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ORIGINAL PAPER

Ontogenetic dietary changes of green turtles (*Chelonia mydas*) in the temperate southwestern Atlantic

G. M. Vélez-Rubio[1,2,3] · L. Cardona[4] · M. López-Mendilaharsu[1,5] · G. Martínez Souza[1,6] · A. Carranza[3,7] · D. González-Paredes[1,8] · J. Tomás[2]

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Abstract The present study combines esophageal lavage ($n = 74$), stomach content ($n = 52$) and stable isotope analysis ($n = 126$) to understand the ontogenetic dietary shift of green turtles (*Chelonia mydas*) inhabiting the temperate waters off Uruguay. Based on esophageal and stomach analysis, green turtles in the region start consuming macroalgae soon after recruiting to neritic habitats; however, gelatinous macrozooplankton is still a major component of the diet of neritic juvenile green turtles measuring of less than 45 cm in curved carapace length (CCL). Conversely, turtles larger than 45 cm CCL were predominantly herbivores, with a gradual increase in the occurrence of macroalgae with size. Stable isotope analysis confirmed the dietary pattern revealed by esophageal lavage and stomach contents analysis, and also revealed that most of the green turtles smaller than 50 cm CCL found in Uruguayan waters had moved from Brazil only a few months ago. This conclusion is based on the large differences in the $\delta^{15}N$ values of potential prey from southern Brazil and Uruguay and on a strong signal from Brazilian macrophytes in the skin of most green turtles from Uruguay. Turtles larger than 50 cm CCL, conversely, made a more prolonged use of Uruguayan foraging grounds. Furthermore, according to the stable isotope ratios in their skin, some turtles remained year round in Uruguayan coastal waters. The overall evidence indicates that green turtles inhabiting the coastal waters off Uruguay exhibit a rapid, but not abrupt, dietary shift after recruiting to neritic habitats and are best described as omnivores than as pure herbivores, with a relevant role of gelatinous macrozooplankton in their diets. Furthermore, most of the turtles spend only short periods in the area and their primary foraging grounds are in Brazil.

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✉ G. M. Vélez-Rubio
gabriela.velez@uv.es

[1] Karumbé NGO, Av. Rivera 3245, C.P.11600 Montevideo, Uruguay

[2] Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology (ICBIBE), University of Valencia, Aptdo. 22085, E-46071 Valencia, Spain

[3] Grupo de Investigación y Formación de Recursos Humanos en Biodiversidad, Centro Universitario Regional del Este (CURE), Universidad de la República, Campus de Maldonado, 20100 Punta del Este, Uruguay

[4] Biodiversity Research Institute (IRBio) and Department of Animal Biology, Faculty of Biology, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

[5] Fundação Pró-TAMAR, Rio Vermelho, Salvador, Bahia 2219, Brazil

[6] Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Campus Carreiros - Av. Itália, km 8., Rio Grande, Rio Grande do Sul 96203-900, Brazil

[7] Área Biodiversidad y Conservación, Museo Nacional de Historia Natural, C.C. 399, C.P. 11000 Montevideo, Uruguay

[8] Hombre y Territorio Association, c/ Betania 13, 41007 Seville, Spain

Introduction

Ontogenetic dietary shifts are frequent in aquatic vertebrates because of age-related changes in habitat, body size and characteristics of the feeding organs (e.g., Bethea et al. 2007; Costalago et al. 2012; Vales et al. 2015). As a consequence, species fill different trophic niches during their life cycles, which results into food webs more complex than expected on species richness only (McCann 2012).

Green turtles (*Chelonia mydas*) exhibit an oceanic–neritic developmental pattern (Bolten 2003). Early research suggested that green turtles shift from an omnivorous diet during their juvenile, oceanic stage of their early life to a primarily herbivorous diet when they are 3–6 years old and recruit to neritic habitats (Bjorndal 1985, 1997; Seminoff et al. 2002). Such an ontogenetic dietary shift to herbivory was described as abrupt and irreversible in some tropical regions (Bjorndal 1997; Reich et al. 2007; Arthur et al. 2008). However, recent research has revealed regional differences in the timing of this process (Cardona et al. 2010), with high levels of omnivory after recruitment (e.g., Cardona et al. 2009, Burkholder et al. 2011, Lemons et al. 2011, Russell et al. 2011, Gonzalez Carman et al. 2013) and the persistence of a carnivorous diet in adults that forage in the open ocean throughout their life (Hatase et al. 2006; Kelez 2011; Parker et al. 2011). Such intraspecific variability can be expected for a species inhabiting a wide diversity of habitats in tropical and warm-temperate waters around the globe (Wallace et al. 2010), as trophic plasticity will ensure survival on a wide range of local conditions (Santos et al. 2015).

Green turtles in the southwestern Atlantic nest primarily on tropical islands (Almeida et al. 2011; Bellini et al. 2013; Weber et al. 2014 and references therein) and forage along the coast of mainland South America. According to stomach content analysis and direct observations, juvenile green turtles from southern Brazil often have omnivorous diets, but the relative abundance of vegetal material increases quickly with turtle size (Nagaoka et al. 2012; Reisser et al. 2013; Morais et al. 2014). Conversely, stomach content and stable isotope analysis indicate that juvenile green turtles captured during the warm season off Argentina feed primarily on gelatinous plankton (Gonzalez Carman et al. 2013). Juvenile green turtles occur in Uruguayan waters year round, but their abundance peaks are in late austral summer and early austral fall (López-Mendilaharsu et al. 2006; Gonzalez Carman et al. 2012; Vélez-Rubio et al. 2013; Martinez Souza 2015), probably because of a seasonal latitudinal migration (Gonzalez Carman et al. 2012). The main foraging grounds of green turtles off Uruguay are found in the rocky outcrops scattered along the east coast (López-Mendilaharsu et al. 2006; Vélez-Rubio et al. 2013), but little is known about their diet. Previous research suggested that green turtles inhabiting warm-temperate regions with wide fluctuations in sea surface temperature (SST) exhibit a delayed ontogenetic dietary shift and high levels of omnivory (Cardona et al. 2009, 2010), a pattern recently confirmed also for the species in Brazil (Santos et al. 2015). A similar situation may occur off Uruguay, where SST ranges annually 9–27 °C (Acha et al. 2004).

Esophageal lavage and stomach contents analysis provide with direct information about diet composition, although they are often highly biased and represent only a short time window (days to weeks), which usually results in an incomplete description of an animal's diet (Hyslop 1980; Burkholder et al. 2011; Gonzalez Carman et al. 2013). On the other hand, stable isotope analysis of tissues with a low turnover rate integrates dietary information during longer periods (Hobson 1999; Dalerum and Angerbjörn 2005). Furthermore, stable isotope analysis gives information about assimilated prey and not only on those consumed (Post 2002). Dietary inference from stable isotope analysis of animal tissue is possible because animal tissue isotopic composition is ultimately derived from that of its complete diet over time, plus the effect of trophic discrimination from predator to prey (DeNiro and Epstein 1981; Arthur et al. 2008). Stable isotope ratios in sea turtle skin integrate diet during two or three months, and the prey-to-consumer trophic discrimination factor for the epidermis of green turtles has been determined experimentally (Seminoff et al. 2006). This offers an opportunity not only to study the ontogenetic dietary change in green turtles occurring off Uruguay, but also to test hypothesis about the movements of green turtles between southern Brazil and Uruguay, namely that (1) most turtles migrate from southern Brazil to coastal waters off Uruguay to forage during the warm season and (2) some turtles stay year round in Uruguayan waters, possibly overwintering along coastal habitats. In this paper, we used esophageal lavage, stomach contents analysis and stable isotope analysis to test these hypotheses.

Materials and methods

Study area

The Uruguayan coast (710 km length) is part of a complex hydrological system that comprises the frontal zone of the Rio de la Plata estuary and the Atlantic Ocean (Fig. 1). This system is characterized by a strong salinity gradient, affected by seasonal and episodic variations in the outflow of the estuary (Ortega and Martínez 2007; Campos et al. 2008; Horta and Defeo 2012). The estuarine plume flows from the west and mixes with the oceanic waters

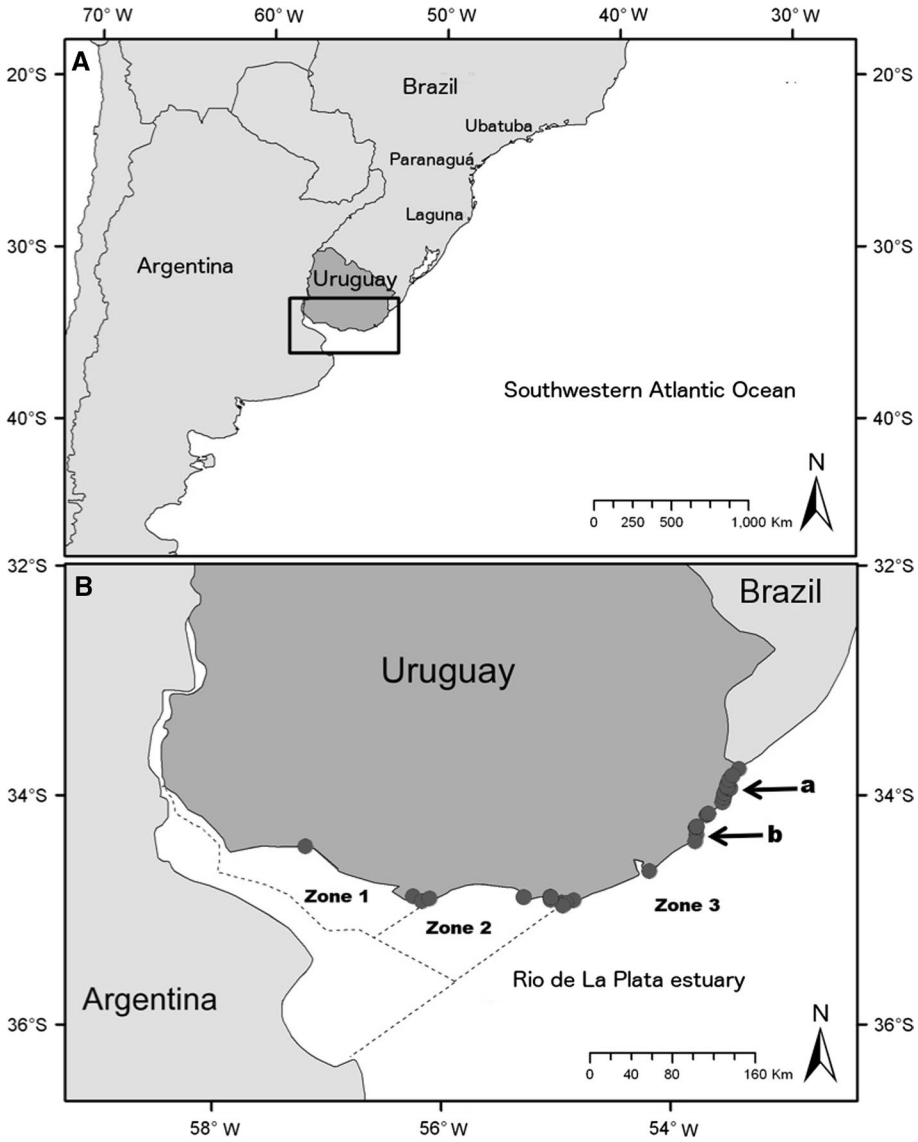


Fig. 1 **a**, Map of the southwestern Atlantic and the study area indicated (*solid line*); **b**, map of the Uruguayan coast. *Dark circles* indicate the stranding locations of turtles included in the study ([Zone 1] inner estuarine zone, [Zone 2] outer estuarine zone and [Zone 3] oceanic zone). The *arrows* indicate the locations where green turtles were captured for the study (**a**, Coastal-Marine Protected Area of Cerro Verde e Islas de La Coronilla and **b**, Coastal-Marine Protected Area of Cabo Polonio)

Table 1 Resume of the three methodologies for green turtle diet analysis in the present study

Analysis	Period	Season	Zone	N	Type of record	CCL range (cm)
Esophageal lavage	2003–2005	Summer/fall	CMPA Cerro Verde/Cabo Polonio	74	Captured	32.6–58.4
Stomach contents	2009–2013	All year	All Uruguayan coast	52	Stranded	29.8–62.0
Stable isotopes	2012–2013	All year	All Uruguayan coast	126	Captured/stranded	27.8–66.8

of the subtropical convergence zone. The cold Falkland/Malvinas current influences this zone in the austral winter and the warm Brazilian current during the austral summer. This results in SST variations greater than 15 °C (range 9–27 °C) throughout the year (Acha et al. 2004). Three zones can be distinguished in Uruguayan coastal waters (Fig. 1b) based on the differences in hydrological characteristics (e.g., Defeo et al. 2009): the inner estuarine zone (Zone 1) and the outer estuarine zone (Zone 2) are influenced directly by the Rio de la Plata discharge, whereas the oceanic zone (Zone 3) has a remarkable but variable oceanic regime. The Uruguayan coast is a succession of sandy beaches of variable extension (2–20 km long) separated by rocky outcrops, scarcer in the estuarine zones than in the oceanic zone. As in waters of the southeastern coast of Brazil, Uruguayan coastal waters host few brown algae and not allow the development of seagrass meadows probably due to a high turbidity (Oliveira 1984; Coll and

Oliveira 1999). The most common macroalgae species in shallow rocky bottoms are the chlorophytes *Ulva lactuca*, *Ulva fasciata*, *Codium cf. decorticatum*, *Chaetomorpha* sp., *Cladophora* cf *vagabunda* and the rhodophytes *Grateloupia* cf *filicina*, *Grateloupia cuneifolia*, *Chondracanthus teedei*, *Pterocladiella capillacea*, *Criptopleura ramosa*, *Corallina officinalis*, *Polysiphonia* sp., *Hypnea musciformis* and *Rhodymenia* sp. (Coll and Oliveira 1999). Mussels (*Mytilus edulis*) are the most conspicuous invertebrates in the community (Borthagaray and Carranza 2007).

Field data collection and process of samples

From 2003 to 2005, 74 green turtles were captured for esophageal lavage in two Coastal-Marine Protected Areas (CMPA) of Uruguay (Cerro Verde e Islas de La Coronilla and Cabo Polonio, Fig. 1b, Table 1), as part of the long-term study on abundance and habitat use of the species in eastern Uruguay conducted by the nongovernment organization (NGO) Karumbé. Turtles were captured alive over rocky bottoms in very shallow waters. Set nets (nylon monofilament, 50 m length × 3 m depth, 30 cm stretched mesh size) were deployed perpendicular to wave direction and were monitored constantly to avoid drowning of turtles caught. Curved carapace length (CCL, notch to tip) was measured for each turtle using a flexible tape (±0.1 cm). All turtles were tagged with inconel flipper tags (Style # 681, National Band and Tag, Kentucky, USA) before release in the same site of capture. Since marine turtles from different populations may reach sexual maturity at different sizes, we used the minimum size of nesting females from the closest nesting colonies (CCL = 90 cm in Trindade island Brazil; Almeida et al. 2011) to classify the turtles captured as putative juveniles or putative adults. The turtles included in the present study are really small compared to those putative adults, as clarified latter in the results section.

Esophageal lavage was used to provide a sample of food resources that the turtles had ingested in the previous hours before being captured (Forbes and Limpus 1993). Methodology for esophageal lavage followed Makowski et al. (2006) protocol. Turtles were placed on their carapace, with the head positioned downward, keeping the mouth open with a pry bar. The water injection tube had 4 mm ID (internal diameter) with a wall thickness of 1.5 mm and 3 m in length. The tube was lubricated with vegetable oil and passed down the esophagus until resistance was met and clean seawater was gently pumped into the esophagus. The lavage was sieved (0.2 mm) to retrieve food items regurgitated by the turtle. All food material obtained was preserved in a 4 % formalin solution with seawater.

Samples for stable isotope analysis were collected from 126 green turtles captured in the same locations in 2012 and 2013 (Fig. 1b, Table 1). Skin biopsies were taken from the dorsal side of the inguinal region of the left hind flipper. The skin area was cleaned for disinfection and to remove ectoparasites. A thin layer of epidermal tissue (~1 cm^2) was collected with a scalpel and preserved in a NaCl solution until analysis.

Stomach content samples and skin samples were also collected from the carcasses of dead turtles stranded along the entire Uruguayan coast during the period 2009–2013 (Fig. 1b, Table 1). These stranded turtles were recorded during beach surveys conducted by technicians of the marine turtle stranding network (see Vélez-Rubio et al. 2013). Fifty-two digestive track contents were analyzed and preserved separately in esophagus, stomach and intestine sections. Stomach contents were rinsed and preserved in a 4 % formalin solution in seawater. For skin samples, we used the same protocol as with the captured green turtles (see above). All the turtles were freshly dead as a result of bycatch, and controlled experiments have demonstrated that $\delta^{13}C$ and $\delta^{15}N$ values in the skin of sea turtles do not change during the first weeks after death (Payo-Payo et al. 2013).

Samples of macroalgae were collected in Uruguay (17 species; in CMPA of Cerro Verde and CMPA of Cabo Polonio) and southern Brazil (5 species; in Santa Catarina). Sampling was designed to collect most of the species more frequently in the gut contents of green turtles off Uruguay (present study) and southern Brazil (Reisser et al. 2013). Furthermore, two species of gelatinous macrozooplankton were collected: *Chrysaora lactea* from Uruguay and *Velella velella* from Brazil. All the samples were rinsed with water and frozen until analysis or stored dry as herbarium samples. The description of the variability of the stable isotope values of the turtles and their prey within the $\delta^{13}C$–$\delta^{15}N$ space, hereafter called the isospace, was complemented with data from the literature about the squid *Loligo sanpaulensis* from Brazil and Uruguay (Drago et al. 2015) and the seagrass *Halodule wrightii* from Brazil (Lazzari 2012).

Gut contents analysis

Dietary items retrieved by esophageal lavage and stomach content analysis were identified to the lowest possible taxonomic level. Dietary item groups were quantified by frequency of occurrence (FO) and relative volume (RV) (Hyslop 1980). For relative volume measure, the entire sample volume and the relative sample volume of each diet group were calculated by means of water displacement in a graduated cylinder. Any item with a relative volume >5 % in at least one sample was considered a major diet component (Garnett et al. 1985; López-Mendilaharsu et al. 2005).

To determine the variation in diet between individual animals, RV of each major prey group was calculated for each individual turtle as follows:

$$\%\mathrm{RV}_i = \frac{\text{Total volume of diet item in each turtle}}{\text{Total volume of each turtle}} \times 100$$

FO of each dietary species and RV of each major dietary item group (macroalgae, gelatinous macrozooplankton and marine debris) were determined as follows:

$$\%\mathrm{FO} = \frac{\text{Number of samples containing diet item}}{\text{Total mumber of samples}} \times 100$$

$$\%\mathrm{RV} = \frac{\text{Total volume of diet item in all samples}}{\text{Total volume of all samples}} \times 100$$

We estimated the importance of the main dietary groups through the Index of Relative Importance (%IRI, Pinkas et al. 1971) as follows:

$$\mathrm{IRI} = \%\mathrm{RV} \times \%\mathrm{FO}$$

$$\%\ \mathrm{IRI} = \left(\frac{IRI}{\sum_{n-1}^{n} IRI}\right) \times 100$$

We studied the ontogenetic dietary shift of green turtles through stomach contents analysis (collected during 2009–2013, $N = 52$) and for esophagus analysis (collected during 2003–2005, $N = 74$) of specimens classified in three size classes A: CCL < 35 cm; B: 35 ≥ CCL < 45 cm; and C: CCL ≥ 45 cm (Table 1).

Stable isotope analysis

A total of 126 epidermis green turtle samples were available for stable isotope analysis. Samples were dried at 60 °C for three days, grounded to a fine powder, and lipids extracted with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959). Lipids are depleted in ^{13}C in comparison with other molecules, which could bias $\delta^{13}C$ values; it is thus desirable to remove lipids prior to stable isotope analysis (DeNiro and Epstein 1978). After the chloroform/methanol treatment, the C:N ratio of turtle epidermis was always lower than 4, thus confirming that lipids had been removed efficiently or were naturally scarce. Macroalgae are often covered with epibionts containing carbonates, which may also bias $\delta^{13}C$ values. Accordingly, macroalgae samples were split into two subsamples. One of them was treated with O.5 N hydrochloric acid (HCl), to remove carbonates and with a chloroform:methanol (2:1) solution to remove lipids. However, acidification may modify the $\delta^{15}N$ value, so the second bulk subsample was used to determine the $\delta^{15}N$ value (Cardona et al. 2012). Samples were weighed into tin cups with a microbalance (0.3 mg for skin and animal prey samples and 0.5 mg for algae samples, because they differ in their N contents), combusted at 1000 °C, and analyzed in continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan) at the Centres Científics i Tecnològics of the Universitat de Barcelona (Spain).

Stable isotope abundances were expressed in δ notation according to the following expression:

$$\delta X = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] \times 10^3$$

where X is ^{13}C or ^{15}N, Rsample is the heavy to light isotope ratio of the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), respectively, and Rstandard is the heavy to light isotope ratio of the reference standards, which were VPDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. International isotope secondary standards of known $^{13}C/^{12}C$ ratios, as given by the IAEA (International Atomic Energy Agency IAEA), namely polyethylene (IAEA CH_7, $\delta^{13}C = -31.8$ ‰), graphite (IAEA USGS24, $\delta^{13}C = -16.1$ ‰) and sucrose (IAEA CH_6, $\delta^{13}C = -10.4$ ‰), were used for calibration at a precision of 0.2 ‰. For nitrogen, international isotope secondary standards of known $^{15}N/^{14}N$ ratios, namely $(NH_4)_2SO_4$ (IAEA N1, $\delta^{15}N = +0.4$ ‰ and IAEA N_2, $\delta^{15}N = +20.3$ ‰) and KNO_3 (IAEA NO_3, $\delta^{15}N = +4.7$ ‰), were used to a precision of 0.3 ‰.

Statistical analysis

Results are reported as mean ± standard deviation, unless otherwise stated.

The relationship between $\delta^{13}C$ and $\delta^{15}N$ values and turtle size (curved carapace length, CCL), date of capture or stranding (Julian day), distance to the inner part of Rio de la Plata Estuary, and type of record (captured or stranded) were modeled using generalized additive models (GAM) with package *mgcv* (package version 1.7–5, Wood 2011) in R 2.11 (R Development Core Team 2010). This method is based on the use of nonparametric smoothing functions that allow flexible description of complex species responses to environment (Leathwick et al. 2006). The GAM approach is an extension of the generalized linear model (GLM). This technique enables robust analysis of regression models with nonlinear covariate functional form and a range of nonnormal error terms (Hastie and Tibshirani 1990). The degree of smoothness of model terms was estimated as part of fitting using penalized cubic regression splines. A Gaussian error model was used in the GAM analysis, with a link identity function. Model selection was guided by Akaike information criterion (AIC). For GAMs plots, the y-axis is a relative scale, with a positive value on the plots, indicating a positive effect of that explanatory variable on the dependent variable and a negative *y*-value indicating a negative effect of that variable. The range of the smoothed function indicates the relative importance of each predictor.

One-way ANOVA and Tukey's post hoc test were used to compare the stable isotope ratios of macroalgae species simultaneously collected from Brazil and Uruguay and to test for differences in the isotope baseline of both regions.

We used the Bayesian stable isotope mixing model in the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010) to estimate the relative contributions of the different dietary items (macroalgae, cnidarians, seagrasses and squids) from Uruguay and Brazil to turtle diet. SIAR assumes that the variability associated with food sources and trophic enrichment is normally distributed (Parnell et al. 2010). To better restrict our model, we used elemental concentrations (%C and %N) measured for each organic basal source in this study (Coelho Claudino et al. 2013). Although SIAR incorporates uncertainty about diet–tissue isotopic discrimination factors in the form of standard deviation, we conducted a sensitivity analysis running SIAR for green turtle epidermis with diet–tissue isotopic discrimination factors of $+0.17 \pm 0.03$ for $\delta^{13}C$ and $+2.80 \pm 0.11$ $\delta^{15}N$ (Seminoff et al. 2006). The criteria to include putative dietary items into the model were that they (1) had been observed in the gut contents in Brazil (Reisser et al. 2013) or Uruguay (this study) and (2) are common species in the Uruguayan coast (Coll and Oliveira 1999; Borthagaray and Carranza 2007). Accordingly, the model included the macroalgae *Ulva* sp. and *Grateloupia* sp., the cnidarian *Chrysaora lactea* and the squid *Loligo sanpaulensis* from Uruguay and the macroalgae *Ulva* sp. and *Codium decorticatum*, the seagrass *Halodule wrightii*, the cnidarian *Velella velella* and the squid *Loligo sanpualensis* from southern Brazil.

Results

Gut content analysis

Seventy-four esophageal lavage samples were collected from healthy green turtles that were captured while feeding off east Uruguay. All turtles were considered juvenile, ranging from 32.6 to 58.4 cm CCL (mean $\pm$ SD $= 41.6 \pm 5.8$ cm, $N = 74$). We identified 18 types of dietary items: 10 species of rhodophytes, 4 species of chlorophytes, 2 mollusk species and 2 cnidarian species (Table 2). Macroalgae occurred in all the samples and represented the bulk of the dietary items collected from the esophagus lavages.

All the stranded turtles sampled for stomach contents analysis were considered juvenile, as they ranged 29.8–62.0 cm CCL (mean $\pm$ SD curved carapace length $= 40.0 \pm 7.0$ cm, $N = 52$). These turtles presented an omnivorous diet, with a high occurrence of both macroalgae (FO = 69.2 %) and gelatinous macrozooplankton (FO = 48.1 %). Furthermore, marine debris occurred in 51.9 % of the stomach contents analyzed (Table 2). Relative volume data revealed a similar picture, with macroalgae prevailing on the average diet (RV $= 46.6 \pm 44.6$ %), followed by gelatinous macrozooplankton (23.0 ± 30.8 %) and marine debris (13.3 ± 19.5 %). However, the broad standard deviations associated with these means revealed a large variability of individual diet. The IRI values revealed that the relevance of gelatinous zooplankton decreased and the relevance of macroalgae increased with turtle size (Fig. 2). Actually, macroalgae were the only significant food item (%IRI > 96.3) for turtles larger than 45 cm CCL. Although we identified twenty different species of macroalgae (Table 2), five taxa occurred in more than 35 % of the samples: *Ulva* sp. (FO = 82.5 %), *Grateloupia* sp. (FO = 62.5 %), *Chondracanthus* sp. (FO = 47.5 %), *Codium decorticatum* (FO = 40.0 %) and *Pterocladiella capillacea* (FO = 40.0 %).

Stable isotope values

The macroalgae species collected in Brazil and Uruguay (*Codium decorticatum*, *Pterocladiella capillacea* and *Ulva* sp.) differed significantly in their $\delta^{15}N$ values (ANOVA, $F = 69.31$, df $= 2$, p value < 0.001), with those from Uruguay enriched in ^{15}N as compared with those from Brazil. Conversely, the $\delta^{13}C$ values of macroalgae from Brazil and Uruguay were not statistically different (ANOVA, $F = 0.95$ df $= 2$, p value $= 0.400$). Hence, differences in the $\delta^{15}N$ baseline existed for both areas.

CCL of green turtles for stable isotope analysis ranged 27.8–66.8 cm (mean $\pm$ SD CCL $= 39.4 \pm 6.4$ cm, $N = 126$). The stable isotope ratios in the epidermis of turtles ranged from -18.2 to -13.6 ‰ for $\delta^{13}C$ and from 6.7 to 15.6 ‰ for $\delta^{15}N$ (Table 3). Figure 3 shows the distribution of green turtle isotope values within the isospace defined by the potential prey from Uruguay and Brazil, after accounting for the trophic discrimination factor.

Based on the AIC values of competing models, the GAM that best explained the variability of $\delta^{13}C$ values in turtle epidermis with the lowest AIC value also included CCL, Julian day and distance from the estuary as the best explanatory variables (Table 4, Fig. 4a). The explanatory power of this model was 45.1 %, and the adjusted R-square was 0.36. There was a nonlinear relationship between epidermis $\delta^{13}C$ and CCL: positive for turtles smaller than 35 cm CCL, negative for turtles between 35 and 40 cm CCL and almost linear for turtles larger than 40 cm CCL. Epidermis $\delta^{13}C$ values were not affected by Julian day from January to July (summer, fall and early winter) and negatively from August to December (late winter to spring). Finally, the relationship between the distance from the estuary and epidermis $\delta^{13}C$ values was nonlinear, with peaks of the $\delta^{13}C$ values

Table 2 Comparison of the main dietary groups found in the digestive track analysis of esophageal lavage ($N = 74$) and stomach contents ($N = 52$)

Dietary species	Esophageal lavage groups (CCL)			Stomach contents groups (CCL)		
	<35 cm ($n = 4$)	≥35 < 45 cm ($n = 51$)	≥45 cm ($n = 20$)	<35 cm ($n = 10$)	≥35 < 45 cm ($n = 29$)	≥45 cm ($n = 8$)
	FO (%)	FO (%)	FO (%)	FO (%)	FO (%)	FO (%)
Macroalgae	**100**	**100**	**100**	**70.0**	**69.0**	**75.0**
Chlorophyta						
Chaetomorpha sp.	0	0	0	20.0	10.3	13.0
Cladophora sp.	33.3	21.6	15.8	30.0	17.2	25.0
Codium sp.	0	3.9	5.26	30.0	31.0	25.0
Ulva sp.	100	96.1	89.5	60.0	58.6	50.0
Rhodophyta						
Amphiroa beauvoisii	0	0	0	22.2	10.5	5.2
Ceramium sp.	83.3	41.2	36.8	0	17.2	25.0
Chondracanthus sp.	100	76.5	94.7	20.0	34.5	50.0
Corallina officinalis	0	7.8	5.26	30.0	10.3	0
Criptopleura ramosa	16.7	27.4	36.8	10.0	13.8	38.0
Grateloupia sp.	50.0	49.0	31.6	30.0	44.8	38.0
Hypnea musciformis	0	0	0	0	13.8	13.0
Jania rubens	50.0	29.4	26.3	18.9	12.4	8.3
Polysiphonia sp.	33.3	51.0	84.2	10.0	31.0	31.0
Pterocladiella capillaceae	16.7	23.5	10.5	20.0	31.0	25.0
Rhodymenia sp.	0	0	0	10.0	10.3	25.0
Phaeophyta						
Sarggassum sp.	0	0	0	10.0	6.9	0
Gelatinous macrozooplankton	**0**	**2.9**	**5.3**	**24.1**	**16.9**	**28.6**
Hydrozoa	0	0	0	10.0	0	0
Cephalopoda (beaks)	0	0	0	10.0	0	33.3
Gasteropoda	0	0	0	3.3	0	0
Crustacea	0	0	0	0	25.0	0
Fanerogams	0	0	0	23.3	25.0	0
Marine debris	**0**	**0**	**0**	**25.4**	**12.6**	**9.3**

Turtles sampled were divided into three groups according to size

FO frequency of occurrence of the dietary items

Note that esophageal lavages were carried out in the east of Uruguay (in warmer months), while stomach contents analyzed were collected from strandings along the entire Uruguayan coast (during all the year)

at approximately 375 and 575 km from the estuary and a decreasing trend in between them.

The GAM that best explained the variability of $\delta^{15}N$ values in turtle epidermis included CCL, Julian day and distance from the estuary as the best explanatory variables (Table 5, Fig. 4b). The explanatory power of this model was 45.0 %, and the adjusted *R*-square was 0.41. Epidermis $\delta^{15}N$ values increased linearly with CCL and nonlinearly with distance to the estuary and were higher in late winter and spring than the rest of the year (Fig. 4b).

Diet reconstruction with SIAR

According to the GAMs, turtles were divided for diet reconstruction into two seasons (late winter/spring vs. rest of the year) and four size classes (<35 cm CCL, 35–40 cm CCL, 40–50 cm and more than 50 cm). No turtle less than 35 cm CCL was collected in spring.

According to SIAR, turtles less than 50 cm CCL captured/stranded in summer, fall and early winter had omnivorous diets based primarily on *Pterocladiella capillacea* and *Codium decorticatum* from Brazil and with a

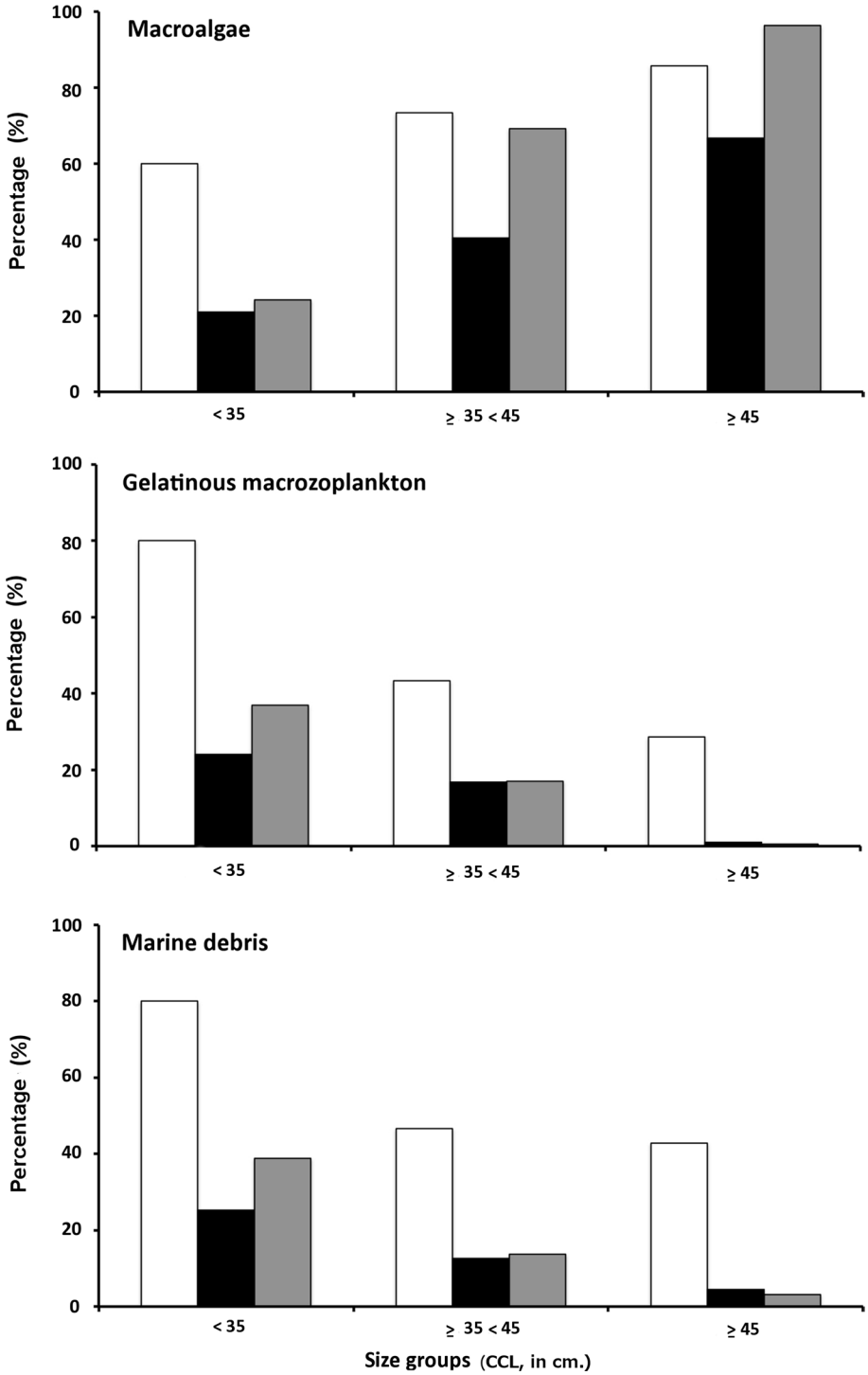


Fig. 2 Ontogenetic dietary change in green turtles. Frequency of occurrence, FO, (*white bars*), relative volume, RV, (*black bars*), and Index of Relative Importance, IRI, (*gray bars*), of the main dietary categories of items (macroalgae, gelatinous macrozooplankton and marine debris) in the stomach contents of stranded green turtles from Uruguayan according to size classes (curved carapace length (CCL): <35 cm ($n = 10$); ≥35 <45 ($n = 29$); and ≥45 ($n = 8$))

contribution of animal prey up to 30 % of the assimilated nutrients (Fig. 5). Turtles larger than 50 cm CCL also had a macroalgae-based omnivorous diet, but prey from Brazil and Uruguay made similar contributions. The turtles captured or stranded in late winter and spring also had a macroalgae-based omnivorous diet, independently of their carapace length, but with a more balanced contribution of prey from Brazil and Uruguay (Fig. 6).

Discussion

The results of the gut contents analysis reported here revealed a macroalgae-based omnivorous diet for the juvenile green turtles occurring year round at the foraging grounds off Uruguay. Green turtles present a rapid, but not abrupt, dietary shift after recruiting to neritic habitats. This shows that green turtles in the region start consuming

Table 3 Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in the epidermis of green turtles from Uruguay and their potential prey from Uruguay and Brazil

Group/species (n)	$\delta^{13}C$ (mean ± SD)	$\delta^{15}N$ (mean ± SD)
Green turtles		
Summer to fall		
CCL < 35 cm (20)	−15.5 ± 0.6	7.5 ± 1.3
35 ≤ CCL < 40 cm	−15.6 ± 0.6	10.4 ± 1.6
40 ≤ CCL < 50 cm (42)	−15.7 ± 0.7	9.7 ± 2.3
≥50 cm (6)	−14.8 ± 0.5	11.3 ± 1.2
Spring		
35 ≤ CCL < 40 cm	−17.1 ± 1.3	13.4 ± 1.2
40 ≤ CCL < 50 cm (6)	−16.3 ± 0.5	13.3 ± 1.0
≥50 cm (1)	−14.9	11.9
Macroalgae		
Brazil		
Codium sp. (5)	−14.9 ± 1.9	6.9 ± 0.4
Pterocladiella capillaceae (5)	−14.3 ± 1.0	8.4 ± 0.4
Sargassum sp. (5)	−17.4 ± 2.9	6.6 ± 1.6
Ulva sp. (5)	−17.1 ± 0.2	7.7 ± 0.2
Uruguay		
Anphiroa anastomasans (1)	−18.7	9.6
Ceramium sp. (1)	−16.9	11.6
Chondracanthus sp. (3)	−13.1 ± 0.6	10.6 ± 0.8
Cladophora sp. (2)	−15.7 ± 0.5	10.9 ± 0.7
Codium sp. (3)	−13.8 ± 4.6	10.6 ± 1.6
Corallina officinalis (1)	−11.1	9.0
Criptopleura ramosa (2)	−14.7 ± 2.0	11.7 ± 2.0
Grateloupia sp. (5)	−14.8 ± 1.1	11.2 ± 0.5
Haplogonia andersonii (1)	−16.4	10.8
Hypnea musciformis (2)	−14.3 ± 2.2	10.7 ± 1.8
Polysiphonia sp. (4)	−18.9 ± 1.5	9.9 ± 1.3
Pterocladiella capillaceae (1)	−16.4	12.2
Rhodymenia sp. (3)	−14.9 ± 3.1	10.5 ± 0.7
Ulva sp. (4)	−13.0 ± 2.3	10.5 ± 1.1
Cnidaria		
Brazil		
Velella velella (4)	−14.8 ± 1.2	10.3 ± 1.9
Uruguay		
Chrysaora lactea (1)	−16.1	14.2
Cephalopods		
Brazil (Drago et al. 2015)		
Loligo sanpaulensis (2)	−18.1 ± 0.2	10.0 ± 0.5
Uruguay (Drago et al. 2015)		
Loligo sanpaulensis (5)	−16.3 ± 0.3	16.4 ± 0.0
Seagrass		
Brazil (Lazzari 2012)		
Halodule wrightii (3)	–14.2 ± 0.4	3.2 ± 1.7

Values are reported as mean ± standard deviation

Sample size is reported in brackets

macroalgae soon after recruiting to neritic habitats, but continue consuming relevant amounts of gelatinous plankton and some squids, even as late juveniles. It should be noted, however, the sharp differences found between the results revealed by esophageal lavage and stomach contents analysis, as hardly any animal prey was obtained from the esophageal lavages. A possible explanation is that turtles were captured while grazing on rocky outcrops, and hence esophageal lavages revealed only the prey consumed immediately before the capture. Conversely, stomach contents probably integrate diet over previous days and hence informed also about the diet consumed while foraging in other habitats. On the other hand, samples from esophageal lavages and stomach contents were dominated by *Ulva* sp., *Chondracanthus* spp., *Grateloupia* spp. and *Polysiphonia* spp., precisely the four taxa that dominate the macroalgae community along the Uruguayan coastline (Vélez-Rubio et al. *In prep*.). This suggests little selectivity on macroalgae by green turtles, but further research based on controlled experiments is needed before concluding that green turtles graze in a nonselective way.

Cephalopod beaks occurred in 31.5 % of the stomach contents (Vélez-Rubio et al. 2015) analyzed in this study, but they did not necessarily reveal the recent consumption of cephalopods by neritic green turtles. Cephalopod beaks, composed of hardly digestible chitin, are known to accumulate into the gut of marine vertebrates for years (Hernández-García 1995; Tomás et al. 2001; Xavier et al. 2005), which probably explain why most of the beaks recovered from the stomach and intestine of neritic green turtles corresponded to oceanic species (Vélez-Rubio et al. 2015). This conclusion is further supported by the modest contribution of squids to the diet of green turtles according to SIAR and the absence of fresh squids into the stomachs of turtles.

Nevertheless, dietary reconstructions based on stable isotope faced serious limitations due to the complex variations in the isotope baseline within the region. Previous research has demonstrated the existence of a strong latitudinal gradient in the $\delta^{15}N$ baseline across Río de la Plata estuary, with species from southern Brazil typically depleted in ^{15}N as compared to those in Uruguay and northern Argentina (Vales et al. 2014; Drago et al. 2015). Southern Brazil remains away from the Río de la Plata plume because the confluence of the southward Brazil current and the northward Falkland/Malvinas current

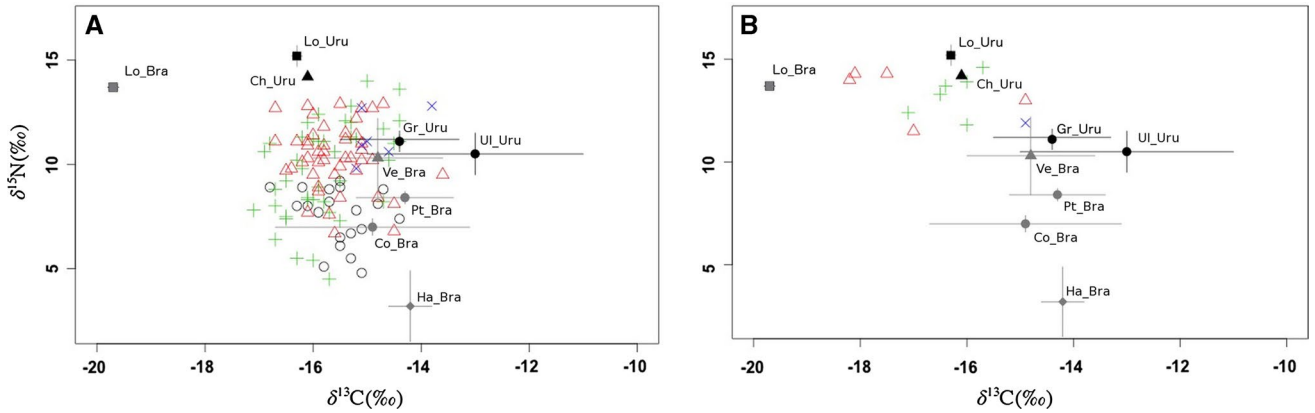


Fig. 3 Values of $\delta^{15}N$ and $\delta^{13}C$ in the epidermis of juvenile *green turtles* (intentionally captured and stranded) from Uruguayan coastal waters, and those of potential prey. Values have been corrected using the trophic discrimination factors from Seminoff et al. (2006). *Symbols* show the average stable isotope ratios of potential dietary species from Uruguay (*black symbols*) and Brazil (*gray symbols*): macroalgae (*circles*; Co: *Codium decorticatum*, Gr: *Grateulopia* sp., Pt: *Pterocladiella capillacea*, Ul: *Ulva* sp.), gelatinous macrozooplankton (*triangle*; Ch: *Chrysaora lactea*, Ve: *Velella velella*), cephalopods (*Loligo sanpaulensis*), seagrass (*diamond*; *Halodule wrightii*). **a**, summer to winter: *green turtles* classified by size ($n = 126$); *colored symbols* represent the stable isotope ratios of turtles for each size group: <35 cm ($n = 20$, *black open circles*); 35 ≥ CCL > 40 cm ($n = 46$, *red open triangles*); 45 ≥ CCL < 50 cm ($n = 42$, *green tails*); >50 cm ($n = 6$, *blue crosses*); **b**, spring: *green turtles* classified by size ($n = 12$); *colored symbols* represent the stable isotope ratios of turtles for each size group: 35 ≥ CCL > 40 cm ($n = 5$, *red open triangles*); 45 ≥ CCL < 50 cm ($n = 6$, *green tails*); >50 cm ($n = 1$, *blue crosses*)

Table 4 Parameter estimates from the best GAM to describe the variation in values of $\delta^{13}C$ of green turtles epidermic tissue as a function of covariate (CCL, day and distance from the estuary)

MODEL – $\delta^{13}C$ ~ s(CCL, bs = "cs") + s(julian, bs="cc") +s(dist, bs="cr")				
Name		Estimate	Std. error	*T* value	*P* (>*t*)
Parametric coefficients				
Intercept		−15.6336	0.05625	−277.9	<0.001
Name	Edf	Ref.df	*F*	*P* value
Approximate significance of smooth				
s(CCL)	5.521	6.385	6.259	<0.001
s(julian)	4.376	8.000	1.177	0.0488
s(dist)	7.178	8.009	2.864	0.0062

Edf array of estimated degrees of freedom for the model terms; *Ref.df* estimated residual degrees of freedom; *Chi.sq* array of test statistics for assessing the significance of model smooth terms

causes an offshore flux and hence drifts away the plume (Longhurst 1998). Nevertheless, such $\delta^{15}N$ gradient is stronger than any regional onshore/offshore gradient and may be related to the sewage input from Buenos Aires and Montevideo (Acha et al. 2004). Such latitudinal gradient had a strong influence on the $\delta^{15}N$ values of the potential prey analyzed here, and we also detected a weak, but significant, effect of sampling location along the estuarine plume of the $\delta^{15}N$ values of green turtles epidermis. Such variability in the $\delta^{15}N$ baseline certainly compromised the performance of SIAR, because the specificity with which mixing models can decipher diet trends is only as powerful as the specificity in the stable isotope values across the various potential diet items. A common solution in this situation is to cluster species with statistically similar stable isotope values, as far as the resulting groups are ecologically meaningful (e.g., Cardona et al. 2012; Zenteno et al. 2015). However, this is hard to undertake in the present situation, because the two species with the largest overall, both in their $\delta^{13}C$ and $\delta^{15}N$ values, were a jellyfish from Brazil (*Velella velella*) and a macroalgae from Uruguay (*Grateulopia* sp.).

As every technique available for dietary studies is somewhat biased, diet description is highly dependent on the method used, with SIA usually revealing a larger contribution of animal prey to the diet of green turtles than gut contents analysis (Table 6). Nevertheless, when the results from this study are combined with previous

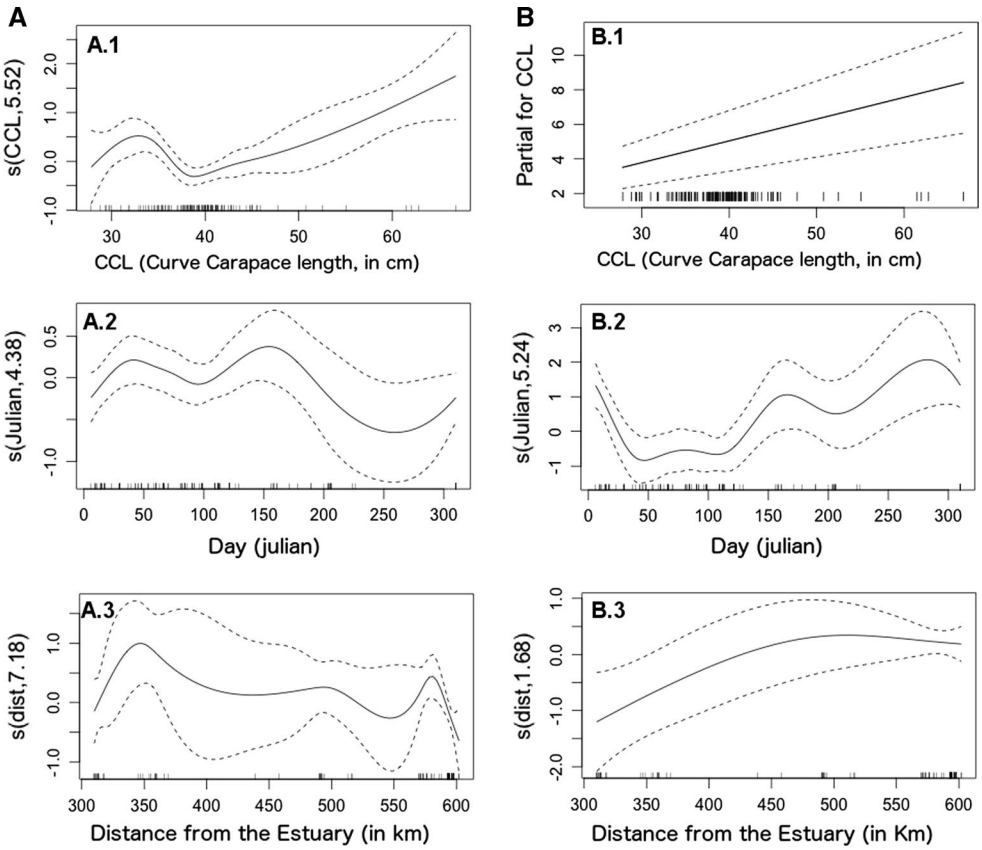


Fig. 4 Model terms for the generalized additive model (GAM) of the variation in values of $\delta^{13}C$ [**A**] and $\delta^{15}N$ [**B**] of green turtles epidermic tissue. Estimated smooth functions (*solid lines*) with 95 % confidence interval (*dashed lines*) are shown for each explanatory variable: **A.1/B.1**, curved carapace length (CCL); **A.2/B.2**: day (in Julian); **A.3/B.3**, distance from the inner part of the Rio de La Plata Estuary (in Km). Y-axis = fitted function with estimated degrees of freedom in parenthesis; x-axis = variable range with rug plots indicating sampled values. Note the difference in y-axes scale

Table 5 Parameter estimates from the best GAM to describe the variation in values of $\delta^{15}N$ of green turtles epidermic tissue as a function of covariate (CCL, day and distance to the estuary)

MODEL – δ15 N ~ CCL + s(julian, bs="cc") + (dist,, bs = "cs")				
Name	Estimate	Std. error	*T* value	*P* (>*t*)
Parametric coefficients				
Intercept	5.8797	0.8832	6.657	<0.001
CCL	0.1261	0.0220	5.730	<0.001
Name	Edf	Ref.df	*F*	*P* value
Approximate significance of smooth				
s(julian)	5.244	8.0	4.306	<0.001
s(dist)	1.676	2.1	3.800	0.0236

Edf array of estimated degrees of freedom for the model terms; *Ref.df* estimated residual degrees of freedom; *Chi.sq* array of test statistics for assessing the significance of model smooth terms

research conducted in southern Brazil (Nagaoka et al. 2012; Reisser et al., 2013; Morais et al. 2014; Santos et al. 2015) and Argentina (Gonzalez Carman et al. 2012, 2013), a clear regional pattern on the ontogenetic dietary shift of green turtles emerges. Green turtles smaller than 45 cm CCL inhabiting the Atlantic south to latitude 27ºS have an omnivorous diet, based on plant material and with gelatinous macrozooplankton as the main animal prey. On the other hand, green turtles larger than 45 cm CCL are primarily herbivores, but they still consume some animal prey. Only in areas with a scarcity of submerged macrophytes, as in the turbid plume of the Río de La Plata estuary, neritic juvenile green turtles may resume a mainly carnivorous diet (Gonzalez Carman et al. 2013). Omnivorous diet and increasing consumption of plant material with carapace length have been reported for green turtles inhabiting other warm-temperate regions (Cardona et al. 2009, 2010; Lemons et al. 2011) and even from some tropical regions (Amorocho and Reina 2007; Russell et al. 2011). As a consequence, the fast shift to herbivory following recruitment, once thought to be typical of green turtles, apparently happens in only some tropical regions

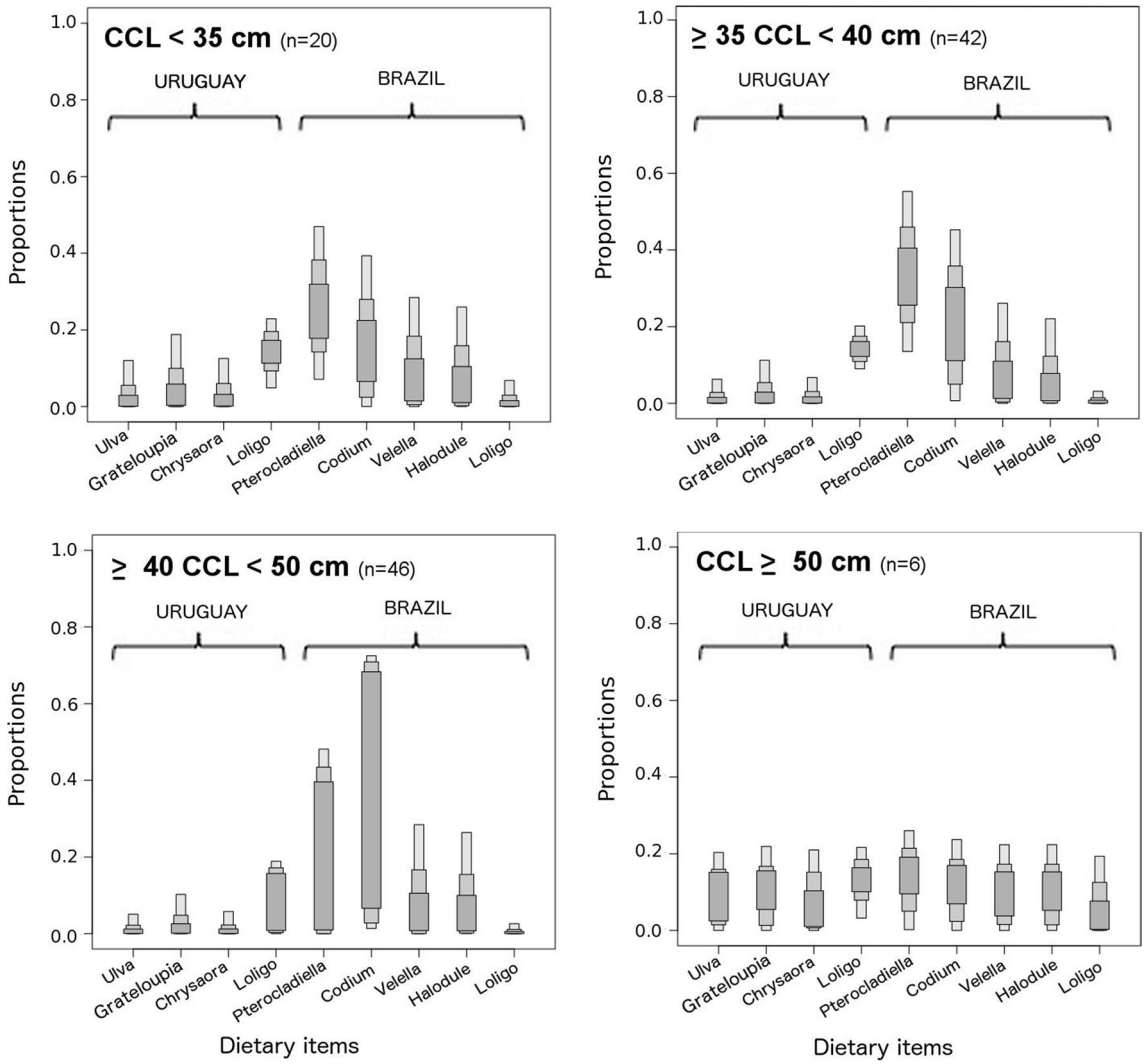
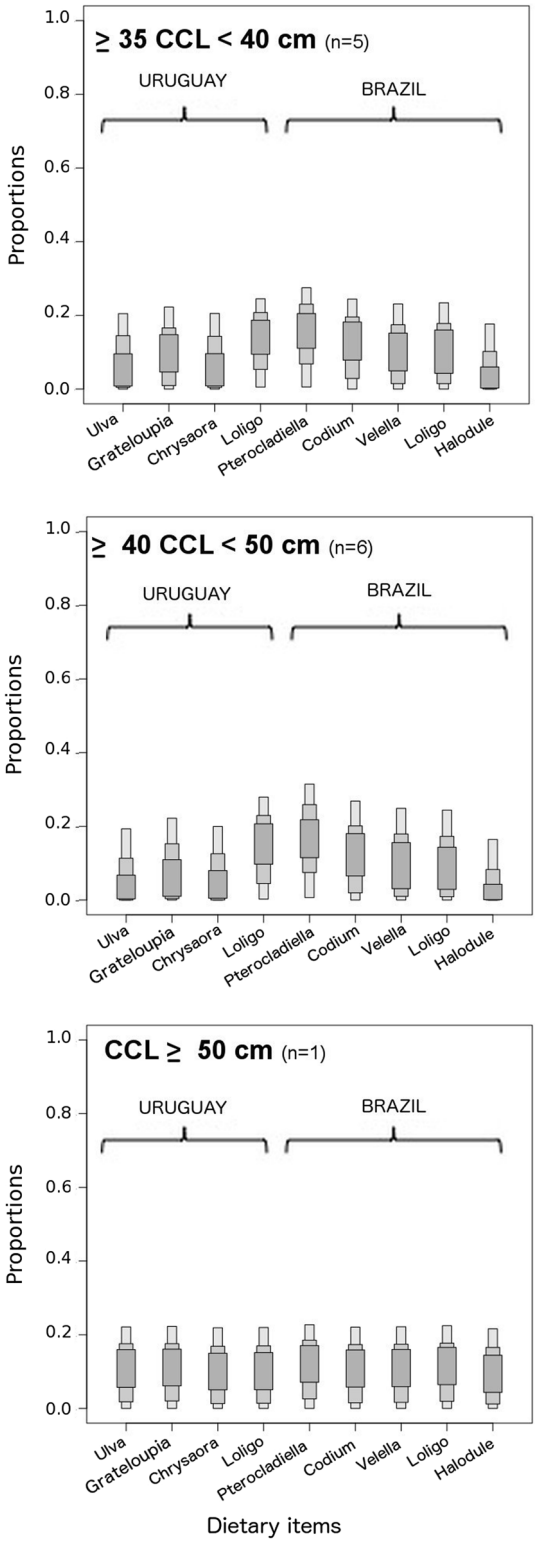


Fig. 5 Feasible contribution of potential prey (macroalgae, gelatinous macrozooplankton, squids and seagrass) from Brazil and Uruguay to the diet of juvenile green turtles sampled in spring according to SIAR (95, 75 and 50 % confidence intervals). Macroalgae: Gr: *Grateloupia* sp., Ul: *Ulva* sp, Pt: *Pterocladiella capillacea*, Co: *Codium decorticatum*. Gelatinous macrozooplankton: Ve: *Velella velella*, Cn: *Chrysaora lactea*. Squids: *Loligo sanpaulensis*. Seagrasses: *Halodule wrightii*

characterized by extensive seagrass meadows (Bjorndal 1997; Reich et al. 2007; Arthur et al. 2008).

Furthermore, the latitudinal variation in the isotope baseline offers a good opportunity to trace the movements of turtles. Stomach contents analysis revealed a decrease in the consumption of animal prey as the turtles grow, thus ruling out the hypothesis that increasing $\delta^{15}N$ values in the epidermis of larger turtles is because of a higher trophic level. Instead, the positive relationship between $\delta^{15}N$ values and turtle size is better explained by a longer residence of larger turtles in Uruguay, where macroalgae, gelatinous macrozooplankton and squids are enriched in ^{15}N as compared with those from Brazil (Vales et al. 2014; Drago et al. 2015; this study). The only possible confounding factor here would be that the amount of gelatinous zooplankton in the stomach contents of the largest turtles may had been underestimated because of its faster digestion, especially in large-size dead stranded individuals.

It has to be kept in mind that stable isotope ratios in turtle skin integrate dietary information during several months, although the exact turnover rate has been assessed experimentally for only smaller juveniles (Reich et al. 2008) and hence the time window integrated by the skin of larger juveniles is unknown. In this scenario, a strong isotopic signal of Brazilian macrophytes in the epidermis of the turtles captured off Uruguay from summer to winter indicates that most of these turtles had spent only a short time (weeks to months) there prior to capture. This interpretation is consistent with previous



◀ **Fig. 6** Feasible contribution of dietary species (macroalgae, gelatinous macrozooplankton, squids and seagrass) from Brazil and Uruguay to the diet of juvenile green turtles sampled in all season (except spring) according to SIAR (95, 75 and 50 % confidence intervals). Turtles were divided into four size groups according to the GAM: ≥ 35 CCL < 40, ≥ 40 CCL < 50, >50 CCL (no turtles of <35 CCL were captured in this season). Macroalgae: Gr: *Grateloupia* sp., Ul: *Ulva* sp, Pt: *Pterocladiella capillacea*, Co: *Codium decorticatum*. Gelatinous macrozooplankton: Ve: *Velella velella*, Cn: *Chrysaora lactea*. Squids: *Loligo sanpaulensis*. Seagrasses: *Halodule wrightii*

satellite telemetry and mark-recapture studies conducted in Uruguay and reflecting seasonal movements along the coastal waters of Brazil, Uruguay and Argentina (López-Mendilaharsu et al. 2006; Gonzalez Carman et al. 2012; Martinez Souza 2015). Nevertheless, the stable isotope ratios in the skin of green turtles occurring off Uruguay in spring clearly show they have been shifting frequently foraging grounds off Uruguay and southern Brazil and hence are not recent immigrants. Compared with the rest of the turtle groups considered here, turtles sampled in spring exploited other macroalgae species, as *Polysiphonia* sp. and some Corallinaceae species, probably because of the dominance of this species in the macroalgae community during winter along the Uruguayan coast (G V–R scuba diving observation). Due to the low SST values recorded during the coldest months of the year, a great proportion of the green turtle aggregation seems to migrate north in austral fall, although some turtles remain in the area overwintering in bays, estuaries (e.g., Valizas river, Andreoni channel) or harbors (e.g., Port of La Paloma), where the SST does not drop below 12–15 °C (Vélez-Rubio et al. 2013; Martinez Souza 2015). These turtles probably exhibit periods of brumation or winter dormancy (Witherington and Ehrhart 1989), as a strategy to survive with these cold temperatures (López-Mendilaharsu et al. 2006; Martinez Souza 2015).

Therefore, according to the present study and other studies conducted in the area (Gonzalez Carman et al. 2012; Martinez Souza 2015), we propose that most of the green turtles occurring off Uruguay from summer to winter had dispersed recently from southern Brazil and spend only a few weeks or months off Uruguay and that only a few turtles overwinter in Uruguayan waters along coastal habitats. Finally, some green turtles may overwinter offshore, as noted by some satellite tracked green turtles from Argentina (Gonzalez Carman et al. 2012). The reason why most juvenile green turtles spend only some weeks foraging off Uruguay might be related to

Table 6 Comparisons of juvenile and adult green turtle diet studies from different populations worldwide

References	Study area	Method	Record	N	CCL ± SD (cm)	Range (CCL, cm)	Diet
Bjorndal (1980)	Bahamas, Caribbean	Feces	IC	12	–	–	Herbivorous (seagrasses)
Seminoff et al. (2002)	Northeast Pacific, USA	SI	IC	50	36.9 ± 3.7	31.0–50.0	Omnivorous
Amorocho and Reina (2007)	Gorgona Island Pacific, Colombia	EL	IC	84	58.4 ± 7.8 (SCL)	37.0–72.9 (SCL)	Omnivorous
López-Mendilaharsu et al. (2008)	East Pacific, Mexico	EL	IC	15	59.9 ± 1.9 (SCL)	48.0–75.6 (SCL)	Herbivorous (macroalgae)
Arthur et al. (2008)	East Australia	SI	IC	64	49.5 ± 9.6.4	6.6–115	Herbivorous (after recruit to neritic area)
Rusell and Balazs (2009)	Hawaii Pacific, USA	DT	St	–	–	–	Herbivorous (seagrasses and macroalgae)
Carrion-Cortez et al. (2010)	Galapagos Islands Pacific, Ecuador	EL	IC	65	≠ Size groups	46.0–95.0	Herbivorous (macroalgae)
Burkholder et al. (2011)	Northwestern Australia	EL/SI	IC	65	≠ Size groups	–	Omnivorous
Lemons et al. (2011)	San Diego Northeast Pacific, USA	SI	IC	86	89.9 ± 21.2	49–115	Omnivorous
Parker et al. (2011)	North central Pacific, USA	DT	Bc	10	≠ Size groups	30–70	Carnivorous
Vander-Zanden et al. (2013)	Caribbean	SI	IC	376	≠ Size groups	31.7–122	Herbivorous (seagrasses)
Williams et al. (2013)	Florida, Gulf of Mexico	DT/SI	St	43	≠ Size groups	22.5–72.7	Omnivorous
Present study	Uruguay, southwestern Atlantic	EL/DT/SI	IC/St	246	39.9 ± 6.2	27.8–66.8	Omnivorous

Methodology: *EL* Esophagus lavage, *DT* Digestive track analysis, *SI* Stable Isotopes analysis; Type of record: *IC* Intentionally captured, *St* Stranded, *Bc* Bycatch

For a previous review of green turtle feeding ecology in the southwestern Atlantic region see Santos et al. (2015)

the latitudinal pattern of SST. The activity of the microbial flora digesting the plant material ingested by young green turtles is temperature dependent (Bjorndal 1980); hence, remaining for longer periods in Uruguayan waters offers no advantage for them than if they can forage into the warmer waters of southern Brazil. Only those turtles resuming a carnivorous diet may gain any benefits from spending longer periods at high latitude (Gonzalez Carman et al. 2013).

In summary, juvenile green turtles occurring off Uruguay are short-term migrants with a macroalgae-based omnivorous diet, with a decreasing contribution of animal prey to the diet when turtles reach 45 cm CCL. Diet variation reflects regional seasonal migrations in the southwestern Atlantic; hence, cooperation between neighboring countries is mandatory for conservation of this endangered species.

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References

Acha EM, Mianzan HW, Guerrero RA, Favero M, Bava J (2004) Marine fronts at the continental shelves of austral South America: physical and ecological processes. J Mar Syst 44:83–105

Almeida AP, Moreira LMP, Bruno SC, Thome´ JCA, Martins AS, Bolten AB, Bjorndal KA (2011) Green turtle nesting on Trindade Island, Brazil: abundance, trends and biometrics. Endanger Species Res 14:193–201

Amorocho DF, Reina RD (2007) Feeding ecology of the East Pacific green sea turtle *Chelonia mydas agassizii* at Gorgona National Park, Colombia. Endanger Species Res 3:43–51

Arthur KE, Boyle MC, Limpus CJ (2008) Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. Mar Ecol Prog Ser 362:303–311. doi:10.3354/meps07440

Bellini C, Santos AJB, Grossman A, Marcovaldi MÂ, Barata PCR (2013) Green turtle (*Chelonia mydas*) nesting on Atol das Rocas, north-eastern Brazil, 1990–2008. J Mar Biol Assoc UK 93:1117–1132

Bethea D, Hale L, Carlon JK, Cortés E, Manire CA, Gelsleichter J (2007) Geographic and ontogenetic variation in the diet and daily ration of the bonnethead shark, *Sphyrna tiburo*, from the eastern Gulf of Mexico. Mar Biol 152:1009–1020

Bjorndal KA (1980) Nutrition and grazing behaviour of the green turtle *Chelonia mydas*. Mar Biol 56:147–154

Bjorndal KA (1985) Nutritional ecology of sea turtles. Copeia 3:736–751

Bjorndal KA (1997) Foraging ecology and nutrition of sea turtles. In: Lutz PL, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Ratón, pp 199–231

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bolten AB (2003) Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. In: Lutz PL, Musick J, Wyneken J (eds) The Biology of Sea Turtles, vol II. CRC Press, Boca Raton, pp 243–257

Borthagaray AI, Carranza A (2007) Mussels as ecosystem engineers: their contribution to species richness in a rocky littoral community. Act Oeco 31:243–250

Burkholder DA, Heithaus MR, Thomson JA, Fourqurean JW (2011) Diversity in trophic interactions of green sea turtles *Chelonia mydas* on a relatively pristine coastal foraging ground. Mar Ecol Prog Ser 439:277–293. doi:10.3354/meps09313

Campos EJD, Piola AR, Matano RP, Miller JL (2008) PLATA: a synoptic characterization of the southwest Atlantic shelf under influence of the Plata River and Patos Lagoon outflows. Cont Shelf Res 28:1551–1555

Cardona L, Aguilar A, Pazos L (2009) Delayed ontogenic dietary shift and high levels of omnivory in green turtles (*Chelonia mydas*) from the NW coast of Africa. Mar Biol 156:1487–1495. doi:10.1007/s00227-009-1188-z

Cardona L, Campos P, Levy Y, Demetropoulos A, Margaritoulis D (2010) Asynchrony between dietary and nutritional shifts during the ontogeny of green turtles (*Chelonia mydas*) in the Mediterranean. J Exp Mar Biol Ecol 393:83–89. doi:10.1016/j.jembe.2010.07.004

Carrión-Cortez JA, Zárate P, Seminoff JA (2010) Feeding ecology of the green sea turtle (Chelonia mydas) in the Galapagos Islands. J Mar Biol Assoc UK 90:1005–1013

Cardona L, Alvarez de Quevedo I, Borrell A, Aguilar A (2012) Massive consumption of gelatinous plankton by Mediterranean apex predators. PLoS ONE 7:e31329. doi:10.1371/journal.pone.0031329

Coelho Claudino M, Abreu PC, Miranda Garcia A (2013) Stable isotopes reveal temporal and between-habitat changes in trophic pathways in a southwestern Atlantic estuary. Mar Ecol Prog Ser 489:29–42. doi:10.3354/meps10400

Coll J, Oliveira EC (1999) The Benthic Marine Algae of Uruguay. Bot Mar 42(1):129–135

Costalago D, Navarro J, Álvarez-Calleja I, Palomera I (2012) Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species. Mar Ecol Prog Ser 460:169–181

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658. doi:10.1007/s00442-005-0118-0

Defeo O, Horta S, Carranza A, Lercari D, de Álava A, Gómez J, Martínez G, Lozoya JP, Celentano E (2009) Hacia un Manejo Ecosistémico de Pesquerías. Áreas Marinas Protegidas en Uruguay, Facultad de Ciencias-DINARA, Montevideo

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Drago M, Franco-Trecu V, Zenteno L, Szteren D, Cardona L (2015) Sexual foraging segregation in South American sea lions increases during the pre-breeding period in the Río de la Plata plume. Mar Ecol Prog Ser 525:261–272

Forbes G, Limpus C (1993) A non-lethal method for retrieving stomach contents from sea turtles. Wildlife Res 20:339–343

Garnett ST, Pirce IR, Scott FJ (1985) The diet of the green turtle, *Chelonia mydas* (L.), in Torres Strait. Aust Wildlife Res 12:103–112

Gonzalez Carman V, Falabella V, Maxwell S, Albareda D, Campagna C, Mianzan H (2012) Revisiting the ontogenetic shift paradigm: the case of juvenile green turtles in the SW Atlantic. J Exp Mar Biol Ecol 429:64–72. doi:10.1016/j.jembe.2012.06.007

Gonzalez Carman V, Botto F, Gaitán E, Albareda D, Campagna C, Mianzan H (2013) A jellyfish diet for the herbivorous green turtle *Chelonia mydas* in the temperate SW Atlantic. Mar Biol 161(2):339–349. doi:10.1007/s00227-013-2339-9

Hastie TJ, Tibshirani RJ (1990) Generalized additive models. Monog Stat Appl Prob 43, Chapman and Hall, London

Hatase H, Sato K, Yamaguchi M, Takahashi K, Tsukamoto K (2006) Individual variation in feeding habitat use by adult female green sea turtles (*Chelonia mydas*): are they obligately neritic herbivores? Oecologia 149:52–64

Hernández-García V (1995) The diet of the swordfish Xiphias gladius Linnaeus, 1758, in the central east Atlantic, with emphasis on the role of cephalopod s. Fish Bull 93:403–411

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326

Horta S, Defeo O (2012) The spatial dynamics of the whitemouth croaker artisanal fishery in Uruguay and interdependencies with the industrial fleet. Fish Res 125–126:121–128

Hyslop EJ (1980) Stomach contents analysis-a review of methods and their application. J Fish Biol 17:41–429

Kelez S (2011) Bycatch and foraging ecology of sea turtles in the eastern Pacific. PhD dissertation, Duke University, Durham, NC

Lazzari L (2012) Isótopos estáveis de carbono e nitrógeno aplicados ao estudio da ecología trófica do peixe-boi marinho (*Trichechus manatus*). Master Thesis, Programa de Pós-graduaçao em Oceanografia Biológica, Universidad Federal do Rio Grande

Leathwick JR, Elith J, Hastie T (2006) Comparative performance of generalized additive models and multivariate adaptive regression splines for statistical modelling of species distributions. Ecol Mod 199:188–196

Lemons G, Lewison R, Komoroske L, Gaos A, Lai CT, Dutton P, Eguchi T, LeRoux R, Seminoff JA (2011) Trophic ecology of green sea turtles in a highly urbanized bay: insights from stable isotopes and mixing models. J Exp Mar Biol Ecol 405:25–32. doi:10.1016/j.jembe.2011.05.012

Longhurst A (1998) Ecological geography of the sea. Academic Press, San Diego

López-Mendilaharsu M, Gardner SC, Seminoff JA, Riosmena-Rodriguez R (2005) Identifying critical foraging habitats of the green

turtle (*Chelonia mydas*) along the Pacific coast of the Baja California peninsula, Mexico. Aquat Conserv Mar Freshw Ecosyst 15:259–269

López-Mendilaharsu M, Estrades A, Caraccio MN, Calvo V, Hernández M, Quirici V (2006) Biología, ecología y etología de las tortugas marinas en la zona costera uruguaya. In: Menafra R, Rodríguez-Gallego L, Scarabino F, Conde D (eds) Bases para la conservación y el manejo de la costa uruguaya. Vida Silvestre Uruguay, Montevideo, pp 247–257

López-Mendilaharsu M, Gardneri SC, Riosmena-Rodriguez R, Seminoff JA (2008) Diet selection by immature green turtles (Chelonia mydas) at Bahia Magdalena foraging ground in the Pacific Coast of the Baja California Penin- sula, Mexico. J Mar Biol Assoc UK 88:641–647

Makowski C, Seminoff J, Salmon M (2006) Home range and habitat use of juvenile Atlantic green turtles (*Chelonia mydas* L.) on shallow reef habitats in Palm Beach, Florida, USA. Mar Biol 148:1167–1179

Martinez Souza G (2015) Caracterizaçao populacional de juvenis de tartaruga-verde (*Chelonia mydas*) em duas áreas do Atlântico Sul Ocidental. PhD Thesis, Programa de Pós-graduaçao em Oceanografia Biológica, Universidad Federal do Rio Grande, Brazil

McCann KS (2012) Food webs. Princeton University Press, Princeton

Morais RA, dos Santos RG, Longo GO, Yoshida ETE, Stahelin GD, Horta PA (2014) Direct evidence for gradual ontogenetic dietary shift in the green turtle *chelonia mydas*. Chelonian Conserv Biol 13(2):260–266

Nagaoka S, Martins A, Santos R, Tognella M, Oliveira Filho E, Seminoff JA (2012) Diet of juvenile green turtles (*Chelonia mydas*) associating with artisanal fishing traps in a subtropical estuary in Brazil. Mar Biol 159:573–589. doi:10.1007/s00227-011-1836-y

Oliveira EC (1984) Brazilian mangal vegetation with special emphasis on the seaweeds. In: Por FD, Dor I (eds) Hydrobiology of the Mangal. W. Junkers Publ, The Hague, pp 55–65

Ortega L, Martínez A (2007) Multiannual and seasonal variability of water masses and fronts over the Uruguayan shelf. J Coast Res 23:618–629

Parker D, Dutton PH, Balazs GH (2011) Oceanic diet and distribution of haplotypes for the green turtle, *Chelonia mydas*, in the central North Pacific. Pac Sci 65(4):419–431

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source Partitioning Using Stable Isotopes: coping with Too Much Variation. PLoS ONE 5(3):e9672. doi:10.1371/journal.pone.0009672

Payo-Payo A, Ruiz B, Cardona L, Borrell A (2013) Effect of tissue decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba* and loggerhead sea turtles *Caretta caretta*. Aquat Biol 18:141–147

Pinkas L, Oliphant MS, Iverson ILK (1971) Food habits of albacore, bluefin tuna, and bonito in California waters. Fish Bull 152:1–105

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83:703–718

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. Accessed 20 Jan 2014

Reich KJ, Bjorndal KA, Bolten AB (2007) The 'lost years' of green turtles: using stable isotopes to study cryptic life stages. Biol Lett 3:712–714. doi:10.1098/rsbl.2007.0394

Reich KJ, Bjorndal KA, Martinez del Rio C (2008) Effects of growth and tissue type on the kinetics of 13C and 15 N incorporation in a rapidly growing ectotherm. Oecologia 155:651–663

Reisser J, Proietti M, Sazima I, Kinas P, Horta P, Secchi E (2013) Feeding ecology of the green turtle (*Chelonia mydas*) at rocky reefs in western South Atlantic. Mar Biol 160:3169–3179

Russell DJ, Balazs GH (2009) Dietary shifts by green turtles (Chelonia mydas) in the Kane'ohe Bay region of the Hawaiian Islands: a 28-year study. Pac Sci 63:181–192

Russell DJ, Hargrove S, Balazs GH (2011) Marine sponges, other animal food, and nonfood items found in digestive tracts of the herbivorous marine turtle *Chelonia mydas* in Hawaii. Pac Sci 65:375–381

Santos RG, Silva Martins A, Batista MB, Horta PA (2015) Regional and local factors determining green turtle Chelonia mydas foraging relationships with the environment. Mar Ecol Prog Ser 529:265–277

Seminoff JA, Resendiz A, Nichols WJ (2002) Diet of East Pacific green turtles (*Chelonia mydas*) in the central Gulf of California, Mexico. J Herpetol 36:447–453

Seminoff JA, Jones TT, Eguchi T, Jones DR, Dutton PH (2006) Stable isotope discrimination (δ13C and δ15N) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. Mar Ecol Prog Ser 308:271–278

Tomás J, Aznar FJ, Raga JA (2001) Feeding ecology of the loggerhead turtle *Caretta caretta* in the western Mediterranean. J Zool 255(04):525–532

Vander Zanden HB, Arthur KE, Bolten AB, Popp BN, Lagueux CJ, Harrison E, Campbell CL, Bjorndal KA (2013) Trophic ecology of a green turtle breeding population. Mar Ecol Prog Ser 476:237–249

Vales DG, Saporiti F, Cardona L, De Oliveira LR et al (2014) Intensive fishing has not forced dietary change in the South American fur seal *Arctophoca* (= *Arctocephalus*) *australis* off Río de la Plata and adjoining areas. Aquat Conserv Mar Freshw Ecosyst 24:745–759

Vales DV, Cardona L, García NA, Zenteno L, Crespo EA (2015) Ontogenetic dietary changes in male South American fur seals *Arctocephalus australis* in Patagonia. Mar Ecol Prog Ser 525:245–260

Vélez-Rubio GM, Estrades A, Fallabrino A, Tomás J (2013) Marine turtle threats in Uruguayan waters: insights from 12 years of stranding data. Mar Biol 160:2797–2811

Vélez-Rubio G, Tomás J, Míguez-Lozano R, Xavier JC, Martinez Souza G, Carranza A (2015) New insights in southwestern Atlantic Ocean Oegopsid squid distribution based on juvenile green turtle (*Chelonia mydas*) diet analysis. Mar Biodiver 45:701–709. doi:10.1007/s12526-014-0272-x

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB et al (2010) Regional Management Units for Marine Turtles: a Novel Framework for Prioritizing Conservation and Research across Multiple Scales. PLoS ONE 5(12):e15465

Weber SB, Weber N, Ellick J, Avery A, Frauenstein R, Godley BJ, Broderick AC (2014) Recovery of the South Atlantic's largest green turtle nesting population. Biodivers Conserv 23(12):3005–3018

Williams NC, Bjorndal KA, Lamont MM, Carthy RR (2013) Winter Diets of Immature Green Turtles (*Chelonia mydas*) on a Northern Feeding Ground: integrating Stomach Contents and Stable Isotope Analysis. Estuaries Coasts 37(4):986–994. doi:10.1007/s12237-013-9741-x

Witherington BE, Ehrhart LM (1989) Hypothermic stunning and mortality of marine turtles in the Indian River lagoon system, Florida. Copeia

Wood S (2011) gamm4: Generalized additive mixed models using mgcv and lme4. R package version 0.1–2

Xavier JC, Croxall JP, Cresswell KA (2005) Boluses: a simple, cost-effective diet method to assess the cephalopod prey of albatrosses? Auk 122:1182–1190

Zenteno L, Crespo E, Vales D, Silva L, Saporiti F, Oliveira LR, Secchi ER, Drago M, Aguilar A, Cardona L (2015) Dietary consistency of male South American sea lions (*Otaria flavescens*) in southern Brazil during three decades inferred from stable isotope analysis. Mar Biol 162:275–289