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# Habitat use of nesting female olive ridley turtles (*Lepidochelys olivacea*) inferred by stable isotopes in eggs

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## ABSTRACT

Olive ridley sea turtles (*Lepidochelys olivacea*) can use a vast number of different habitats and food sources throughout their life cycle. This species is one such organism that changes both the environment and diet during different life stages. Based on stable isotope analysis ( $\delta^{13}$ C and  $\delta^{15}$ N) of the components of fresh eggs (yolk, albumen, and shell) and unhatched eggs (contents and shell), the habitat use of females nesting in the eastern Atlantic Ocean, Brazil, was elucidated. As the yolk is formed months before migration to the nesting areas, it was possible to infer that they originated from both high- and low-latitude feeding areas. For albumen and shell, both carbon and nitrogen isotopic values indicated either the neritic environment at the latitude of the breeding area, tissues of turtles catabolized due to fasting during breeding, or differences in tissue-specific metabolic routing. The contribution of potential prey such as jellyfish for yolk and demersal prey for both albumen and shell demonstrated the plasticity of habitat use of this population and the use of both pelagic and neritic waters. High individual variability further reinforces the need for preservation of the habitats utilized by olive ridley turtles in both neritic and oceanic environments over a vast area of the tropical ocean up within 20 degrees south and north of Equator.

## 1. Introduction

The habitat use of migratory animals is essential to understanding the ecology, different behavioral habits, and possible intraspecific variation of these organisms as well as supporting conservation actions over vast areas. There is an urgency to understand the connectivity of these habitats utilized by marine megafauna due to factors such as bycatch in fisheries, climate change, marine pollution and habitat degradation, which directly affect sea turtle populations (Lutcavage et al., 1997; Sales et al., 2008). Stable isotope analysis (SIA) has been used to understand the linkage of different environments used by these animals, including sea turtles, in their migration between feeding and breeding areas (Hobson and Norris, 2008; Ceriani et al., 2014).

Stable isotopes function as intrinsic markers when animals move between habitats with distinct isotopic baseline values (Hobson and Norris, 2008) due to the different photosynthetic cycles of producers at the base of the trophic chain or the nitrogen sources (Fry, 2006). These changes can be assessed, for example, by carbon and nitrogen values ( $\delta^{13}$ C and  $\delta^{15}$ N, respectively) in animal tissues, which have been used to infer habitat and diet (Fry, 2006; Hobson and Norris, 2008). In marine ecosystems,  $\delta^{13}$ C values vary with latitude in both oceanic and neritic waters, so the higher the latitude is, the lower the  $\delta^{13}$ C value (Groericke and Fry, 1994). Such dynamics are related to the sea surface temperature, which is negatively related to the amount of carbon dioxide dissolved in seawater and thus increases the amount of <sup>13</sup>C entering the trophic chain through the photosynthesis of the primary producers (Groericke and Fry, 1994). For nitrogen isotopic values, as the trophic level along the trophic chain increases, higher values of  $\delta^{15}$ N are found, estimated to be within 3 to 5‰ at each level (DeNiro and Epstein, 1978, 1981; Peterson and Fry, 1987). Enriched nitrogen values could also

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originate from external sources, such as river runoff, upwelling and sewage, as it has a large amount of nitrogen nutrient input (Minagawa and Wada, 1984).

Stable isotope values in animal tissues can be permanently maintained in inert tissues such as feathers, scute and bones or integrated for some time in metabolically active tissues. In sea turtles, blood plasma has been shown to have the most recent isotopic signatures due to rapid turnover, followed by red cells, epidermis, and scute (Seminoff et al., 2006, 2009; Reich et al., 2008). However, for the components of sea turtle eggs, such as yolk, albumen and eggshell, the turnover time is unknown. The yolk is known to form four to nine months before the turtle migrates to breeding areas, and albumen and eggshell are probably formed after fertilization of eggs that occurs near the nesting area in neritic waters (Roastal, 2007). For this reason, the isotopic values of the volk may indicate values linked to feeding areas (Hobson and Norris, 2008). Yolk formation is essential for the preparation of these animals for reproduction and consists of the nutrient deposition of reserves in the oocytes for subsequent formation of the yolk to feed the embryo (Roastal, 2007). Although this metabolic process begins approximately seven months prior to migration to the breeding grounds, it takes five months to be completed; thus, vitellogenesis ends before migration to the breeding areas (Roastal, 2007). According to Roastal et al. (1998), the Kemp's ridley sea turtle (Lepidochelys kempii), endemic to the Gulf of Mexico, carries out yolk formation before migration, and thus, it is possible that the vitellogenesis of the olive ridley sea turtle (Lepidochelys olivacea) is similar, as they are the closest living relative sea turtle species (Villaça et al., 2022).

Based on stable isotope analysis of the egg volks of female loggerhead sea turtles (Caretta caretta) in Japan, an oceanic-neritic dichotomy in habitat use during the nonnesting period was observed (Hatase et al., 2002). Smaller individuals originated from oceanic waters ( $\delta^{13}$ C <-18% and  $\delta^{15}$ N < 12%) and the largest individuals from neritic waters  $(\delta^{13}C \ge -18\% \text{ and } \delta^{15}N \ge 12\%)$  based on carbon and nitrogen values (Hatase et al., 2002). For loggerhead sea turtles from Florida in the North Atlantic Ocean, the variation in these values was based on the change in latitude between the feeding and breeding areas through stable isotopes of the egg yolk (Ceriani et al., 2012). Green turtle (Chelonia mydas) females sampled from the oceanic island of Atol da Rocas in Brazil had consistent variation in both habitat and feeding, based on SIA of the egg yolk and the scute tissue (Agostinho et al., 2021). In addition, a strong relationship between carbon and nitrogen values was observed among egg components and nesting female tissue for loggerhead and leatherback (Dermochelys coriacea) turtles in Australia and in the Mediterranean Sea, respectively (Zbinden et al., 2011; Carpentier et al., 2015).

In addition to intrinsic markers such as stable isotopes, external markers, e. g. satellite tracking, has contributed substantially to elucidating the levels of behavioral plasticity in populations of sea turtles. For instance, in the Eastern Pacific Ocean, adult female olive ridley sea turtles head to the open ocean soon after reproduction, remaining in oceanic areas (Beavers and Cassano, 1996). In contrast, females nesting in northern Australia remain mostly in the neritic environment (McMahon et al., 2007; Whiting et al., 2007). In the East Atlantic Ocean, on the other hand, female olive ridleys stayed in neritic environments



Fig. 1. Study area where olive ridley sea turtle (*Lepidochelys olivacea*) eggs were sampled at Abaís beach, Sergipe state, Brazil. Darker hatched areas in the Atlantic Ocean are indications of feeding areas based on satellite telemetry studies (Silva et al., 2011; Santos et al., 2019); RJ: Rio de Janeiro state; SE: Sergipe State.

during the mating period and returned to the open sea after nesting (Maxwell et al., 2011). Finally, the population nesting in northeastern Brazil was defined as holding three postnesting destinations: moving to oceanic waters (towards the north coast of Africa), moving northwards along the Brazilian coast to neritic waters along the north and northeastern Brazilian coast, and moving southward neritic waters, e.g., along the Rio de Janeiro coast reaching the Cabo Frio upwelling area (Fig. 1; Silva et al., 2011; Santos et al., 2019).

The olive ridley sea turtle is the most abundant sea turtle species worldwide (Reichart, 1993) and is distributed in tropical waters in all three oceans (Villaça et al., 2022). However, it is also the least studied due to its oceanic life cycle coupled with limited funding for research in comparison to that available for other species. After hatching, olive ridley sea turtles move to the open sea, where all juvenile stages occur (Plotkin, 2010); when this species matures in the western Atlantic Ocean (~17 years old, Petitet et al., 2015), it returns regularly to coastal waters for nesting (Plotkin, 2010). Adults can be either oceanic or neritic during the nonnesting period (Plotkin, 2010) or use both habitats in variable proportions.

The olive ridley turtle is on the global red list of threatened species, classified as "Vulnerable" (IUCN, 2021). Threats to this species include incidental capture in fisheries, such as trawling in neritic waters and pelagic longlines in oceanic waters (Castilhos et al., 2011). The most important breeding area for this species in Brazil is in the state of Sergipe (Fig. 1; Silva et al., 2010), which is also the second largest rookery for the Atlantic Basin (Castilhos et al., 2022); however, strandings of both dead females and males adjacent to this important nesting site are usually recorded and attributed to fishing (Silva et al., 2010; Castilhos et al., 2011), which directly threatens this population. In addition to these threats, there are other threats, such as climate change and habitat degradation; thus, knowledge of habitat use during its whole life cycle is useful and urgently needed to understand the ecology and demography of this species (Ceriani et al., 2014). Studies of this nature can also contribute to conservation and environmental awareness efforts in nesting areas and therefore help in the conservation of these threatened animals, both during breeding and nonbreeding periods.

Thus, the present study aims to infer the habitat use of breeding female olive ridley turtles based on SIA of their egg components (yolk, albumen and shell). Because each component is formed at different times, it is possible to deduce the visited habitats and potential diet of these animals over time, back to nonbreeding foraging areas where nutrients are accumulated. Furthermore, regressions were applied between the available tissues (fresh eggs and unhatched eggs), and a stable isotope mixed model was built to evaluate the contributions of oceanic and neritic dietary sources and infer the habitat use of the population and individuals to complement the analyses from each egg component. Finally, the use of nonviable eggs and eggshells aims to test the use of nondestructive sampling in substitution for yolks in viable eggs. Therefore, we hypothesize that female olive ridley turtles from northeastern Brazil will have isotopic values in egg yolk consistent with variable migration patterns in both neritic and oceanic areas and different latitudes, mirroring satellite telemetry data (Santos et al., 2019).

## 2. Materials and methods

#### 2.1. Sampling

The study was conducted in partnership with the *Fundação Projeto TAMAR* (Brazilian Sea Turtle Conservation Program) at Abaís beach, in the state of Sergipe, Brazil, which is a 36 km beach monitored during the nesting period (Fig. 1; Silva et al., 2007). This state is located in the tropical zone and has a dry summer (De Blij and Muller, 1993) with high-energy sandy beaches, several estuaries and the absence of rocky shores (Silva et al., 2007). The coast of the state of Sergipe is the main Brazilian breeding area for solitary (i.e., non-*arribada*) olive ridley sea

turtles, where there are  $\sim$ 12,700 nests per year (2018/2019 nesting season; Castilhos et al., 2022), and with an increasing number over the years (Silva et al., 2007).

During TAMAR monitoring in the study area in November 2014 and January 2015, female olive ridley turtles were approached at the time of egg laying, and one egg per nest was sampled, totaling 29 eggs from 29 different nests. For each female, the curved carapace length (CCL) was measured from the nuchal notch of the carapace to the posterior end of the posterior margin and identified with metal tags attached to both anterior flippers. The sampled turtle nests were marked, and after the expected hatching period (45 to 60 days) (Silva et al., 2007), those that did not achieve 100% hatching success, 3 unhatched eggs, without rupture and without developed embryos, were collected for further comparison with fresh eggs ( $n_{nest} = 15$ ;  $n_{egg} = 45$ ). Then, 3 other unhatched eggs were collected from each different nest, without prior knowledge of the respective female olive ridley turtle ( $n_{nest} = 10$ ;  $n_{egg} = 30$ ).

Based on Colman et al. (2014), potential neritic prey for olive ridley sea turtles were collected in the study area from incidental shrimp trawl catches for a previous study (Petitet and Bugoni, 2017). Neritic prey consisted of crustaceans and demersal fishes. Isotopic values of two types of jellyfish (Scyphozoa and Hydrozoa), which occur in the South Atlantic Ocean, from Dodge et al. (2011) and González-Carman et al. (2014) were chosen as references for the oceanic prey.

## 2.2. Stable isotope analysis

The yolk, albumen and shell of fresh eggs were separated manually and dried in an oven at 60 °C for 48 h, 24 h and 24 h, respectively. The full content of unhatched eggs was used, because the yolk and albumen could not be separated, and was dried in an oven at 60 °C for 48 h. After drying, the components of fresh (yolk and albumen) and unhatched eggs (whole content) had lipids extracted using a Soxhlet apparatus with a 2:1 solution of chloroform:methanol for 8 h (Ceriani et al., 2014). Then, the samples were dried at 60 °C in an oven for 24 h to remove the residual solvent. After lipid extraction, for the samples that demonstrated a C:N ratio > 3.5, regarded as lipid-rich samples, a mathematical correction from Post et al. (2007) was applied using Eq. (1):

$$\delta^{13}C_{extracted} = \delta^{13}C_{nonextracted} - 3.32 + (0.99*C:N_{value})$$
(1)

Lipids were not extracted from the eggshell, assuming that the amount of lipids was minimal, which was later confirmed by the C:N ratio < 3.5. After drying, calcium carbonate was extracted from the eggshells by acidification with 10% HCl using the "drop by drop" technique (Jacob et al., 2005) until no bubbles were produced (Medeiros et al., 2015). Then, the samples were washed with distilled water and dried in an oven at 60 °C for another 24 h. All tissues were then ground, homogenized, weighed (~0.7 mg) and placed in tin capsules for further analysis.

All samples were analysed by a continuous-flow isotope-ratio mass spectrometer (CF-IRMS, Thermo Finnigan Delta Plus XP, Bremen, Germany) coupled with an elemental analyser (Costech ECS 4010, Milan, Italy) at the Stable Isotope Laboratory at Washington State University, School of Biological Sciences, Pullman, Washington, USA. Stable isotope ratios were expressed in  $\delta$  notation as parts per thousand (‰) deviation from the international standards Vienna Pee Dee Belemnite limestone (carbon) and atmospheric air (nitrogen), as in Eq. (2):

$$\delta(\%) = \frac{R_{sample}}{R_{standard}} - 1 \tag{2}$$

where  $R_{sample}$  and  $R_{standard}$  are the corresponding ratios of heavy to light isotopes ( $^{13}C/^{12}C$  and  $^{15}N/^{14}N$ ) in the sample and standard, respectively.

## 2.3. Statistical analysis

A generalized linear model (GLM) was applied between the carbon and nitrogen isotopic values of all components of fresh eggs (yolk, albumen, and shell) and unhatched eggs (contents and shell) to provide cross-tissue conversion equations (Vander Zanden et al., 2014). In this analysis, we used the mean carbon and nitrogen values of the unhatched eggs, as up to 3 samples/eggs were obtained from each nest. Bayesian multilevel regression models (Bürkner, 2017) were also applied between the carbon and nitrogen values of the unhatched eggs to analyse whether there were differences between eggs from the same female. Unhatched eggs from unknown females were included in this analysis. The mean intercept standard deviation was used as a proxy for variation between individuals, and sigma ( $\sigma$ ) was used for variation within individuals to analyse variation between unhatched eggs from the same female.

Bayesian stable isotope mixed models (SIMM), based on MixSIAR GUI (Moore and Semmens, 2008; Semmens et al., 2009; Stock and Semmens, 2013), were used to estimate the contribution of potential prev sources of turtles. The trophic discrimination factor (TDF) for olive ridley sea turtle egg components is unknown. Thus, the TDF used for SIMM in the present study was from blood plasma of a juvenile loggerhead turtle ( $\delta^{13}C = -0.38\% \pm 0.21$  and  $\delta^{15}N = 1.50\% \pm 0.17$ ; Reich et al., 2008) due to internal organs and blood plasma having high isotopic assimilation rates compared to other tissues (Boecklen et al., 2011). In addition, loggerhead and olive ridley sea turtles have similar diets and habitats (Bugoni et al., 2003; Colman et al., 2014), both with an oceanic juvenile phase based on gelatinous prey and a demersal diet based on fish and crustaceans (Bolten and Witherington, 2003; Bugoni et al., 2003; Behera et al., 2014; Colman et al., 2014). Neritic prey (crustaceans and demersal fish species) and oceanic prey (gelatinous species) were used in the mixed models. The SIMM was not intended to infer the diet of female olive ridley turtles but rather to evaluate the different contributions of oceanic vs. neritic food sources among egg components (yolk, albumen, and shell). Therefore, these tissues were used as a proxy for temporal contribution, as sea turtle yolk formation begins four to nine months before migration to the nesting area, and this process lasts five months (Roastal et al., 1998, 2001; Hamann et al., 2002, 2003). After fertilization near the nesting area, the albumen and shell are formed, thus probably with the contribution of prey from neritic waters (Roastal, 2007) or even from the turtles' own tissue due to fasting during the breeding period (Roastal, 2007). During the breeding season, many females reduce or even cease foraging activity (Hochscheid et al., 1999; Hays et al., 2002; Schofield et al., 2006; Fossette et al., 2008, 2012). Among 12 female olive ridley turtles sampled for a diet study in our area, 10 had eggs formed in their oviduct, and only 4 had food in their digestive tract (Colman et al., 2014); thus, this species may feed opportunistically during breeding.

Bayesian statistical framework inferences (Ellison, 2004) were used for the GLM, with the package 'rstanarm' (Gelman and Hill, 2007; Muth et al., 2018), for the multilevel regression model, with the package 'brms' (Bayesian regression models using 'stan'; Bürkner, 2017) and for the SIMM, with the package MixSIAR GUI (Hopkins-III and Ferguson, 2012). All analyses were performed with R software (R Core Team, 2017), Stan (Carpenter et al., 2017; Stan Development Team, 2020) and JAGS programs (Plummer, 2013) to specify models and perform the Bayesian analysis (Gilks et al., 1994). Model diagnostics were based on leave-one-out cross-validation (LOO; Gelfand et al., 1992; Ionides, 2008; Vehtari et al., 2017), in which the model is classified as good, ok, bad or very bad. Further diagnostics were also counted with the Rhat value, which provides information on the convergence of the algorithm (Rhat >1, the model is not well fitted).

# 3. Results

Female olive ridley turtles whose eggs were sampled ranged in size from 66.0 to 80.0 cm CCL (mean  $\pm$  SD = 72.92  $\pm$  2.89 cm). After the egg

incubation period, unhatched eggs were collected from the nests of 28 females, but only eggs from 15 nests ( $n_{nest} = 15$ ) were used in the present study because they contained three eggs without rupture and/or developed embryos. Thus, the total was 40 unhatched eggs ( $n_{egg} = 40$ ) processed for stable isotope analysis due to the loss of five unhatched eggs from different nests during sample processing (Table 1). In addition to these samples, 3 unhatched eggs ( $n_{egg} = 28$ ) were used in this analysis due to the loss of two eggs during processing (Table 1).

The  $\delta^{13}$ C values increased in the following tissue sequence: yolk (mean  $\pm$  SD = -18.05  $\pm$  1.80‰) < albumen (mean  $\pm$  SD = -17.79  $\pm$ 1.69‰) < shell (mean  $\pm$  SD  $= -16.93 \pm 1.$  68‰), while the  $\delta^{15}$  N values had a different tissue sequence: yolk (mean  $\pm$  SD = 11.24  $\pm$  1.35‰)  $\approx$ shell (mean  $\pm$  SD = 11.06  $\pm$  1.31‰) < albumen (mean  $\pm$  SD = 12.86  $\pm$ 1.32‰) for the fresh eggs (Fig. 2a; Table 1). For the unhatched eggs, carbon values followed the same sequence as fresh eggs: contents (mean  $\pm$  SD = -18.27  $\pm$  0.46‰) < shell (mean  $\pm$  SD = -17.56  $\pm$  0.45‰), while nitrogen values were the opposite: shell (mean  $\pm$  SD = 11.82  $\pm$ 1.12%) < contents (mean  $\pm$  SD = 13.61  $\pm$  0.91‰) (Fig. 2a; Table 1). For the unhatched eggs of different female olive ridley turtles, both  $\delta^{13}$ C and  $\delta^{15}$ N values followed the same sequence as unhatched eggs: content (mean  $\pm$  SD =  $-18.46 \pm 0.44$ %) < shell (mean  $\pm$  SD =  $-17.56 \pm$ 0.46‰) and shell (mean  $\pm$  SD = 12.07  $\pm$  1.31‰) < content (mean  $\pm$  SD =  $13.68 \pm 1.10\%$ ) for carbon and nitrogen, respectively (Fig. 2b; Table 1).

The cross-tissue conversion equations demonstrated a relationship for all tissues; however, the GLM fit better between the components of fresh eggs (yolk, albumen, and shell) (Fig. S1) and between the components of unhatched eggs (contents and shell) (Fig. S2) than between the components of fresh eggs and unhatched eggs together (Figs. S3 and S4). Furthermore, multilevel Bayesian regression showed that there was more variation between different nests than within nests (except SD  $\pm$  SD = 0.48  $\pm$  0.08 and sigma  $\pm$  SD = 0.15  $\pm$  0.02 for carbon; intercept SD  $\pm$  SD = 1.03  $\pm$  0.16 and sigma = 0.36  $\pm$  0.04 for nitrogen), showing that there was no difference between unhatched eggs from the same nest (Table 2).

The largest contribution to all fresh egg components was jellyfish (Fig. 3; Table 3), but both albumen and shell showed a significant contribution from demersal fish and crustaceans, respectively (Fig. 3; Table 3).

## Table 1

Stable isotope values ( $\delta^{13}$ C and  $\delta^{15}$ N) of egg components of female olive ridley turtles (*Lepidochelys olivacea*) from northeastern Brazil. The  $n_{nest} = 29$  are the components of fresh eggs (yolk, albumen, and shell); the  $n_{nest} = 15$  and  $n_{egg} = 40$  are the components of unhatched eggs (contents and shell) from 15 previous fresh egg nests sampled; The  $n_{nest} = 10$  and  $n_{egg} = 28$  are the components of unhatched eggs (contents and shell) from unknown female olive ridley turtles.

Tissue	n <sub>nest</sub>	n <sub>egg</sub>	$\delta^{13}$ C (‰) (mean $\pm$ SD)	Range (‰)	$\delta^{15}$ N (‰) (mean $\pm$ SD)	Range (‰)
Yolk	29	29	$\begin{array}{c} -18.05 \pm \\ 1.80 \end{array}$	-19.23 to -10.07	$\begin{array}{c} 11.24 \pm \\ 1.35 \end{array}$	7.10 to 14.62
Albumen	29	29	$-17.79 \pm 1.69$	-19.04 to -10.20	$\begin{array}{c} 12.86 \pm \\ 1.32 \end{array}$	8.84 to 16.01
Shell	29	29	$-16.93 \pm 1.67$	-17.87 to -9.28	$\begin{array}{c} 11.06 \pm \\ 1.31 \end{array}$	6.94 to 14.34
Unhatched content	15	40	$\begin{array}{c} -18.27 \pm \\ 0.46 \end{array}$	-19.17 to -17.28	$\begin{array}{c} 13.61 \pm \\ 0.91 \end{array}$	12.04 to 16.31
Unhatched shell	15	40	$-17.56 \pm 0.45$	-18.17 to -16.35	$\begin{array}{c} 11.82 \pm \\ 1.12 \end{array}$	8.98 to 15.28
Unhatched content	10	28	$\begin{array}{c}-18.46 \ \pm \\ 0.44\end{array}$	-19.17 to -17.41	$\begin{array}{c} 13.68 \pm \\ 1.10 \end{array}$	12.04 to 16.31
Unhatched shell	10	28	$-17.56 \pm 0.46$	-18.28 to -16.44	$\begin{array}{c} 12.08 \pm \\ 1.31 \end{array}$	10.71 to 15.96



**Fig. 2.** Carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) values of eggs from olive ridley turtles (*Lepidochelys olivacea*) from northeastern Brazil. The first graph shows the data from fresh eggs (yellow dots = the yolk, grey dots = the albumen and red dots = the shell) and the respective unhatched eggs (blue dots = the contents and green dots = the shell), while the second graph shows the data from unhatched eggs of unknown females. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### Table 2

Statistical summary of the Bayesian multilevel regression model to test for differences in  $\delta^{13}$ C and  $\delta^{15}$ N values between unhatched eggs (n = 2 or n = 3) from the same nest of female olive ridley turtles (*Lepidochelys olivacea*) ( $n_{nest} = 25$  and  $n_{egg} = 68$ ) from northeastern Brazil. CI: credible interval; SD: standard deviation; LOO: leave-one-out validation.

Model	Variable	Estimate	SD	95% CI	Rhat	LOO validation (Good + OK validation)	
Unhatched content							
	Intercept	-18.31	0.10	-18.50 to -18.13			
$\delta^{13}C \sim id$	SD Intercept	0.48	0.08	0.36 to 0.65	1.00	89.4%	
	Sigma	0.15	0.02	0.12 to 0.19			
	Intercept	13.72	0.21	13.30 to 14.14			
$\delta^{15}$ N ~ id	SD Intercept	1.03	0.16	0.76 to 1.40	1.00	95.5%	
	Sigma	0.36	0.04	0.29 to 0.45			
Unhatched shell							
$\delta^{13}$ C $\sim$ id	Intercept	-17.54	0.09	−17.71 to −17.36			
	SD Intercept	0.42	0.07	0.30 to 0.59	1.00	92.5%	
	Sigma	0.25	0.03	0.20 to 0.31			
$\delta^{15}$ N ~ id	Intercept	11.98	0.25	11.48 to 12.49			
	SD Intercept	1.23	0.21	0.88 to 1.70	1.00	97.0%	
	Sigma	0.59	0.07	0.47 to 0.74			

# 4. Discussion

In the present study, habitat use and potential prey contributions to the synthesis of eggs in female olive ridley turtles in northeastern Brazil were elucidated by SIA of egg components. This seems to be the first study of SIA in the eggs of olive ridleys globally.

Unhatched eggs are preferred for sampling to avoid damage to embryo development or destruction of the egg, providing results similar to viable eggs. Although in all cases the relationship between fresh egg and unhatched egg tissues, given by the slope in models, was close to 1, the intercepts were greater than zero for nitrogen and less than zero for carbon. This result demonstrates that it is necessary to use the crosstissue conversion equations from one tissue to another for comparative purposes, e.g., the egg yolk that indicates isotopic values from feeding areas. Ceriani et al. (2014) demonstrated a strong relationship among fresh yolk and unhatched eggs for both carbon and nitrogen values (slope relationship close to 1 and intercept close to 0) in loggerhead sea turtles from the North Atlantic Ocean. This difference may be due to the decomposition of egg content and the shell of unhatched eggs, since the summer in northeastern Brazil at 10°S is warmer than in Florida at 18°N. Despite these slight variations,  $\delta^{13}$ C values were shown to be somewhat depleted when comparing fresh egg contents (yolk and albumen) with unhatched egg contents, as well as between fresh eggshell and unhatched eggshell. However, for the nitrogen values, the opposite occurred, i.e., enriched in all tissues in unhatched eggs. These results were similar to those found in female loggerhead turtles from the North Atlantic Ocean and Mediterranean Sea, which even with these differences, it was concluded that sampling unhatched eggs is sufficient to represent isotopic values of a turtle nest (Zbinden et al., 2011; Ceriani et al., 2014).

In our study, no clustering occurred regarding the precise carbon and nitrogen values of the egg yolk, as in Hatase et al. (2002), with enriched and depleted values of the two parameters, classifying turtles originating from neritic and oceanic feeding areas, respectively. Instead, seven of the twenty-nine eggs sampled in our study had enriched carbon, which can be linked to latitude, i.e., individuals with higher carbon values may originate from feeding areas in northern Brazil, which is one of the feeding destinations after the breeding season, for females of the same population, studied by satellite telemetry (Santos et al., 2019). Climate and ocean currents influence water temperature, and the higher the water temperature, the less carbon dioxide is dissolved and thus  $\delta^{13}\mathrm{C}$ values will be higher due to the lower fractionation (Groericke and Fry, 1994; Rubenstein and Hobson, 2004; Fry, 2006). Moreover, four of these seven turtles presented enriched  $\delta^{15}$ N values ( $\geq 12$ ‰), which can be linked to high primary productivity in northern Brazil due to Amazon River outflows, while individuals with low  $\delta^{15}$ N values (< 12‰) could be using areas with limited river discharge at low latitudes, where phytoplankton have higher carbon and lower nitrogen isotopic values than in higher latitudes (Graham et al., 2010; Newsome et al., 2010).

Similar to tracking data (Santos et al., 2019), where only 4 female olive ridley turtles were tracked out of the 40 tagged (10%) and migrated to northern Brazil after nesting, we had 24% (7 out of 29) with



**Fig. 3.** Isospace plot (a) of potential prey items and proportional potential food sources from MixSIAR analysis (b – yolk, c – albumen, d – shell) of each egg component of olive ridley sea turtles (*Lepidochelys olivacea*) from northeastern Brazil. In graph (a), blue dots refer to  $\delta^{13}$ C and  $\delta^{15}$ N values from the yolk, red dots from the albumen and green dots from the shell. Stable isotope values from jellyfish (Hydrozoa and Scyphozoa) prey are from Dodge et al. (2011) and González-Carman et al. (2014), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Stable isotope mixed model (MixSIAR) results with predicted diet proportions (5th and 95th percentile) of each potential prey item compared to  $\delta^{13}$ C and  $\delta^{15}$ N mixture values for olive ridley sea turtle (*Lepidochelys olivacea*) components of eggs (yolk, albumen and shell). Mean values are in parentheses. n = sample size. Values in bold are the highest prey item contribution.

Egg component	n	Jellyfish	Crustacean	Demersal fish
Yolk	29	0.69–0.91 ( <b>0.81</b> )	0.01–0.20 (0.08)	0.03–0.21 ( <b>0.11</b> )
Albumen	29	0.53–0.73 ( <b>0.63</b> )	0.02–0.16 (0.08)	0.18–0.40 ( <b>0.29</b> )
Shell	29	0.58–0.84 ( <b>0.71</b> )	0.07–0.37 ( <b>0.22</b> )	0.02–0.15 (0.07)

enriched carbon, potentially originating from coastal areas northward. Tracked loggerhead turtles elsewhere showed the same dynamics, with depleted carbon values in blood samples from high latitudes (Ceriani et al., 2012). The remaining egg samples in our study (n = 22) showed low carbon values (mean  $\delta^{13}C = -18.67\%$ , range -19.23% to -18.06%) and variable nitrogen, both low and high values (mean  $\delta^{15}N = 11.21\%$ , range 8.03‰ to 12.30‰). Thus, these are individuals

attributed to high latitudes in south/southeast Brazil approximately 10°S of the nesting area in northeast Brazil or the west coast of Africa. The high nitrogen values match olive ridley feeding areas under the influence of the Cabo Frio upwelling along the coast of Rio de Janeiro, where there is a large input of nitrogenous nutrients, mainly nitrate, from deep waters, which causes an increase in  $\delta^{15}$ N values due to the low discrimination of this isotope. The low  $\delta^{15}$ N values may be related to the oceanic environment (west coast of Africa, according to Santos et al., 2019), where nitrogenous nutrients come mostly from atmospheric nitrogen fixation which causes a greater fractionation and thus decrease of  $\delta^{15}$ N values by primary producers. Moreover, as a complement to these low  $\delta^{15}$ N values, olive ridley turtles in this environment feed on pelagic prey such as jellyfish that have low trophic levels. In addition, epidermal tissue of loggerhead turtles demonstrated a variation in carbon values in samples from different latitudes in the neritic environment in the North Atlantic Ocean (Pajuelo et al., 2012). Furthermore, plasma and red blood cells of female olive ridley turtles showed no variation in carbon values between oceanic and neritic individuals at the same latitude in the North Atlantic Ocean and Pacific Ocean in central Mexico

(McClellan et al., 2010; Carpena-Catoira et al., 2022). Finally, several other marine organisms demonstrated latitudinal gradients as the main factor in isotopic carbon variation, as in cephalopods (Takai et al., 2000), penguins (Cherel and Hobson, 2007), North Pacific humpback whales, *Megaptera novaeangliae* (Witteveen et al., 2009), Cory's shearwater, *Calonectris borealis* (Roscales et al., 2011) and albatrosses (Jaeger et al., 2010).

Factors influencing  $\delta^{15}$ N values are related to the type of nitrogenous nutrient that enters a given environment, which can be from nitrogen fixation, nitrate input from deep waters and anthropogenic inputs (sewage or soil fertilizer runoff) (Fry, 2006; Michener and Kaufman, 2007). Therefore, the analysis of habitat use from this parameter alone becomes more difficult because of the lack of knowledge about what type of nitrogenous nutrient is being supplied in a given feeding area of this species (Fry, 2006; Michener and Kaufman, 2007). Unlike the nitrogen value, carbon values have only one type of compound that enters the trophic chain, carbon dioxide, which will vary depending on its tendency to dissolve in water and, therefore, will cause a high or low demand for this nutrient, which leads to a low or high fractionation, respectively, influencing  $\delta^{13}$ C values (Fry, 2006; Michener and Kaufman, 2007).

The fresh volk had lower  $\delta^{13}$ C values than both albumen and eggshell, probably due to the vitellogenesis that occurs approximately seven months before the migration to nesting beaches (Roastal et al., 1998). As the main breeding area of this olive ridley population is located in northeastern Brazil (Silva et al., 2007), this <sup>13</sup>C depletion could be due to the difference in latitudinal gradient between breeding and feeding areas, since the albumen and eggshell are formed after fertilization of the egg when the turtle mates near the nesting area (Roastal, 2007). The  $\delta^{15}$ N values of albumen were higher than those of yolk and eggshell. However, such an increase in albumen values is probably not related to dietary change, as there are many reports that the turtle is under fasting conditions during reproduction and that the albumen is formed during the late stage of egg formation. Thus, the increase in  $\delta^{15}$ N values in albumen and eggshell may be the result of catalysis of turtle tissues due to fasting, being used as an energy source during the nesting period (Roastal, 2007). In this way, the increase in isotopic nitrogen values may be due to nutritional stress and thus mobilization of body proteins (Hobson and Clark, 1992) towards tissues being synthesized during prolonged fasting.

Extended fasting is a natural component of the reproductive period of sea turtles, despite opportunistic feeding occurring (Colman et al., 2014). Therefore, food ingestion is physiologically directed towards reproduction and may contribute to egg formation. In line with this interpretation, the MixSIAR modelling showed a higher contribution of jellyfish to all egg components (yolk, albumen, and shell), although albumen and shell also had minor contributions from potential prey, such as demersal fish and crustaceans, respectively. Similarly, female olive ridley turtles from French Guiana and Indonesia showed diving behaviour compatible with bottom feeding, which indicated that turtles were feeding during the internesting period (Chambault et al., 2016; Fukuoka et al., 2022). Other sea turtle species, such as the hawksbill (Eretmochelys imbricata) from northeastern Brazil, had high levels of the satiety-inducing hormone during the internesting period (Goldberg et al., 2013). Finally, green turtles from Ascension Island showed body mass loss during the breeding period, indicating prolonged fasting (Hays et al., 2002). Thus, these benthic prey contributions are from either the opportunistically eaten real prey or the turtle's own tissue being used as an energy source. In fact, both possibilities should occur since this group of reptiles is usually opportunistic. These results are in agreement with previous studies, which demonstrated the contribution of jellyfish to olive ridley turtle scute samples (Petitet and Bugoni, 2017), as it is a tissue reflecting synthesis that occurred months before sampling (Reich et al., 2008), similar to yolk. In addition, the same study showed the contribution of benthic prey to plasma (Petitet and Bugoni, 2017), which generally has a faster turnover (Reich et al., 2008), thus aligning

with the albumen and eggshell studied here. Furthermore, Petitet et al. (2023) classified the same olive ridley turtle population as opportunistic with specialist individuals, based on isotopic values in sequential growth lines of humeri bones, which is in agreement with the present study due to the variation in the contribution of demersal prey to albumen and eggshell tissues.

## 5. Conclusion

This study demonstrated that female olive ridley turtles have a dichotomy on migration to feeding grounds, with high and low latitudes, but with limited distinction between oceanic vs. neritic habitats, based on the variation in carbon values from the yolk of fresh eggs. As this egg component is produced months before migration to the breeding area, the carbon and nitrogen values reflect the habitat and diet at the time of vitellogenesis, which occurs in feeding areas far away from rookeries. Thus, the habitat use of turtles nesting in Brazil involves both neritic and oceanic waters, covering a range of up to  $40^\circ$  of latitude. Due to its complex life cycle and a population composed of individuals with distinct foraging grounds, conservation actions are required in several areas with different threats. Such threats are related to bycatch by shrimp and finfish trawlers in Brazilian neritic waters, which already cause deaths of adult olive ridley turtles, as well as pelagic longline fishing that affect all age groups, which requires multiple conservation approaches (Hawkes et al., 2006). Thus, although olive ridley is the most abundant sea turtle species globally, it is one the least studied with a range of threats in different areas along their annual cycle.

# Compliance with ethical standards

This study was carried out in accordance with Brazilian law and ethical standards on animal care. The Brazilian Federal Environmental Agency (Instituto Chico Mendes de Conservação da Biodiversidade -ICMBio) approved the study through licence No. SISBIO 46238–1. The Animal Ethics Committee (CEUA-FURG) was informed on procedures with vertebrate eggs.

# CRediT authorship contribution statement

Pâmela Soares de Castro Echevenguá: Conceptualization, Investigation, Data curation, Funding acquisition, Writing - original draft. Roberta Petitet: Methodology, Software, Validation, Formal analysis, Visualization, Funding acquisition, Conceptualization, Data curation, Investigation, Supervision, Writing - review & editing. Jaqueline C. Castilhos: Resources, Writing – review & editing. Fábio Lira C. Oliveira: Resources, Investigation, Writing - review & editing. Leandro Bugoni: Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All data generated and analysed during this study are available in IsoBank datasource https://isobank.tacc.utexas.edu/.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2023.151911.

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