



# Trophic ecology of juvenile green turtles in the Southwestern Atlantic Ocean: insights from stable isotope analysis and niche modelling

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**ABSTRACT:** Determining the diet and trophic status of endangered species can provide insights into their key habitats and predator–prey relationships, both essential for conservation. Integrating dietary information from live-captured and dead-stranded turtles from the same area can also shed light on their ecological diversity. Here, diet intake and prey diversity are determined for juvenile green turtles *Chelonia mydas* from a mixed stock foraging aggregation in the Southwestern Atlantic Ocean (SWAO) using stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis. Skin samples were collected from 117 live-captured (LC) turtles and 66 dead-stranded (DS) individuals along the Paraná coast, southern Brazil. Bayesian isotope ellipse space was determined using the program SIBER. A larger isotopic niche space was found among DS turtles, probably because it included long-term resident turtles and recently settled individuals, the latter of which presented low  $\delta^{15}\text{N}$  values compared to larger putative local turtles. The Bayesian stable isotope mixing model MixSIAR was used to determine dietary contributions, using isotopic values of putative prey. MixSIAR results indicated invertebrates and macroalgae as primary prey groups, showing the omnivorous strategy of green turtles in the SWAO. Moreover, many of the smallest DS turtles had  $\delta^{15}\text{N}$  values suggestive of seagrass consumption, which may reflect individual- and/or life-stage-based diet variability. These findings underscore the value of integrating stranding monitoring protocols with research on live green turtles to evaluate habitat use and diet intake assembled in a neritic foraging area. The data also further knowledge about green turtle foraging ecology and are relevant for species and habitat conservation throughout the SWAO.

**KEY WORDS:** *Chelonia mydas* · Foraging ecology · SW Atlantic · Isotopic ratio · Mixing model · Bayesian inference

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## 1. INTRODUCTION

Understanding diet composition and predator–prey relationships of endangered species can inform the identification of key feeding areas and provide in-

sights into the foraging ecology of the species (James et al. 2005, McClellan & Read 2007, Nagaoka et al. 2012). This information is crucial to determine priority habitat for conservation and management plans, and to determine which areas offer the greatest

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resources for consumers (Fuentes et al. 2006). When implemented in management frameworks, knowledge about dietary needs and habitat use may support better decisions during the development of marine protected areas, fisheries, management practices, and mitigation strategies for habitat stressors such as marine debris (Schuyler et al. 2014, Hays et al. 2019, Haywood et al. 2019). However, despite the value of this information for wildlife conservation, the elusive and cryptic nature of many animals, particularly marine species, makes it difficult to study them in the wild and obtain direct information on their foraging ecology (Reich et al. 2007).

Stable isotope analysis (SIA) of bulk animal tissue is a useful tool to study the diet and habitat use of elusive species (Haak et al. 2019, Hobson et al. 2019, Roswag et al. 2019). This is because stable isotope values of animal tissues reflect their diet and the ecosystem within which they forage (Hobson 1999). Body tissue such as skin or blood can provide insights about consumer's assimilated nutrients and trophic status that are otherwise not attainable with conventional diet analysis techniques such as esophageal lavage or digestive tract inspection (Knoff et al. 2008, Litz et al. 2017). Stable carbon isotope ( $\delta^{13}\text{C}$ ) values of consumer tissues are generally used to determine the origins of nutrients, with a continuum of progressive  $^{13}\text{C}$  depletion in tissues of marine species as they forage farther from the coast (Gannes et al. 1998, Jones & Semionoff 2013). The  $\delta^{13}\text{C}$  values in consumer tissues may also be influenced by the photosynthetic pathways 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. *Avicennia schaueriana*), which are marine angiosperms that derive carbon from terrestrial-based systems, will have substantially lower  $\delta^{13}\text{C}$  values relative to seagrass and marine macroalgae located only a few meters away (Lepoint et al. 2004). Conversely, stable nitrogen isotope ( $\delta^{15}\text{N}$ ) analysis of body tissues provides information on the trophic level of a consumer due to the preferential retention of the heavier  $^{15}\text{N}$  during nutrient assimilation, which results in a step-wise increase in  $\delta^{15}\text{N}$  from prey to consumer (Semionoff et al. 2006, Reich et al. 2007, Bisi et al. 2013).

Sea turtles are among the taxa for which SIA has proven useful for elucidating foraging ecology of individuals and populations (Godley et al. 1998, Arthur et al. 2008, Burkholder et al. 2011, Gillis et al. 2018, Vélez-Rubio et al. 2018). All sea turtles are migratory and have complex life histories during which they occupy distinct habitats and consume dif-

ferent diets depending on their life stage (i.e. ontogenetic habitat and diet shifts observed for green turtles; Cardona et al. 2009, González Carman et al. 2012, Gama et al. 2016, Vélez-Rubio et al. 2018). As dietary nutrients are assimilated, the isotopic values of these foods are reflected in consumer body tissues (Semionoff et al. 2006). Thus, stable isotope values can provide information about individual diet differences as well as the previous location of organisms that move among spatially discrete food webs that are isotopically distinct (often termed 'isoscares') (Semionoff et al. 2002, Vander Zanden et al. 2012, Turner Tomaszewicz et al. 2017a, 2018). Stable isotope data from individuals of differing size and from multiple years in the same area can provide insights about ecological diversity (e.g. behavior, habitat use) across life stages, and when examined in the context of baseline isotope values, these data can provide information about residency patterns among studied turtles (Newsome et al. 2010, Vélez-Rubio et al. 2013, 2018, Turner Tomaszewicz et al. 2018).

The Southwestern Atlantic Ocean (SWAO) hosts a regional management unit (RMU) of green turtles *Chelonia mydas* (Wallace et al. 2011). This ocean region contains expansive neritic foraging areas for green turtles, especially juveniles, and stable isotope analysis has been used to evaluate diet composition at several sites (e.g. González Carman et al. 2014, Vélez-Rubio et al. 2018). Within the SWAO, in southern Brazil, the Paraná coast is used mainly by immature green turtles that are present year-round (Andrade et al. 2016, Gama et al. 2016, Coelho et al. 2018), but seasonally migrate within the region in search of warmer waters and areas with greater food availability (Marcovaldi & Santos 2011, Reisser et al. 2013, Gama et al. 2016, Vélez-Rubio et al. 2018). Throughout the SWAO and particularly along the Paraná coast, many conservation challenges exist for this species. For example, more than 1000 dead-stranded green turtles have been recorded per year since 2015, with bycatch in fisheries gear being a major cause of mortality (Cantor et al. 2020). Additional threats such as coastal habitat degradation, contamination, dredging, and urbanization are also present (Domiciano et al. 2017, Fuentes et al. 2020), which may affect diet, habitat use patterns, and health condition of local green turtles.

In this study, the foraging ecology of juvenile green turtles along the Paraná coast of southern Brazil is examined via stable isotope analysis ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of both live-captured (LC) and dead-stranded (DS) green turtles, to test if this analysis can help us to

understand: (1) the relationship between item consumption and assimilation for regional green turtles; (2) if latitudinal movement might be assessed by differences in diet intake and stable isotope measures obtained in animals from the same foraging ground; and (3) if we can correlate diet and trophic niche with specific development stages of juveniles. The main goal was to determine the recruitment histories of individual turtles and characterize the diet intake and isotopic niche (i.e. proxy for trophic niche) of the regional green turtle population. This was achieved by evaluating the stable isotope values of green turtle bulk skin tissue in the context of local and regional isoscape patterns. Diet composition of LC and DS turtles was determined using a Bayesian stable isotope mixing model that uses turtle and putative prey bulk tissue isotope values to model diet, and niche was determined with Bayesian isotope ellipse models that explored isotope variability among individuals in each group. Our results contribute to a better understanding of the foraging ecology and habitat use of green turtles, which is crucial to support marine habitat conservation efforts throughout the SWAO.

## 2. MATERIALS AND METHODS

### 2.1. Study site

This study was conducted along the coastline of the state of Paraná, southern Brazil (25° 20' to 25° 35' S, 48° 17' to 48° 42' W, Fig. 1). The study area includes numerous bays and the Paranaguá Estuarine Complex (PEC), a 612 km<sup>2</sup> semi-enclosed inlet characterized by mangroves, sandy beaches, seagrass meadows, and rocky shores, and classified as a World Heritage Site (Angulo 1992, Lana et al. 2001, UNESCO 2010). This is a subtropical region, influenced by the Brazil current (warm, summer currents) and the Falklands current (cold, winter currents), resulting in seasonal variation in sea surface temperature (18–30°C), local habitat conditions, and food resource availability (Matano et al. 2010). High marine productivity in the region provides rich food sources and promotes the presence of green turtles year-round (Guebert-Bartholo et al.

2011, Andrade et al. 2016, Gama et al. 2016, Coelho et al. 2018, Fuentes et al. 2020).

### 2.2. Live-capture turtle sampling

A green turtle monitoring and health assessment program has been conducted in the PEC region since 2016 (Domiciano et al. 2019). As part of this program, intentional capture efforts were conducted during 58 field days in 2016 (May, June, September, October), 2017 (February, March), and 2018 (March, June). Turtle capture was conducted using a 50 m tangle net, with 3 m height and 0.30 m knot-to-knot mesh size. The net was checked continuously during the capture efforts and entangled green turtles were promptly removed and brought to the research boat (26 foot vessel with two 150 hp outboard motors). Onboard, body measurements such as curved carapace length (CCL,  $\pm 0.1$  cm) and body weight ( $W$ ,  $\pm 0.1$  kg) were obtained using a flexible tape measure and hanging balance (Pesola AG, PHS100), respectively. All LC individuals were tagged with Inconel flipper tags (National Band and Tag Company, Style 681) and released at the site of capture within 2 h.

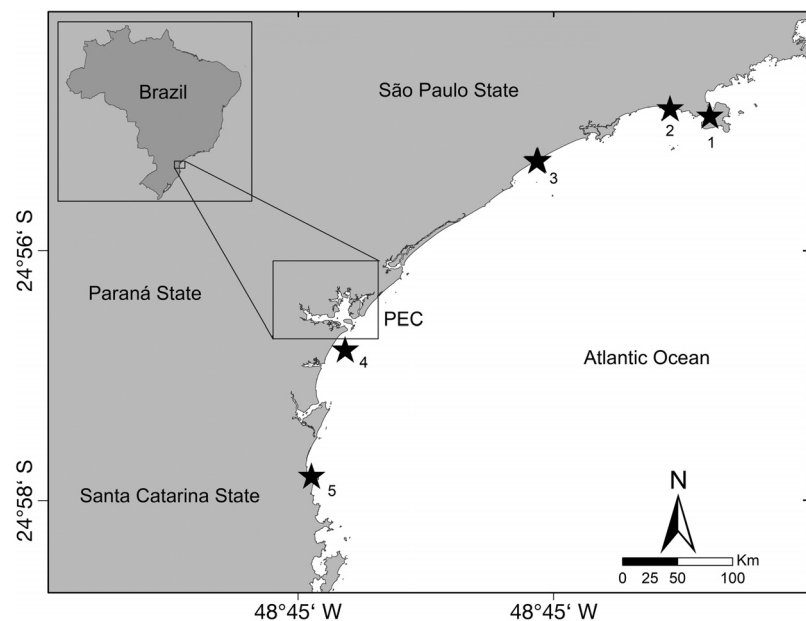


Fig. 1. Marine algae sampling areas (★) along the coast of the Southwestern Atlantic Ocean. 1: Ilha das Cabras; 2: Portinho, in Ilha Bela (São Paulo State); 3: Cama de Anchieta and Praia dos Sonhos, in Itanhaém (São Paulo State); 4: Currais in Paraná State; 5: Penha, in Santa Catarina State. The zoomed square encompasses the Paraná coast of Brazil, including the Paranaguá Estuarine Complex (PEC), where the beach surveys were performed and dead-stranded green turtles were recovered, and the live-capture turtles were sampled

### 2.3. Dead-stranded turtle sampling

DS green turtles were collected within the PEC and along the Paraná coast (~100 km) (Fig. 1) during daily beach surveys conducted by personnel from the Ecology and Conservation Laboratory (Federal University of Paraná, UFPR) as part of the Project to Monitor the Beaches of Santos Bay (PMP-BS). The selected individuals to be sampled were obtained between July to September 2017 and April to June 2018, following the seasonal pattern. More than 2000 juvenile green turtles were found dead-stranded during this interval, but only a subset of 66 specimens was analyzed due to financial and logistical constraints. All the turtle specimens were identified to species and their decomposition stage was ranked (from 2 to 5; adapted from Geraci & Lounsbury 2005); only carcasses classified as 2 and 3 (i.e. the freshest decomposition states) were sampled for this study. For each DS turtle, CCL ( $\pm 0.1$  cm) was recorded.

### 2.4. Habitat sampling

Habitats were sampled to determine the isotope values of macroalgae that are potentially consumed by green turtles. Macroalgae were collected in 6 different areas along the SWAO coast from Ilha das Cabras (23° 49' S, 45° 23' W) in São Paulo State, to Praia da Armação (26° 46' S, 48° 38' W) in Santa Catarina State (Fig. 1). These sites included the PEC, as well as other neritic habitats that have similar oceanographic influences and are part of the migratory corridor of this species (González Carman et al. 2012, Fuentes et al. 2020). Habitat sampling was conducted from December 2017 to February 2018, which corresponds with the austral summer, and from June to July 2018, which is during the austral winter. The most reported macroalgae in the diet of local green turtles (Nagaoka et al. 2012, Reisser et al. 2013, Gama et al. 2016) was collected at each site. All samples were stored in plastic 'zip lock' bags with clean seawater, and frozen until analysis. Taxonomic identification was conducted at the Phycology and Marine Water Quality Laboratory (Paraná State University, UNESPAR) in Paranaguá, Brazil. Each sample was separated by histological sectioning, and morphological and reproductive features were examined via stereomicroscope when permissible based on sample quality. The most abundant samples were rinsed, lyophilized, and homogenized prior to isotopic analysis; these included Chlorophyta (*Caulerpa racemosa*, *Chaetomorpha antennina*, *Ulva fasciata*,

*U. lactuca*, *U. intestinalis*), Rhodophyta (*Aglaothamnion felipponei*, *Amphiroa* sp., *Asparagopsis* sp., *Chondria* sp., *Hypnea spinella*, *H. pseudomusciformis*, *Jania crassa*, *Laurencia* sp., *Spyridia* sp.), Ochrophyta (*Chnoospora minima*, *Dictyota ciliolata*, *Sargassum cymosum*), and the cyanobacteria *Lyngbya majuscula*.

### 2.5. Tissue sampling and stable isotope analysis

Epidermis samples (0.10–0.25 g; hereafter referred to as skin) were collected from both LC and DS turtles with a razor blade from the dorsal surface of the neck and frozen until analysis as per Gillis et al. (2018). Special care was taken to collect surface epidermal tissue without any underlying connective tissue.

Approximately 1.0 mg of skin from both LC and DS green turtles, and 0.5 mg of each forage item sample, was loaded into tin capsules and analyzed by an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) at the São Paulo University Oceanographic Institute (IO-USP) in São Paulo, Brazil. Prior studies have indicated that lipid extraction is unnecessary when the sample lipid content is  $\leq 5\%$  and/or when C:N  $< 3.5$  (Post et al. 2007, Turner Tomaszewicz et al. 2017b); therefore, we did not remove lipids from turtle tissues or food resource samples.

Analyses were undertaken for %C, %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ . Sample stable isotope ratios relative to an isotope standard are expressed in the following conventional delta ( $\delta$ ) notation, measured in parts per thousand (‰):

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) (1000) \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the corresponding ratios of heavy to light isotopes ( $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$ ) in the sample and standard, respectively. All carbon isotopic results are expressed in standard delta notation relative to Vienna Pee Dee Belemnite (or VPDB). All nitrogen isotopic results are expressed in standard delta notation relative to the atmospheric air (or AIR). All analytical runs included the certified USGS-40 (United States Geological Survey) and IAEA-600 (International Atomic Energy Agency) reference materials, which were inserted after every 40 samples to calibrate the isotopic results and to estimate the precision of the analysis. Replicate assays of standard materials indicated recoveries of the method of 96 to 100%, and analytical uncertainty of 0.18 and 0.05‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  measurements, respectively.

## 2.6. Statistical analysis

The differences in the isotopic means for DS and LC turtles among the years were tested using a Tukey post hoc test. To test for isotope differences between size classes (groupings made within 10 cm CCL size groups; Size Group 1: 26.0–36.0 cm; Size Group 2: 36.1–46.0 cm; Size Group 3:  $\geq 46.1$  cm; following Gama et al. 2016), an ANOVA was performed for DS and LC turtles. Linear regression was conducted to detect correlation between turtle CCL and isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). All statistical analyses were conducted using the software R 3.6.1, where  $p < 0.05$  was used to define statistical significance. Only raw, corrected isotope data were used for analyses; no data transformation was conducted. The analysis of normality, homoscedasticity, and standardized residuals showed the adequacy of the ANOVA assumptions (Sokal & Rohlf 1969).

The Stable Isotope Bayesian Ellipses in the package in R (SIBER) (Jackson et al. 2011) was used to generate and compare the bivariate ellipses for DS and LC juvenile green turtles based on their skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. The convex hull total area (CHA) and the standard ellipse area (SEA) were calculated to determine the isotopic niche space for DS and LC (Jackson et al. 2011). The Bayesian mixing model MixSIAR (Stock & Semmens 2016) was used to determine the prey contributions to the diet of juvenile green turtles. Model input data included  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of live green turtles and putative prey species, as well as the values for dead green turtles and their prey species. Because of the limited dietary specificity revealed by MixSIAR when using numerous prey groups (Stock & Semmens 2016), here the analyses were limited to 5 groups ('red algae', 'green algae', 'seagrass', 'mangrove', 'invertebrates'), with prey items grouped based on similarity in life-history traits and position in the food web (Lemons et al. 2011, Gillis et al. 2018). The 'red algae' group was composed of *Hypnea spinella*, *Hypnea* sp., and *H. pseudomusciformis*.

The 'green algae' group was composed of *Ulva fasciata*, *Ulva* sp., and *U. lactuca*. Both algae groups were composed of species collected in this study and in adjacent areas with similar values to the ones collected by us, to reduce the noise in our models (Table 1). For 'seagrass', the 2 isotope values for *Halodule wrightii* (which is the only species that occurs along the Paraná coast) are from sites with different environmental conditions along the Brazilian coast; however, they were used for model inputs because they were the only data available (Lazzari 2012, Corbisier et al. 2014). It should be noted that one of the studies found high nitrogen isotope values for seagrass, fitting to the category of oceanic-type prey, which includes the seagrass itself, jellies, and other prey items that our model cannot distinguish due to their similarities.

The 'mangrove' group consisted of *Avicennia schaueriana*, which is one of the most common mangrove species in the diets of green turtles from Paranaguá Estuarine Complex, Paraná State in southern Brazil (i.e. Guebert-Bartholo et al. 2011). Mangrove isotope values were obtained by values presented in the literature from Guaratuba Bay, also in the state of Paraná (Larcher et al. 2016). The 'invertebrate' group reflects mean isotope values of 32 species from the region and obtained from the literature, including depositivorous (5), carnivorous (7) and suspensivorous (20) species (Table A1 in the Appendix; Corbisier et al. 2006, Vinagre et al. 2018).

We further explored green turtle diet characterization in MixSIAR by separating turtles into the 3 size groups (Size Group 1: 26.0–36.0 cm; Size Group 2: 36.1–46.0 cm; Size Group 3:  $\geq 46.1$  cm) that reflect the entire size range (CCL) of turtles examined. For all models, we applied turtle-prey trophic discrimination factors (TDFs) from Seminoff et al. (2006;  $0.17 \pm 0.03$  ‰ for  $\delta^{13}\text{C}$ ;  $2.8 \pm 0.11$  ‰ for  $\delta^{15}\text{N}$ ), as these were derived for green turtles of similar size (i.e. life-history phase) to individuals included in this study.

Table 1. Stable isotope values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of the food items considered in our study for juvenile green turtles, which were included in the MixSIAR models. Values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are means  $\pm$  SD (‰)

Prey group	N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Reference
Green algae	10	$-16.70 \pm 0.77$	$7.40 \pm 1.23$	This study, Corbisier et al. (2006), Vélez-Rubio et al. (2016)
Red algae	6	$-17.20 \pm 2.62$	$6.00 \pm 1.06$	This study, Corbisier et al. (2006), Vinagre et al. (2018)
Seagrass	4	$-13.80 \pm 0.50$	$3.70 \pm 0.70$	Lazzari (2012), Corbisier et al. (2014)
Mangrove	3	$-28.30 \pm 0.20$	$3.10 \pm 0.40$	Larcher et al. (2016)
Invertebrates	32	$-15.40 \pm 0.50$	$9.70 \pm 1.40$	Corbisier et al. (2006), Vinagre et al. (2018)



### 3. RESULTS

#### 3.1. Live-capture and dead-stranded turtle samples

A total of 117 individuals were captured alive (LC) during 2016 ( $n = 8$ ), 2017 ( $n = 14$ ), and 2018 ( $n = 89$ ), with 6 turtles recaptured in multiple years. Curved carapace length ranged from 30.6 to 63.2 cm (mean  $\pm$  SD =  $39.6 \pm 6.9$  cm). For DS turtles, a total of 66 individuals were sampled, with CCL ranging from 28.2 to 59.0 cm (mean =  $36.6 \pm 6.3$  cm).

#### 3.2. Isotopic values of turtle samples and forage items

The mean  $\delta^{13}\text{C}$  value of LC turtles was  $-16.9 \pm 1.3\text{‰}$  (range:  $-20.2$  to  $-12.6\text{‰}$ ), whereas that for DS turtles was  $-17.4 \pm 1.4\text{‰}$  (range:  $-25.2$  to  $-15.1\text{‰}$ ). The mean  $\delta^{15}\text{N}$  value of LC turtles was  $10.3 \pm 1.0\text{‰}$  (range:  $8.3$  to  $12.8\text{‰}$ ), and that for DS turtles was  $8.7 \pm 1.8\text{‰}$  (range:  $4.9$  to  $12.9\text{‰}$ ). Further details on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and the elemental concentrations (%C and %N) of LC and DS green turtles are presented in Table 2. Differences between the size groups were observed (ANOVA,  $p \leq 0.05$ ), but there was no relationship between turtle size (CCL) and  $\delta^{13}\text{C}$  for either group. However, there was a significant positive relationship between CCL and  $\delta^{15}\text{N}$  for both LC and DS green turtles ( $R^2 = 0.18$ ,  $p \leq 0.05$ ), with larger turtles presenting higher  $\delta^{15}\text{N}$  values (Fig. 2). Among years, a Tukey post hoc comparison showed significant variability in  $\delta^{13}\text{C}$  values between LC turtles from 2016 and LC turtles from 2018, and between LC turtles from 2017 and 2018. There was no difference in the isotope values of DS turtles among all the years. When looking at the differences between LC and DS groups, there was significant

inter-annual variability within and between the LC and DS turtle groups for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Table 3).

Among forage items, the mean isotope values ranged from  $-28.3 \pm 0.2$  to  $-13.8 \pm 0.5\text{‰}$  for  $\delta^{13}\text{C}$ ; and from  $3.1 \pm 0.4$  to  $9.7 \pm 1.4\text{‰}$  for  $\delta^{15}\text{N}$  (Table 1). The 'seagrass' prey group had the highest mean  $\delta^{13}\text{C}$  value ( $-13.8 \pm 0.5\text{‰}$ ) and the 'mangrove' group had the lowest mean  $\delta^{13}\text{C}$  ( $-28.3 \pm 0.2\text{‰}$ ). For  $\delta^{15}\text{N}$ , the 'mangrove' and 'invertebrates' prey groups had the lowest ( $3.1 \pm 0.4\text{‰}$ ) and the highest ( $9.7 \pm 1.4\text{‰}$ ) mean values, respectively (Table 1). Among local macroalgae, *Hypnea spinella* had the lowest  $\delta^{13}\text{C}$  value ( $-20.1\text{‰}$ ) and *Hypnea* sp. the highest ( $-13.8\text{‰}$ ), whereas *H. pseudomusciformis* had the lowest  $\delta^{15}\text{N}$  value ( $4.9\text{‰}$ ) and *Ulva lactuca* had the highest ( $9.1\text{‰}$ ; Table A1).

#### 3.3. Turtle isotope ellipse space

SIBER analyses yielded a CHA area of 47.8 for DS turtles and of 25.8 for LC turtles. The standard ellipse areas (SEA) were 7.9 (DS) and 4.0 (LC) (Fig. 3). DS green turtles had a greater range in  $\delta^{15}\text{N}$  values and a larger isotopic niche space than live turtles (Fig. 3).

#### 3.4. Mixing model outputs

Our model including 5 dietary prey groups (Fig. 4A,B) indicated that 'invertebrates' were the most consumed prey group by LC turtles, with a mean probable dietary contribution of 41.9% (range: 14.6 to 62.2%; Fig. 4A). For DS juvenile green turtles, the most dominant prey groups were 'green algae' (24.8% mean probable contribution, range: 2.6 to

Table 2. Mean stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) values and elemental concentrations (%C, %N) for live-captured and dead-stranded juvenile green turtles found along the Paraná coast, southern Brazil, from 2016 to 2018. The capture year for 6 specimens is not provided, because they were recaptured in multiple years.  $n$  = number of individuals for which stable isotope values were available

Year	n	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		%C		%N	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Live-captured									
2016	7	$-15.0 \pm 1.8$	$-17.0$ to $-12.6$	$10.9 \pm 0.9$	9.7 to 12.1	$50.7 \pm 10.1$	44.6 to 73.4	$15.4 \pm 2.9$	12.7 to 21.7
2017	14	$-16.0 \pm 0.9$	$-17.9$ to $-14.5$	$10.5 \pm 1.0$	9.1 to 12.5	$44.4 \pm 7.6$	20.0 to 50.7	$13.3 \pm 2.3$	5.5 to 14.8
2018	61	$-17.5 \pm 1.2$	$-20.2$ to $-14.9$	$10.4 \pm 1.0$	8.3 to 12.8	$40.2 \pm 15.9$	2.5 to 52.0	$14.2 \pm 0.7$	12.5 to 16.9
Dead-stranded									
2017	38	$-17.2 \pm 1.0$	$-19.7$ to $-15.8$	$9.1 \pm 1.8$	4.9 to 13.0	$42.0 \pm 6.6$	30.8 to 65.3	$17.3 \pm 5.0$	9.6 to 38.7
2018	28	$-17.5 \pm 1.8$	$-25.2$ to $-15.1$	$8.3 \pm 1.7$	4.9 to 12.0	$41.9 \pm 4.0$	31.3 to 49.0	$16.8 \pm 2.8$	13.9 to 28.3

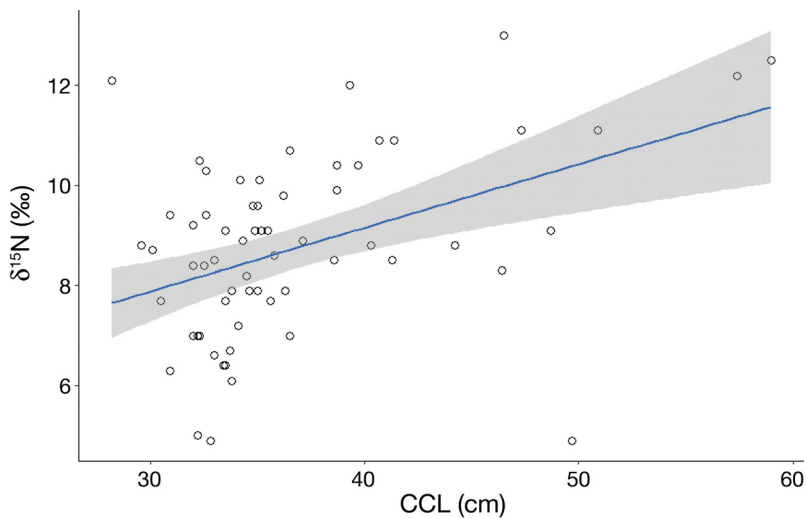


Fig. 2. Linear regression ( $R^2 = 0.18$ ,  $p < 0.05$ ) showing a significant relationship between curved carapace length (CCL) and stable nitrogen isotope values of 66 dead-stranded juvenile green turtles recovered along the Paraná coast of southern Brazil between 2017 and 2018

Table 3. Results from the Tukey post hoc comparison test for mean annual skin tissue  $\delta^{13}\text{C}$  (top) and  $\delta^{15}\text{N}$  (bottom) of the live-captured (LC) and dead-stranded (DS) juvenile green turtles encountered along the Paraná coast, southern Brazil. 'Abs (Dif)-LSD': absolute value of the difference between group means minus Fisher's least significant difference. Values in **bold** indicate pairs of means that are significantly different at  $p < 0.05$

Abs (Dif)-LSD	Difference in means	Lower values	Upper values	p
$\delta^{13}\text{C}$				
LC <sub>2017</sub> – LC <sub>2016</sub>	-1.00	-2.63	0.62	0.430
LC <sub>2018</sub> – LC <sub>2016</sub>	-2.25	-3.63	-0.87	<b>&lt;0.001</b>
DS <sub>2017</sub> – LC <sub>2016</sub>	-2.23	-3.68	-0.78	<b>&lt;0.001</b>
DS <sub>2018</sub> – LC <sub>2016</sub>	-2.48	-3.96	-0.99	<b>&lt;0.001</b>
LC <sub>2018</sub> – LC <sub>2017</sub>	-1.24	-2.26	-0.23	<b>0.007</b>
DS <sub>2017</sub> – LC <sub>2017</sub>	-1.22	-2.32	-0.12	<b>0.020</b>
DS <sub>2018</sub> – LC <sub>2017</sub>	-1.47	-2.62	-0.32	<b>0.004</b>
DS <sub>2017</sub> – LC <sub>2018</sub>	0.01	-0.66	0.70	0.990
DS <sub>2018</sub> – LC <sub>2018</sub>	-0.22	-0.99	0.54	0.920
DS <sub>2018</sub> – DS <sub>2017</sub>	-0.24	-1.12	0.62	0.930
$\delta^{15}\text{N}$				
LC <sub>2017</sub> – LC <sub>2016</sub>	-0.38	-2.11	1.34	0.970
LC <sub>2018</sub> – LC <sub>2016</sub>	-0.58	-2.05	0.88	0.800
DS <sub>2017</sub> – LC <sub>2016</sub>	-1.84	-3.38	-0.31	<b>0.009</b>
DS <sub>2018</sub> – LC <sub>2016</sub>	-2.65	-4.23	-1.07	<b>&lt;0.001</b>
LC <sub>2018</sub> – LC <sub>2017</sub>	-0.20	-1.27	0.87	0.980
DS <sub>2017</sub> – LC <sub>2017</sub>	-1.46	-2.63	-0.29	<b>0.006</b>
DS <sub>2018</sub> – LC <sub>2017</sub>	-2.26	-3.49	-1.04	<b>&lt;0.001</b>
DS <sub>2017</sub> – LC <sub>2018</sub>	-1.26	-1.99	-0.53	<b>&lt;0.001</b>
DS <sub>2018</sub> – LC <sub>2018</sub>	-2.06	-2.88	-1.25	<b>&lt;0.001</b>
DS <sub>2018</sub> – DS <sub>2017</sub>	-0.80	-1.73	0.12	0.120

57.1%) and 'seagrass' (24.2%, range: 8.4 to 37.6%; Fig. 4B).

Among all green turtles encountered during this study in Paraná, which included both LC and DS in-

dividuals, 'invertebrates' and 'green algae' were the most important prey groups (Fig. 5), with mean probable contributions of 30.5% (range: 5.2 to 60.0%) and 23.2% (range: 3.5 to 59.8%), respectively (Fig. 6A). When separating by turtle size category, 'green algae' was the most prominent diet group consumed by the smallest turtles (Size Group 1), with a 43.7% mean probable diet contribution (range: 8.0 to 86.6%; Fig. 6B). For turtles in Size Group 2 (mid-sized turtles), 'invertebrates' and 'green algae' had the highest mean probable contributions (32.7 and 31.7%, respectively; Fig. 6C). For turtles in Size Group 3 (largest turtles), the 'invertebrates' prey group had the highest mean probable contribution (52.6%, range: 5.1 to 74.1%; Fig. 6D).

#### 4. DISCUSSION

Our study highlights the importance of integrating multiple research approaches to better understand trophic status and the ecological diversity of turtle diet. Here we provide additional insights into the diet consumption and dietary diversity of both LC and DS green turtles encountered along the southern Brazilian coast. Concurrent evaluation of turtles from both origins is infrequent, and our study was successful at depicting isotopic differences between the groups that likely reflect diverse ecological strategies, underscoring the omnivorous foraging strategy of this species in the SWAO.

The stable isotope values of turtles in different size classes indicate differing diets among the groups, and suggest recent recruitment from oceanic to neritic habitats for some—usually among the smallest—individuals. As juvenile green turtles grow and develop, their diet shifts from an algae-

dominant diet to one that includes more coastal invertebrates. When we analyzed LC and DS turtles separately, there was a disparity between groups for the most likely consumed prey items, which is high-

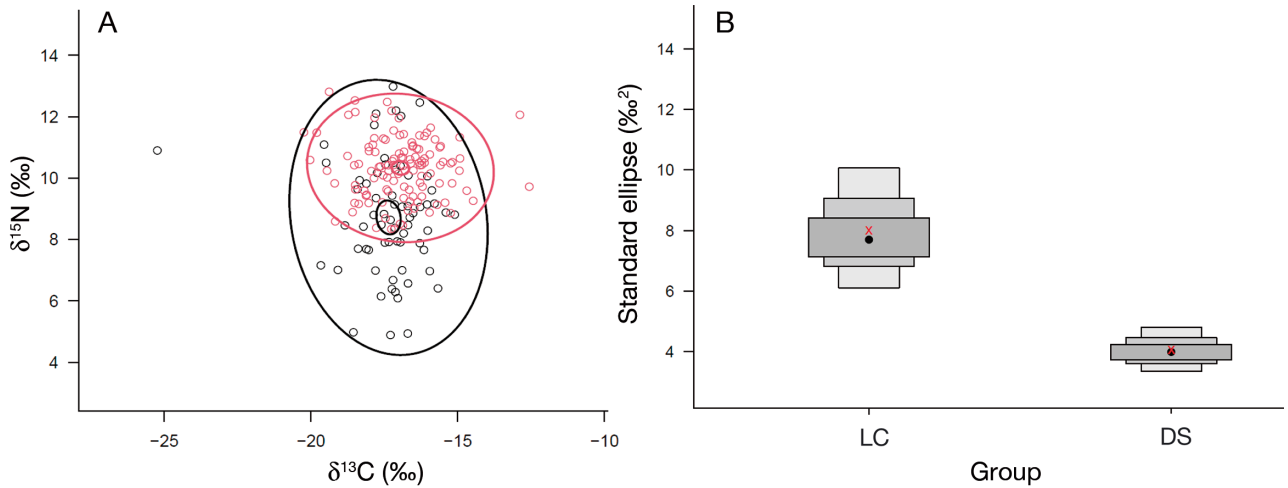


Fig. 3. (A) Standard ellipses, corrected for sample size, calculated using a maximum likelihood approach. (○) Live-captured green turtles; (○) dead-stranded green turtles. (B) Bayesian standard ellipse area (SEA) estimates for dead-stranded (DS) and live-captured (LC) juvenile green turtles. (x) Maximum likelihood estimates of the centroid of a corrected standard ellipse area (SEAc). (●) Mean values; (grey boxes) 50, 75, and 95 % credible intervals

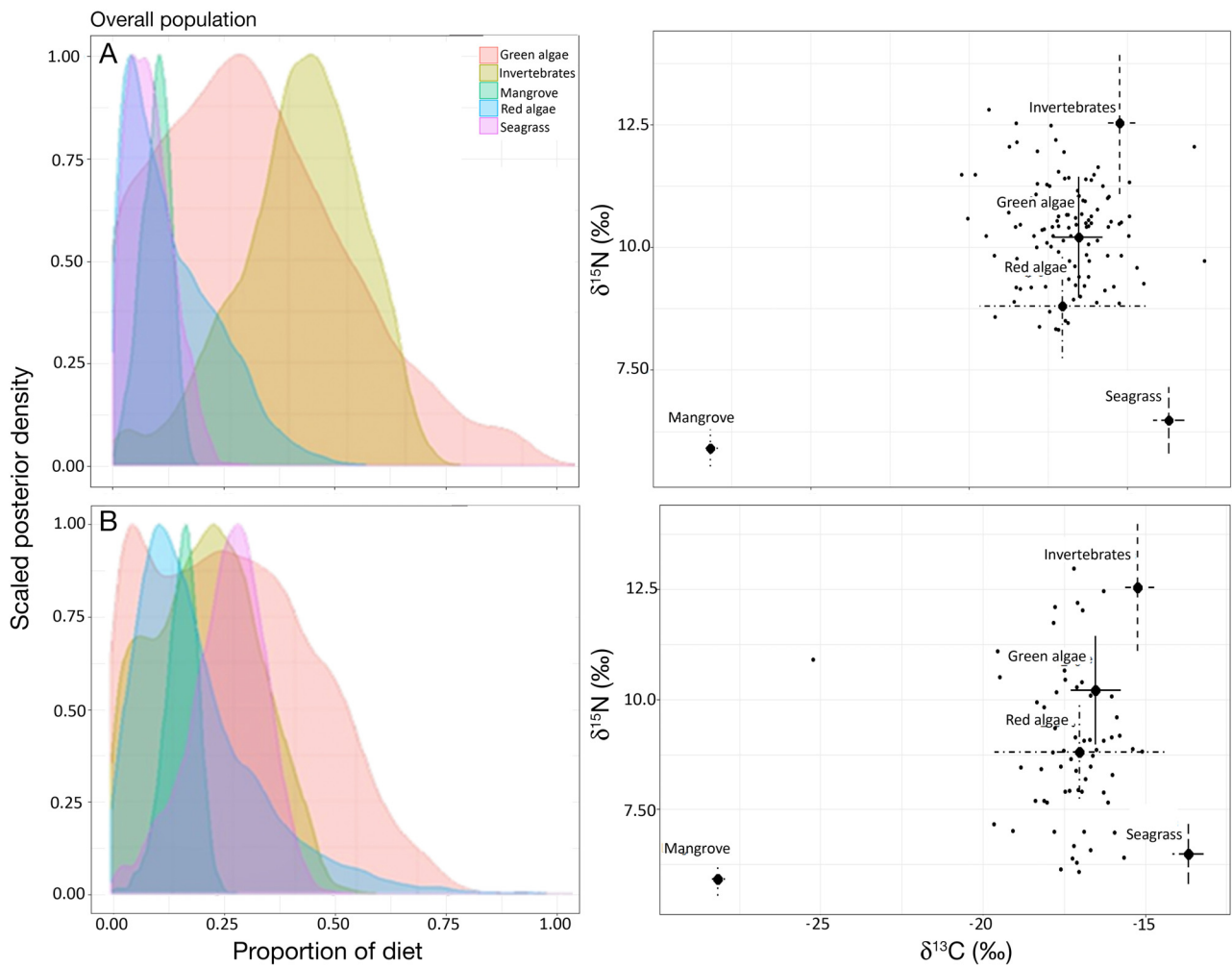


Fig. 4. MixSIAR model outputs for (A) live-captured and (B) dead-stranded juvenile green turtles along the Paraná coast, southern Brazil, with their respective raw data, resulting from the MixSIAR analysis including the isotopic values of their 5 main food items



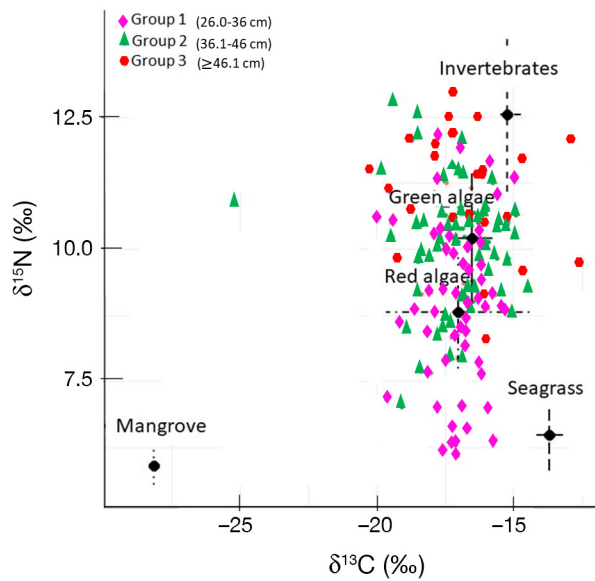


Fig. 5. Raw data for live-captured and dead-stranded juvenile green turtles along the Paraná coast, southern Brazil, among size classes resulting from the MixSIAR analysis, including the isotopic values of their 5 main food items

lighted by the large probable contribution of seagrass only in DS turtles. Whereas the LC turtles mostly consumed foods in the 'invertebrates' and 'green algae' prey groups, DS turtles most frequently ingested 'green algae' and 'seagrass', the latter of which was especially important in the smallest size group. However, it is important to clarify that because the seagrass isotopic values available in the literature are from areas with a significant oceanic water intrusion (Lazzari 2012, Corbisier et al. 2014), the isotope value for 'seagrass' used in MixSIAR models may mimic isotopic values of oceanic prey, as reported elsewhere in the SWAO (Vélez-Rubio et al. 2016). For example, the isotope values attributed in our MixSIAR models to seagrass may reflect the consumption of oceanic items, such as sea jellies, as has been reported for green turtles in Uruguay (Vélez-Rubio et al. 2016). Indeed, the lack of isotopic specificity between neritic seagrasses and oceanic prey makes the mixing model results equivocal and brings some bias to our knowledge about feeding ecology of these smallest stranded turtles. These factors highlight the need for further isotopic study of this green turtle life stage in the SWAO.

SIBER analysis indicated that DS green turtles had a substantially different isotopic niche ellipse space and an overall larger isotopic niche width relative to the LC green turtles included in this study. Both groups had highly similar  $\delta^{13}\text{C}$  ranges, but the DS group had substantially more turtles with low  $\delta^{15}\text{N}$

values, which was responsible for the larger niche space of this group. This result suggests these individuals may be recent arrivals to the Paraná study region, originating from areas that have a lower baseline  $\delta^{15}\text{N}$  than found locally, or perhaps forage at lower trophic positions than their LC counterparts. Considering that most green turtles in the SWAO recruit from offshore areas to coastal waters at  $\leq 35$  cm CCL (Andrade et al. 2016), turtles of this size and smaller would be expected to be oceanic juveniles and thus consume prey of oceanic nutrient pathways, which typically have lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than neritic systems (Hatase et al. 2010, Piovano et al. 2020).

Although the origins of DS turtles are unknown, it is possible that they originate from both offshore and coastal zones. In the South Pacific, Piovano et al. (2020) found a similar result, with 2 groups of turtles at a Fijian foraging habitat, including 1 group of turtles that had different  $\delta^{15}\text{N}$  values to the local habitat and were considered to be recently settled from offshore waters and not yet in isotopic steady state with the coastal habitat. The possibility that at least some of the DS turtles in this study were recent arrivals from offshore waters is also supported by isoscape mapping in the region conducted by Troina et al. (2020), who found higher  $\delta^{15}\text{N}$  values among zooplankton collected along the shelf break in the SWAO relative to plankton collected in offshore waters. If so, then green turtles occupying offshore waters would also be expected to have lower  $\delta^{15}\text{N}$  values than turtles living in coastal shelf habitats.

In addition to offshore origins, it is possible that at least some turtles in this study transitioned to Paraná from other coastal foraging areas in the region (González Carman et al. 2012, Fuentes et al. 2020). Indeed, there is similarity in stable isotope values among green turtles at multiple foraging areas in the SWAO. DS turtles from the Paraná have similar  $\delta^{15}\text{N}$  values to those found for green turtles in Argentina (González Carman et al. 2014) and in northeast Brazil (Bezerra et al. 2015); and their  $\delta^{13}\text{C}$  values are similar to those found for Rio de Janeiro (Di Benedetto et al. 2017) and northeast Brazil (Bezerra et al. 2015). Green turtle  $\delta^{13}\text{C}$  values (range:  $-20.2$  to  $-12.6$ ‰) in Paraná are similar to those for green turtles in northeast and southeast Brazil (Campos & Cardona 2019) (Table 4). For LC turtles in this study, the mean  $\delta^{15}\text{N}$  value ( $10.3 \pm 0.98$ ‰) is similar to values reported for green turtles in northeast and southeast Brazil (Bezerra et al. 2015, Di Benedetto et al. 2017) (Table 4). Likewise, González Carman et al. (2014) reported  $\delta^{13}\text{C}$  values for live green turtles in Argentina ( $-16.5 \pm 0.2$ ‰) that are similar to those of live turtles from our

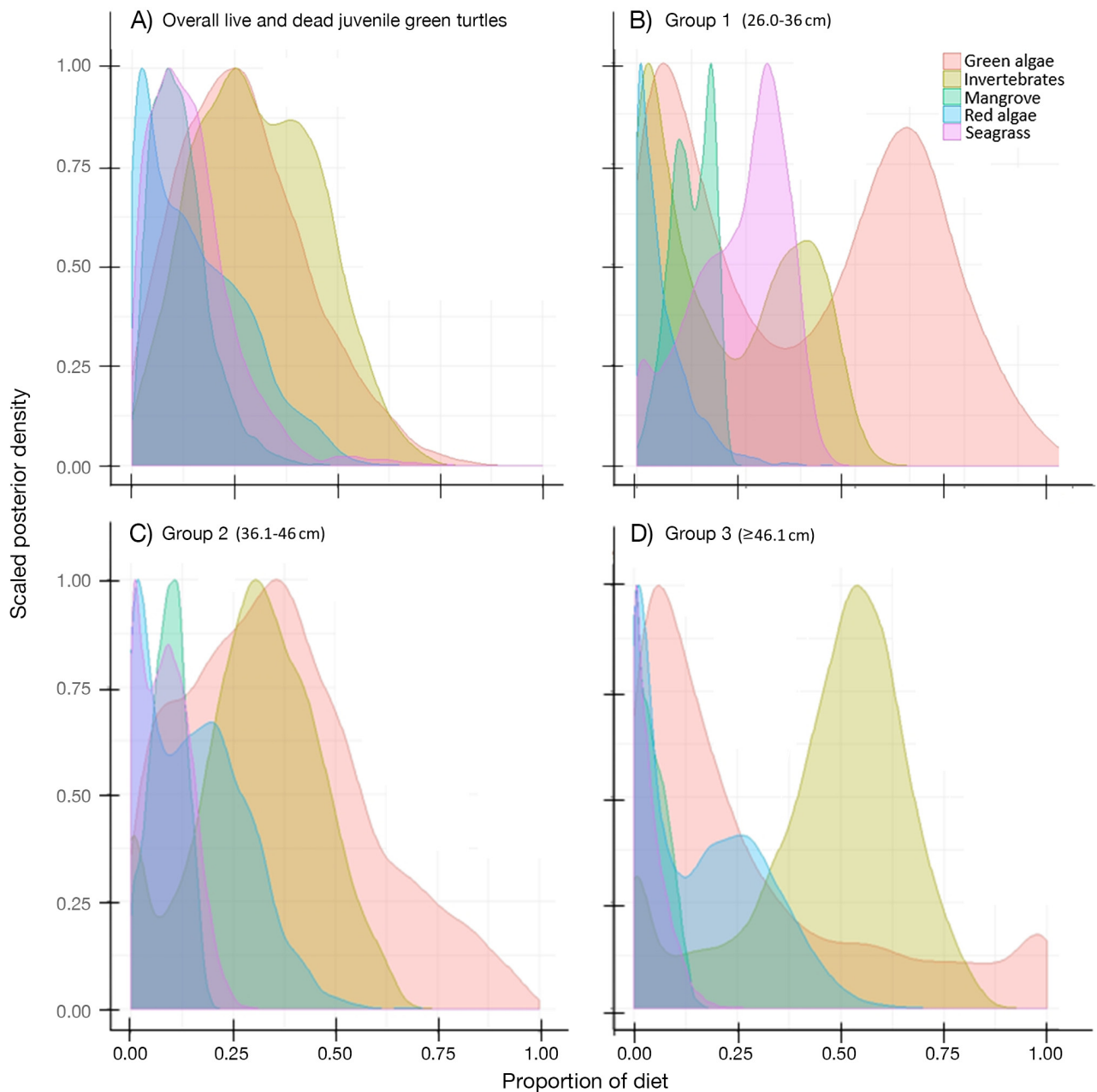


Fig. 6. MixSIAR model outputs for overall and different size classes of live-captured and dead-stranded juvenile green turtles along the Paraná coast, southern Brazil. (A) Overall turtles; (B) Group 1: 26.0–36.0 cm; (C) Group 2: 36.1–46.0 cm; (D) Group 3:  $\geq 46.1$  cm

study in Paraná. The similarity between the isotope values at all these sites, despite their spatial separation by more than 1000 km, suggests a notable level of connectivity among green turtle foraging populations throughout the SWAO. Consistency in isotope values among green turtles assembled in disparate foraging areas of the SWAO could also reflect the relatively small spatial variability in regional  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isoscapes in the SWAO (McMahon et al. 2013). This makes it challenging to assign prior origins of

recently arrived green turtles in the region but does support our use of prey isotope values from areas outside of Paraná in the mixing models.

The presence of algae and invertebrates in the SWAO is reported in all studies that have been conducted in the region; however, for the Paraná coast specifically, the low contribution of seagrass to the diets of LC green turtles may signal impacts of anthropogenic activities that affect the Paraná and wider PEC region. Even the smallest LC turtles did not pres-

Table 4. Stable isotope studies performed with green turtles from along the Southwestern Atlantic Ocean. Study sites are listed from north to south; isotope data are presented as mean  $\pm$  SD or ranges, depending on how data were presented in the source literature

Study site	Sample size (n)	Size range (CCL; cm)	Tissue	$\delta^{13}\text{C}$ (‰) range/ mean	$\delta^{15}\text{N}$ (‰) range/ mean	Reference
Ceará and Bahia, Northeast Brazil	31	27.0–62.0	Muscle Scute	$-17.1 \pm 0.9$ $-18.9 \pm 0.8$	$10.4 \pm 0.22$ $9.6 \pm 0.22$	Bezerra et al. (2015)
Bahia and Ubatuba, Northeast and Southeast Brazil	30	31.1–104	Scute	–21.7 to –13.9	5.3 to 14.0	Campos & Cardona (2019)
Rio de Janeiro, Southeast Brazil	45	27.3–48	Muscle	$-17.4 \pm 0.8$	$9.9 \pm 1.8$	Di Benedetto et al. (2017)
Paraná, South Brazil	66 dead 117 alive	28.2–59.0 33.4–54.0	Skin	$-17.4 \pm 1.4$ $-16.9 \pm 1.3$	$8.7 \pm 1.8$ $10.3 \pm 1.0$	This study
Argentina	7 dead; 7 alive	31.3–52.2 <sup>a</sup>	Skin	$-15.5 \pm 0.7$ $-16.5 \pm 0.2$	$9.5 \pm 1.0$ $11.1 \pm 1.1$	González Carman et al. (2014)
Uruguay	126	27.8–66.8	Skin	–18.2 to –13.6	6.7 to 15.6	Vélez-Rubio et al. (2016)
Uruguay	20	27.8–66.8	Scute	–19.8 to –14.8	5.8 to 15.8	Vélez-Rubio et al. (2018)

<sup>a</sup>Size range reflects animals included in the gut analysis, only a subset of which had stable isotope information

ent seagrass as an important dietary item, which was a common finding of previous diet studies in this area (e.g. Guebert-Bartholo et al. 2011, Gama et al. 2016).

It is also known from previous studies that marine algae are the most frequently consumed food type among green turtles in the SWAO (Santos et al. 2015, Gama et al. 2016, Vélez-Rubio et al. 2016). For instance, in Uruguay 69% of juvenile green turtles with CCL from 35 to 45 cm had consumed macroalgae, with *Ulva* sp. being the most-consumed species (58.6%) (Vélez-Rubio et al. 2016). Along the Paraná coast, *Ulva* sp. comprised 43% of green turtle diet (Gama et al. 2016). This is a macroalgae species that proliferates in polluted areas; thus, its high consumption could be reflective of low habitat quality (Bordean et al. 2016, Bonanno et al. 2020).

The high importance of the ‘invertebrates’ prey group in the LC juvenile green turtle diet may be reflective of changes in juvenile green turtle foraging strategy, specifically along the Paraná coast, where prior gut content analyses have shown that turtles at this life stage most frequently consumed vegetal prey items such as seagrass and macroalgae (Guebert-Bartholo et al. 2011, Gama et al. 2016). Dependence on invertebrate prey species has been reported throughout subtropical and temperate areas of Brazil (González Carman et al. 2014, Vélez-Rubio et al. 2016). These changes could be a consequence of widespread habitat degradation and a resulting lower availability of vegetal prey items.

The study of both LC and DS turtles provided novel insights on potential differences in their diet, habitat use, and foraging behavior. The isotopic similarities found among this and prior SWAO studies suggest that juvenile green turtle foraging ecology is relatively consistent throughout the region and reinforces the importance of this area as a key foraging ground for the species. As a result, every effort should be made to build effective management actions that promote population recovery and habitat protection throughout the SWAO region.

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### Appendix. Additional data on stable isotope values of different taxonomic groups

Table A1. Stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) values ( $\pm\text{SD}$  where available) of marine algae, seagrass, mangrove, and invertebrates from the Southwestern Atlantic Region. SP: Sao Paulo, SC: Santa Catarina

Site	Habitat item	Genus/species	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Reference
Itanhaém/SP, Brazil	Rhodophyta	<i>Hypnea pseudomusciformis</i>	-18.0	4.9	This study
Itanhaém/SP, Brazil	Rhodophyta	<i>Hypnea spinella</i>	-20.1	6.3	This study
Ubatuba/SP, Brazil	Rhodophyta	<i>Hypnea spinella</i>	-17.0	7.3	Corbisier et al. (2006)
Southeast Brazil	Rhodophyta	<i>Hypnea</i> sp.	-13.8	5.4	Vinagre et al. (2018)
Itanhaém/SP, Brazil	Chlorophyta	<i>Ulva fasciata</i>	-17.0	6.6	This study
Penha/SC, Brazil	Chlorophyta	<i>Ulva lactuca</i>	-15.6	9.1	This study
Ubatuba/SP, Brazil	Chlorophyta	<i>Ulva fasciata</i>	-17.2	6.4	Corbisier et al. (2006)
South Brazil	Chlorophyta	<i>Ulva</i> sp.	-17.1	7.7	Vélez-Rubio et al. (2016)
Guaratuba/PR, Brazil	Black mangrove	<i>Avicennia schaueriana</i>	$-28.3 \pm 0.2$	$3.1 \pm 0.4$	Larcher et al. (2016)
Northeast Brazil	Seagrass	<i>Halodule wrightii</i>	$-14.2 \pm 0.4$	$3.2 \pm 1.7$	Lazzari (2012)
Southeast Brazil	Seagrass	<i>Halodule</i> sp.	-13.5	4.2	Corbisier et al. (2014)
Southeast Brazil	Invertebrates	Depositivorous	$-15.9 \pm 2.2$	$9.5 \pm 1.0$	Corbisier et al. (2006)
Southeast Brazil	Invertebrates	Carnivorous	$-14.9 \pm 1.1$	$11.3 \pm 0.8$	Corbisier et al. (2006)
Southeast Brazil	Invertebrates	Suspensivorous	$-15.5 \pm 1.6^a$	$8.4 \pm 0.6^a$	Corbisier et al. (2006), Vinagre et al. (2018)

<sup>a</sup>Results presented as a combination of the 2 referenced studies

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