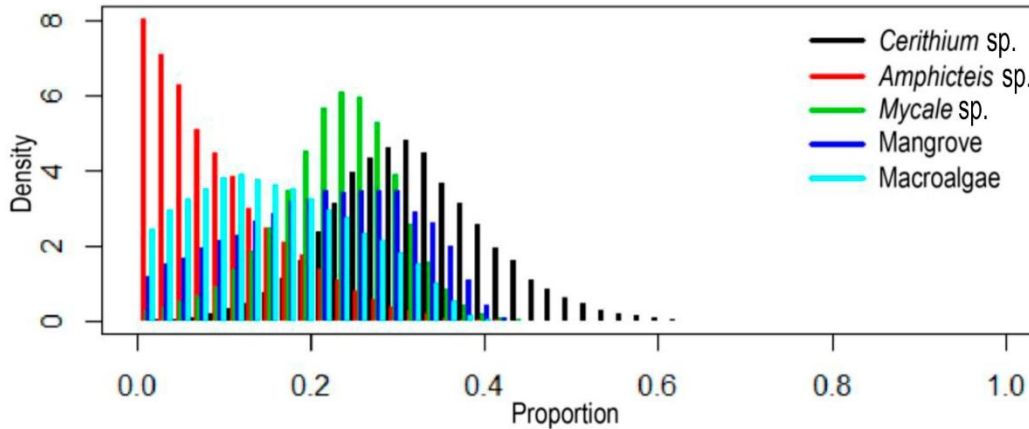
*Cerithium* sp., *Mycale* sp. and *Amphicteis* sp. were relatively consistent in hawksbill diet (25% average contribution). During the same period, the dietary contribution for mangrove decreased from 21 to 18% and for macroalgae decreased from 20 to 15% during the same period (Fig. 11).

## DISCUSSION

This is the first study to combine oesophageal lavage and stable nitrogen and carbon isotope analyses in eastern Pacific sea turtles, and to our knowledge is also the first-ever published stable isotope study for hawksbills in this region. The information obtained in this research contributes to the knowledge of the foraging behaviour and general biology of hawksbills (Gaos *et al.*, 2012b; Carrión *et al.*, 2013; Heidemeyer *et al.*, 2014; Chacón *et al.*, 2015; Llamas *et al.*, 2017), and our data show that coastal waters of Golfo Dulce

offer important shelter and food for this critically endangered species (Gaos *et al.*, 2011; Carrión *et al.*, 2013). Moreover, the hawksbill's apparent fidelity to this area-as evidenced by numerous recaptures-underscores the need to protect the habitats and prey resources present in Golfo Dulce (Chacón *et al.*, 2015).

The CPUE for our study (0.2 turtles/6 h) is higher than that reported in previous research in the area (Chacón *et al.*, 2015) and together with the numerous recaptures indicates a stable resident population of both juveniles and adults. The presence of a 39.5 cm (CCL) individual suggests this site hosts hawksbills that have recently recruited from open ocean habitats. Post-oceanic juveniles have also been reported at other areas along the Pacific coast including Punta Coyote (minimum CCL 36 cm; Carrión *et al.*, 2013), Matapalito Bay (minimum CCL = 31 cm), in the Gulf of Nicoya (Heidemeyer *et al.*, 2014), and Coiba National Park (minimum CCL = 30 cm) (Llamas *et al.*, 2017).



**Figure 9.** SIAR histogram projection incorporating the discrimination factors from Reich *et al.* (2008) in the skin of sampled hawksbill turtles *Eretmochelys imbricata* and the distribution of the contribution of the five possible diet sources for the period of March to August 2015 in Golfo Dulce, Costa Rica.

**Table 6.** Proportional contributions of putative prey species obtained with SIAR.

Species	Plasma		Skin	
	Contribution range (%)	Mean	Contribution range (%)	Mean
<i>Cerithium</i> sp.	1 to 63	24	1 to 63	38
<i>Amphicteis</i> sp.	0 to 59	15	0 to 38	10
<i>Mycale</i> sp.	0 to 59	25	0 to 42	22
Mangrove	1 to 50	30	0 to 41	21
Macroalgae	1 to 45	20	0 to 43	10

Coupled with our data, these observations indicate that eastern Pacific hawksbills recruit to coastal habitats with a CCL  $\geq 30$  cm, and show that the Pacific coast of Costa Rica is an important area for juveniles recruiting to coastal habitats after completing their oceanic juvenile phase.

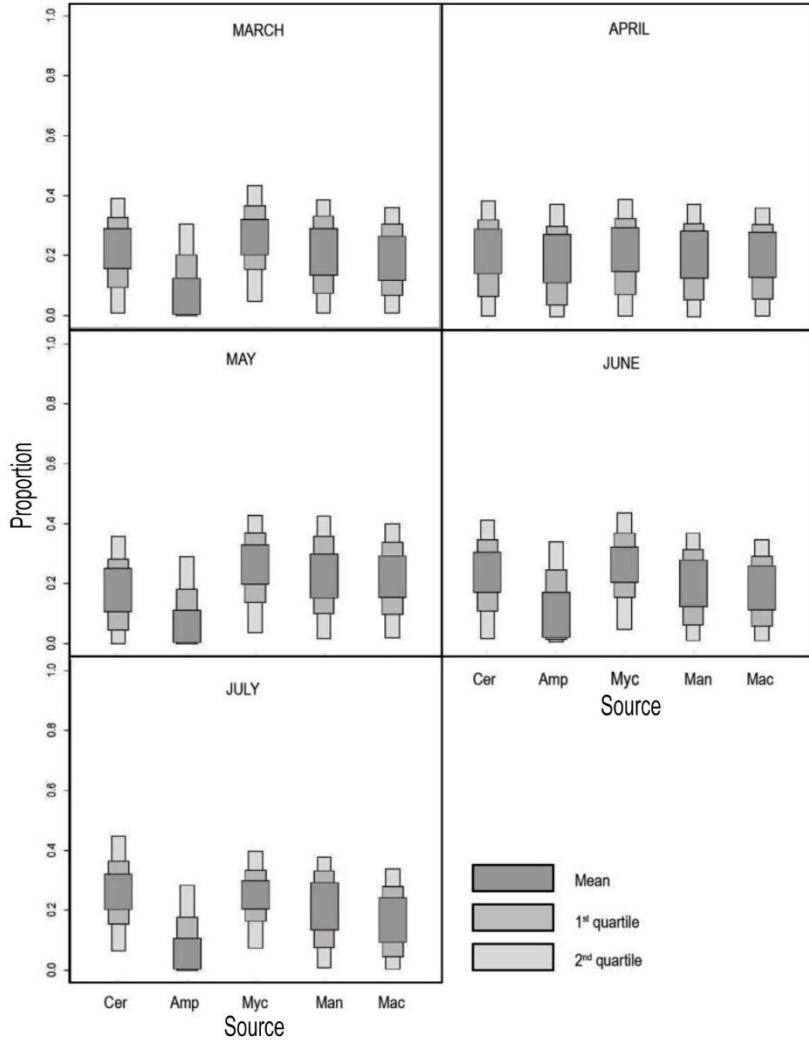
It cannot be established if there is a life stage-based (*i.e.*, juvenile vs. adult) habitat preference. However, our results suggest that coastal estuaries and mangrove forests are especially important for adult hawksbills (Fig. 3), a finding that is consistent with data from El Salvador and Pacific Nicaragua (Gaos *et al.*, 2010, 2011, 2012a, 2012b), as well as from the Guanacaste coast of Costa Rica (Carrión *et al.*, 2013). In Golfo Dulce, the use of mangrove forests may be a way to compensate for the regional progressive loss of coral reef ecosystems (Cortés *et al.*, 2010), as these mangrove forests offer vertical structure that may provide protection from predators, much like coral reefs.

All hawksbills were captured in waters of 7 m or shallower. This pattern has been reported for other sites

in the EP (Diez *et al.*, 2003; Gaos *et al.*, 2012a, 2016; Carrión *et al.*, 2013; Llamas *et al.*, 2017), indicating that they live in shallow areas, likely related to food availability, with greater food densities and diversity in shallow waters (Meylan, 1988; León & Bjorndal, 2002; Carrión *et al.*, 2013).

From our lavage efforts, macroalgae were highlighted as the prey type having the highest frequency of occurrence and importance based on the *Rw*, with *B. moriziana* the most significant diet item (*Rw* = 20.22). This may be related to macroalgae's abundance and association to the rocky substrate where large numbers of invertebrates inhabit (Ward *et al.*, 1999). The consumption of invertebrates such as *Cerithium* sp. and *Amphicteis* sp. is relevant, and these are likely important dietary alternatives where sponges are not abundant (León & Bjorndal, 2002). The fact that both marine algae and invertebrates were found in the lavage samples in more than trace amounts underscores the generalist foraging strategy of hawksbill turtles in Golfo Dulce, which may be consistent with food availability scenarios at other sites in the EP (Gaos *et al.*, 2006, 2010, 2011, 2012a).

Applying stable isotope analyses for the first time ever in EP hawksbills, we were able to confirm that in addition to merely consuming a mixed diet, these turtles are also assimilating the nutrients garnered from these diverse food sources. Indeed, the SIAR mixing model results indicate that all the analysed potential diet items contribute to hawksbill diet and nutrition in Golfo Dulce (Figs. 9-10). This strongly contrasts with hawksbills in the Caribbean that almost exclusively consume sponges and only occasionally forage on invertebrates (Meylan, 1988; León & Bjorndal, 2002; Parker *et al.*, 2009; Bell, 2012).

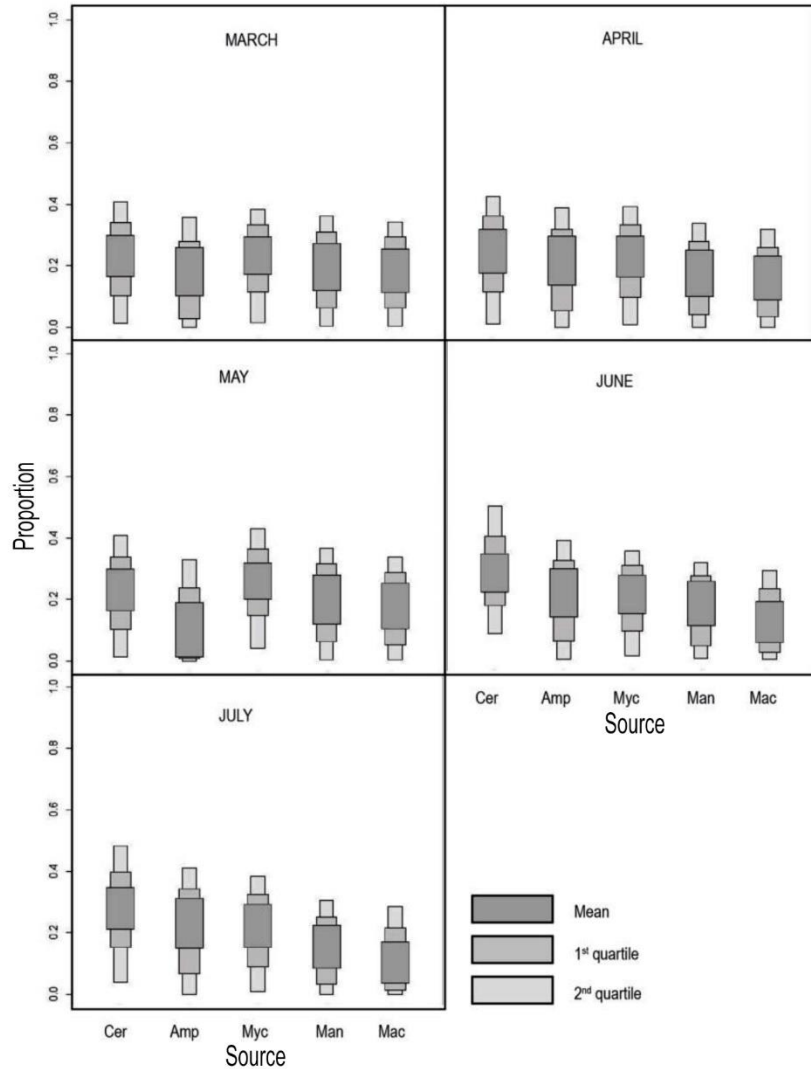


**Figure 10.** SIAR illustration showing the contribution ranges for the diet of hawksbill turtles *Eretmochelys imbricata* by the diet items from March to July 2015 in Golfo Dulce, Costa Rica. Cer: *Cerithium* sp., Amp: *Amphicteis* sp., Myc: *Mycale* sp., Man: mangrove, Mac: macroalgae.

The identification of the sponges *Mycale* sp. and *Geodia* sp. in the oesophageal lavage samples, although not found in great quantity, indicates that hawksbills in this study consumed at least some sponges. However, the SIAR outputs for both blood plasma and skin indicate a greater dietary importance for *Mycale* sp. than suggested by our lavage results. The disparity in the results may be related with the sponge tissue being assimilated during digestive processes to a greater extent than other consumed prey species or perhaps due to difficulty retrieving this prey type via the lavage technique.

The five months of data collection allow us to evaluate temporal consistency in hawksbills trophic status (Figs. 11-12). The sponge *Mycale* sp. and the

snail *Cerithium* sp. were constant diet sources, and the latter was a constant diet component and the biggest contributor to hawksbills diet during this study. Isotope values from bulk skin tissue, which provide a ca. 4- to 5-month dietary retrospective, suggest that prior to our sampling period the intake and contribution of invertebrates was greater than that for algae and plant prey species. For plasma, our isotope results suggest that the consumption of macroalgae and mangrove increased during this study. Our results also suggest that invertebrates became less frequently consumed over the course of our research. Together, these observations may indicate a seasonal shift in the rate of ingestion of different food items, perhaps related to a change in resource abundance prior to, and during, our study.



**Figure 11.** Monthly variation of the contribution ranges for the diet of the hawksbill *Eretmochelys imbricata* by the possible diet elements in the period of March to July 2015 in Golfo Dulce, Costa Rica. Cer: *Cerithium* sp., Amp: *Amphicteis* sp., Myc: *Mycale* sp., Man: mangrove, Mac: macroalgae.

Despite its high occurrence in lavage samples, the least consistent dietary component, as reflected in the plasma isotopes, was the excavating worm *Amphicteis* sp. This suggests relatively low nutrient assimilation from this prey item. The ingestion of *Amphicteis* sp. could be incidental, although its contribution of valuable nutrients for hawksbills cannot be ruled out. However, when gauging the importance of *Amphicteis* sp. and other diet constituents, we also acknowledge that the diet-tissue discrimination values from Reich *et al.* (2008) used in this study may not be ideal and perhaps resulted in the disparity between our lavage results and the SIAR model outputs. We therefore encourage dedicated studies of stable isotope disci-

mination for hawksbill turtles to better inform future isotopic studies of this species in the EP and elsewhere.

Isotopic turnover is defined as the isotopic change due to growth and metabolic tissue replacement associated with a change in diet (MacAvoy *et al.*, 2001), where skin tissue has a relatively slow turnover of 4-5 months (in *Caretta caretta*; Reich *et al.*, 2008) and blood plasma has a turnover rate of approximately 35 days (in *Trachemys scripta*; Seminoff *et al.*, 2007). In this research, when comparing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from hawksbills at the time of their initial capture vs. recapture(s) (Fig. 6), the plasma isotopic values remained constant, thus suggesting that their residency in this foraging area has been ongoing for at least a

month prior to the first capture. Skin isotope values suggest the same pattern for the majority of individuals, signifying their residence for at least four months prior to the start of this study; however, two individuals showed notable changes in these values, suggesting that they may have recently recruited to Golfo Dulce. The individual identified with tag numbers 1999/2000 showed substantial changes in both nitrogen and carbon isotope values between the first and second captures ( $\delta^{15}\text{N}$  from 12.9 to 9.5 ‰,  $\delta^{13}\text{C}$  from -15.1 to -16.8‰). A second turtle (JB240/GL982), also showed different values, albeit to a lesser extent, between its initial and final capture ( $\delta^{15}\text{N}$  from 10.3 to 11.9‰,  $\delta^{13}\text{C}$  from -15.3 to -14.8‰). In both cases, the differing isotope values suggest that at first capture both turtles still possessed isotope values in their tissues that were reflective of habitats occupied prior to arriving in Golfo Dulce.

### CONCLUSIONS

This is the first-ever report on the stable isotope-derived diet profile for hawksbill turtles in the EP, and the results of this study increase our understanding of the nutritional ecology of this endangered species in Golfo Dulce. Indeed, our results, demonstrate that they forage on a large variety of prey species in this area. This generalist foraging tactic, compared to the species' diet in the Caribbean and other coral-dominated habitats, may also be the trophic strategy for hawksbills at other foraging grounds in the EP, particularly those containing mangrove estuaries. This feeding pattern and the fidelity to the Golfo Dulce foraging area, as substantiated via ongoing research in the area (*e.g.*, Chacón *et al.* 2011; 2015), underscores the importance of protecting this region from human impacts, as its ecosystems are vulnerable to threats such as conversion into farm lands (Algeet *et al.*, 2015; Gallmetzer & Schulze, 2015) and climate change (Ghosh *et al.*, 2015). In-water research and conservation efforts have been developing in the area, and the Costa Rican Fisheries and Aquaculture Institute (INCOPESCA) has declared the Golfo Dulce as a Responsible Marine Fisheries Area. This designation prohibits trawling, longlining, and trammel fishing, while at the same time regulates the type and size of hooks used for artisanal hook-and-line fishing. However, involvement of the community in both learning about, and protecting, this ecosystem and the species that inhabit it, are paramount to maintaining the pristine nature of this tropical fjord and its marine inhabitants. In the same light, this research indicates that further stable isotope studies should be conducted on this enigmatic EP population to complement information on habitat use and foraging behaviour, and to establish the predator-prey discrimination factors ( $\Delta_{\text{dt}}$ ) for the species. By providing

additional data for hawksbills in this and surrounding areas, this and future studies will provide a more robust view of hawksbill trophic ecology.

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