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Food preferences and Hg distribution in *Chelonia mydas* assessed by stable isotopes

M.F. Bezerra [a, *], L.D. Lacerda [a], C.E. Rezende [b], M.A.L. Franco [b], M.G. Almeida [b], G.R. Macêdo [c], T.T. Pires [c], G. Rostán [c], G.G. Lopez [c]

[a] Laboratório de Biogeoquímica Costeira, Instituto de Ciências do Mar, Universidade Federal do Ceará, Av. Abolição 3207, Fortaleza, CE, 60165-081, Brazil
[b] Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, Parque Califórnia, Campos dos Goytacazes, RJ, 28013-602, Brazil
[c] Brazilian Center for the Protection and Research of Sea Turtles Foundation (Pro-Tamar Foundation), Salvador, Bahia, Brazil

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ABSTRACT

Mercury (Hg) is a highly toxic pollutant that poses in risk several marine animals, including green turtles (*Chelonia mydas*). Green turtles are globally endangered sea turtle species that occurs in Brazilian coastal waters as a number of life stage classes (i.e., foraging juveniles and nesting adults). We assessed total Hg concentrations and isotopic signatures (^{13}C and ^{15}N) in muscle, kidney, liver and scute of juvenile green turtles and their food items from two foraging grounds with different urban and industrial development. We found similar food preferences in specimens from both areas but variable Hg levels in tissues reflecting the influence of local Hg backgrounds in food items. Some juvenile green turtles from the highly industrialized foraging ground presented liver Hg levels among the highest ever reported for this species. Our results suggest that juvenile foraging green turtles are exposed to Hg burdens from locally anthropogenic activities in coastal areas.

1. Introduction

Pollution by non-degradable contaminants, such as mercury (Hg) and its compounds, is considered one of the most concerning anthropogenic impacts to marine organisms including sea turtles. Efforts to reduce marine pollution are a priority issue for their conservation at global level (Hamann et al., 2010). Although several studies have focused on Hg effects on marine organisms (i.e. fish, marine mammals and seabirds), there is still a large gap regarding Hg impacts on reptiles (Perrault et al., 2013; Schneider et al., 2013).

Mercury is constantly being transformed in oceanic waters by biological, chemical and physical processes. As a result, Hg presents a residence time of approximately 30 years in superficial waters (<200 m) while in greater depths this time could be of ~100 years (UNEP, 2013). In a recent assessment of anthropogenic Hg, Lamborg et al. (2014) estimated the amount of anthropogenic Hg present in the ocean to be 290 ± 80 million moles. In addition, about 2/3 of this amount resides in water shallower than 1000 m, representing three times the amount of Hg present in pre-industrial oceans' surface waters. Therefore, long-lived oceanic organisms, such as sea turtles, are exposed throughout their lives to the legacy of Anthropocene Hg pollution as well as from long-term emissions from natural sources.

Studies have shown that Hg contamination could be related to several sub-lethal effects in marine animals (Clarkson, 2002; Wolfe et al., 1998). In reptiles, including sea turtles, Hg exposure has been related to physiological and behavioral changes, immunosuppression and reduced reproductive success (Day et al., 2007; Komoroske et al., 2011; Perrault et al., 2011; Schneider et al., 2013). Foraging habit and feeding preferences has been linked to Hg biomagnification in a variety of marine organisms (Bezerra et al., 2012; Di Beneditto et al., 2012, 2011; Pereira et al., 2010). A variety of ecological features (i.e. carbon diet sources, prey items and trophic level status) are effectively assessed by using stable isotopes signatures (i.e. ^{13}C and ^{15}N) providing valuable information to identify Hg bioaccumulation pathways in marine food webs (Bergeron et al., 2007; Ferriss and Essington, 2014).

The green sea turtle (*Chelonia mydas* Linnaeus, 1758) is frequent on Brazilian foraging grounds and is currently classified as an endangered species according to IUCN (2014). This species often uses

* Corresponding author.
E-mail address: mmoisesfb@hotmail.com (M.F. Bezerra).

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oceanic islands as nesting areas, and in Brazilian waters nesting females present a minimum curved carapace length (CCL) of 90 cm which could be used as a proxy for size at sexual maturation (Bellini et al., 2012; Almeida et al., 2011). Adult and neritic juvenile green turtles have a predominantly herbivorous diet (Bjorndal, 1980). However, hatchlings and young juveniles present an opportunistic feeding behavior with an omnivorous diet that varies according to the local food availability of each foraging ground (Cardona et al., 2010). In the Ceará coast, northeastern Brazil, the major food items reported for green turtles are marine macro algae (Ferreira, 1968). The same is reported for juvenile green turtles from foraging grounds located more southerly on the northern coast of Sao Paulo state (Sazima and Sazima, 1983). Juvenile green turtles are also frequently found along the northern coast of Bahia. This area hosts an important feeding ground for this species, but information on spatial use, demography and feeding habits is still scarce (Jardim, A. unpublished data). Feeding habit has been linked to Hg concentrations in juvenile green turtles (Bezerra et al., 2012). Considering that juvenile green turtles are mainly omnivorous and that Hg tends to accumulate through several food web levels, individuals at this life stage may be exposed to a higher risk than adults.

In this study we compared Hg concentrations in internal organs and tissues of green turtles from two foraging grounds in NE Brazil, the coast of Ceará and northern coast of Bahia. The former is a nearly pristine region while the latter is located on an industrial area with chemical and petrochemical activities (Marins et al., 2004; Rocha et al., 2012). In addition, the stable isotopes ^{13}C and ^{15}N were measured in order to infer about the species diet and its trophic level, as well as to assess the contribution of food source to their Hg body burdens.

1.1. Study area

Both study areas are located in the South Atlantic Ocean (Fig. 1) and are characterized as foraging grounds for juvenile and adult green sea turtles (Lima et al., 2013; Marcovaldi and Marcovaldi, 1999). The northern coast of Bahia is under influence by a petrochemical industrial complex in the surroundings of Camaçari municipality, and an extensive industrial development along *Todos os Santos Bay* (BTS), which is located in the vicinity of Salvador. As a result, this area has been receiving a large input of heavy metal contaminants in the last several decades, which potentially enhances the exposure of marine species, threatening biodiversity and human safety. For instance, relatively high concentrations of Hg have been reported in fish muscle (Centro de Recursos Ambientais – CRA, 2005) and shellfish from the northern coast of Bahia (Souza et al., 2011). The Ceará coast is characterized as nearly pristine regarding Hg contamination because of low industrial development. Marins et al. (2004) reported Hg concentrations in sediments (<63 μm) from several estuaries, ranging from 1 to 49 ng g^{-1}. These values are comparable to the Hg background for the region which is 15 ng g^{-1}.

2. Materials and methods

All analytical and sampling procedures were in accordance with all protocols for wildlife conservation accepted and authorized by the Ministry of the Environment and Natural Resources of Brazil (MMA) and the Brazilian Institute for Biodiversity Conservation – *Instituto Chico Mendes* (ICMBio) through SISBio License number 21693-1; 21693-2; 21693-3 and 21693-5.

Forty-one C. *mydas* specimens were sampled between 2009 and 2012 along the coast of Ceará (n = 16) and the northern coast of Bahia (n = 25). A technical staff from the Brazilian Marine Turtle Conservation Program (TAMAR) collected samples from dead individuals found stranded on the beach or debilitated animals that died during rehabilitation procedures performed. Approximately 10 g of muscle, kidney and liver were collected from each individual by necropsy and moisture content was determined by lyophilization (79.6%; 81.8% and 76.5%, respectively). Scutes were collected randomly from different areas of the carapace, cleaned with ultrapure water and washed in an ultrasonic bath to remove algae and other residues. Marine macro algae were collected from the intertidal zone and by free diving on the reefs in the two foraging grounds. All samples were collected in acid washed vials and were kept frozen until laboratory analyses.

2.1. Hg analysis

Duplicate samples (0.5 g) were acid digested with 10 mL of concentrated nitric acid (HNO_3 65%) for one hour and then, placed in a microwave furnace for 30 min at 200 °C. Then, 1 mL of hydrogen peroxide was added and the resulting solution was quantitatively transferred to a 100 mL volumetric flask filled with MilliQ® water. All materials that entered in contact with samples were acid washed and duplicates of procedural blanks were included in every digestion bath.

Total Hg (mentioned as T-Hg hereafter) concentrations were determined by CV-AAS using an NIC RA-3 NIPON Hg analyzer. Calibration curves (n = 20) with range varying from 0.5 to 35 ng Hg were performed for each analysis by using Hg standard stock solutions (MERK; 1000 μg mL^{-1}). The mean linearity coefficient (R^2) obtained was 0.9996 ± 0.0002. The limit of detection, calculated according to USEPA (2000), was 1.5 ± 1.2 ng Hg g^{-1}. Duplicates of Standard Reference Materials (NIST 2976 and BCR 060) containing certified T-Hg concentration of 61 ± 3.6 ng g^{-1} and 340 ± 40 ng g^{-1}, respectively, were included in every analysis and presented values of 67.5 ± 9.6 ng g^{-1} and 405 ± 49.1 ng g^{-1}, respectively. T-Hg concentrations are expressed in ng.g^{-1} of dry weight. To compare with literature studies, Hg concentration conversions from wet to dry weight basis were performed using water content data reported by Bezerra et al. (2013).

2.2. Stable isotopes analysis

Elemental (C and N) and isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) were determined for muscle and scute of green turtles. In addition, marine macro algae and invertebrates were also analyzed to investigate diet influence on the isotopic signatures of green turtles from the two different sites. Thus, two marine gastropods (*Stramonita haemostoma* and *Tubinella laevigata*) and different genera of Rhodophyta (*Cryptonemia*, *Bryothamnion*, *Gracillaria*, *Laurencia*, *Hypnea* and *Osmundaria*), Phaeophyta (*Lobophora*, *Padina* and *Sargassum*) and Chlorophyta (*Codium* and *Ulva*) were chosen. Marine algae samples were rinsed with distilled water before lyophilization and homogenization. Approximately 1 mg was weighed and analyzed with a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to an elemental analyzer (Flash 2000). Results are expressed in the conventional delta (δ) notation relative to Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$, according to the equation (1).

$$\delta = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) \times 10^3 \quad (1)$$

where R_{Sample} and $R_{Standard}$ are the corresponding ratios of rare to common isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and international standards, respectively (Peterson and Fry, 1987). In addition, elemental composition (Total Carbon and Total Nitrogen) was

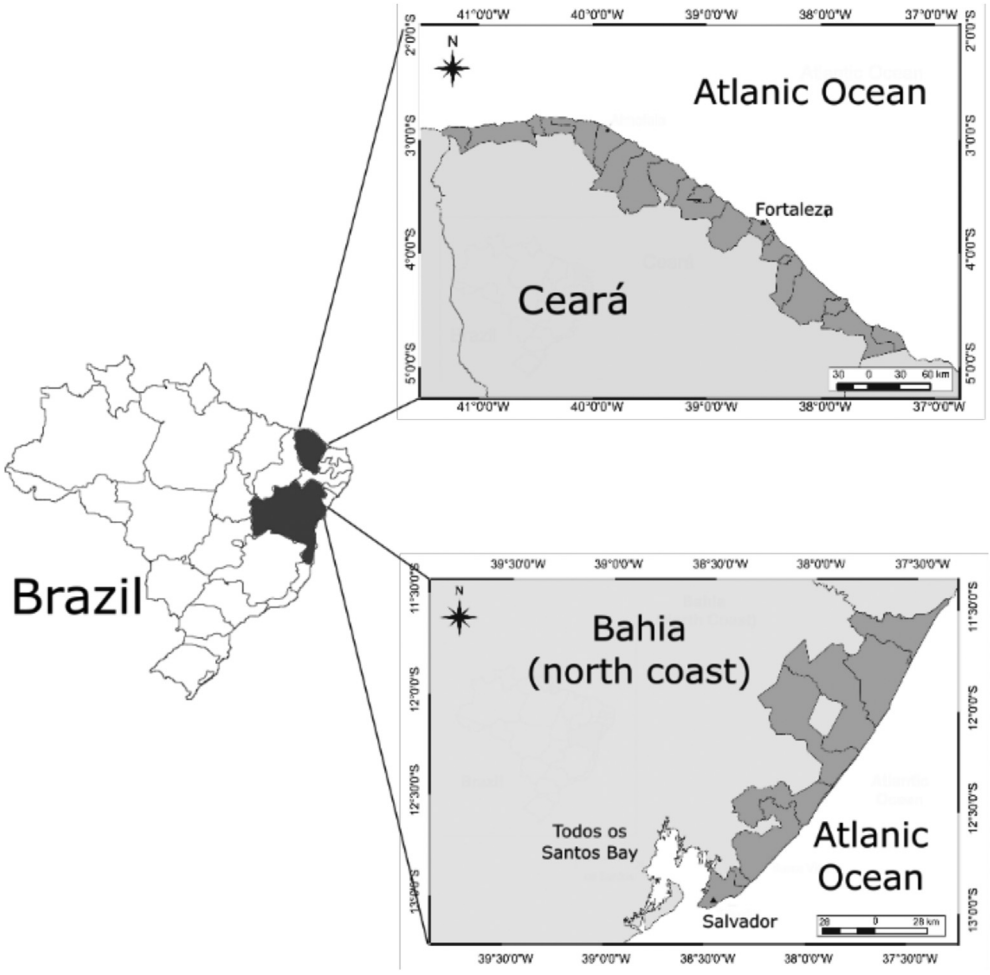

Fig. 1. Map of study areas highlighting the coastal zone of Ceará and Bahia.

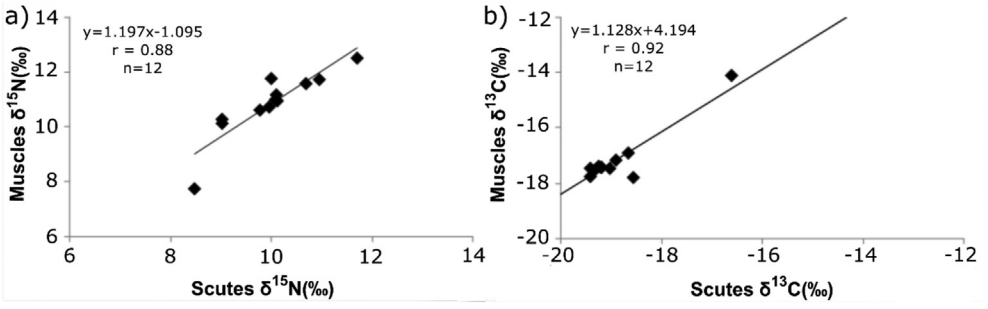

Fig. 2. Linear regressions of (a) $\delta^{15}N$ and (b) $\delta^{13}C$ between scute and muscle of juvenile *C. mydas*.

measured for each diet item and turtle sample. Results were expressed in percentage of dry weight.

Due to insufficient sample mass, it was not possible to determine isotopic composition in muscle samples of green turtle specimens from Ceará. So, prior to data analysis, a linear regression between scute and muscle of 12 green turtles from Bahia was determined according to Vander Zanden et al. (2014) and revealed a significant ($p < 0.001$) correlation of $\delta^{13}C$ and $\delta^{15}N$ showing that the tissues can be converted reliably, allowing a back-calculation of muscle values based on scute signatures (Fig. 2).

To quantify the contribution of different food items (algae and gastropods) to the isotope composition of green turtles, a Bayesian mixing model (SIAR: Stable Isotope Analysis in R) was used. This package provides the probability density distributions, mean proportion and credibility intervals (25%, 75% and 95%) for each source added to the models, incorporating uncertainty linked to elemental concentrations, isotopic signatures and fractionation (Parnell et al., 2010). The Trophic Enrichment Factor (TEF) is defined as the amount of change in isotope ratios between diet and consumer tissue (Phillips and Gregg, 2003) and is part of the routine of mixing models. However, to obtain consistent results the TEF should be applied carefully as several factors may influence the mean enrichment value (Caut et al., 2009; Wolf et al., 2009). Two sets of models with different TEF were developed. For the first set of

models, the TEF proposed by Post (2002) and widely used in the literature was applied ($\Delta^{13}C = 0.4 \pm 1.3‰$; $\Delta^{15}N = 3.4 \pm 1.0‰$). For the second set, therefore, values defined specifically for *Chelonia mydas* were calculated based on a prior study of the green turtle developed by Seminoff et al. (2006), using the equation (2).

$$\Delta dt = \delta_{tissue} - \delta_{diet} \quad (2)$$

where Δdt is the diet-tissue discrimination, δ_{tissue} represents the mean stable isotope ratio ($\delta^{13}C$ or $\delta^{15}N$) among all turtles and δ_{diet} corresponds to the overall mean stable isotope ratio of the main sources defined in the first set of models. Prior to running the models, similar prey items were combined, with the exception of the Rhodophyta algae collected. This group was divided into two statistically different subgroups based on $\delta^{13}C$ values: algae from the genera *Cryptonemia* (group "Cryp") and algae from genera *Bryothamnion*, *Gracillaria*, *Laurencia*, *Hypnea* and *Osmundaria* (group "Red Algae"). The other groups corresponded to "Brown algae" (genera *Lobophora*, *Padina* and *Sargassum*), "Green algae" (genera *Codium* and *Ulva*) and "Gastr" (the sea snails *Stramonita haemostoma* and *Tubinella laevigata*) All models consisted of different marine algae and gastropods as possible sources influencing isotope signatures of muscle and scute of *C. mydas* sampled in Bahia and Ceará.

Table 1
Size (cm), weight (kg) and sex of juvenile *C. mydas* sampled in Ceará and the northern coast of Bahia.

Site	Sample ID	CCL_{min}	CCW	Weight	Sex
Bahia	187 IL	27.0	24.9	1.5	F
	89 SA	29.8	27.0	1.9	F
	250 PF	29.8	27.5	2.6	F
	270 IL	30.6	30.2	2.3	F
	74 SA	31.6	27.7	2.1	F
	198 IL	31.6	29.1	2.5	F
	125 IL	32.0	29.5	2.9	F
	90 SA	33.1	30.7	3.3	F
	290 PF	33.1	29.4	4.1	F
	106 SA	33.3	32.2	3.4	F
	92 SA	33.8	30.4	3.4	F
	295 PF	34.2	29.2	2.9	F
	232 IL	34.8	32.3	3.7	F
	280 IL	34.8	29.9	3.4	F
	147 IL	34.9	32.5	3.6	F
	316 PF	35.0	32.2	5.7	M
	115 SA	35.5	33.5	4.0	F
	304 PF	36.2	35.0	5.6	M
	317 IL	36.2	34.6	4.0	F
	226 PF	38.5	35.3	3.4	F
	273 PF	39.4	35.0	6.4	F
	221 IL	45.2	40.6	7.4	F
	235 PF	47.6	43.3	6.8	M
	103 PF	50.0	46.0	13.0	F
	118 IL	62.0	57.4	24.0	F
Ceará	38/10	25.4	24.8	1.5	F
	35/10	27.0	25.0	2.0	F
	12/10	27.5	25.8	1.7	F
	34/10	28.5	25.2	2.0	F
	05/10	30.0	26.9	2.5	F
	470/09	32.9	30.4	3.0	F
	455/09	33.0	32.8	3.5	F
	458/09	34.1	32.3	4.0	M
	425/09	35.1	34.2	4.0	F
	406/09	35.5	32.7	4.0	M
	454/09	36.4	33.6	3.5	F
	391/09	36.4	30.3	3.5	F
	383/09	39.7	37.5	5.0	F
	24/10	41.0	36.0	6.0	F
	456/09	46.0	41.5	9.0	F
	389/09	55.4	54.7	20.0	F

CCL_{min} – Curved carapace length; CCW – Curved carapace width.

2.3. Statistics

Non parametric tests (Spearman's correlation, Kruskal–Wallis, Dunn and Mann–Whitney) were used to evaluate differences and correlations between biometric data and T-Hg in green turtles tissues and stable isotope data sets since the assumption of normality (Shapiro–Wilks; $p < 0.05$) was not met. A post hoc test (Dunn's test) was used when differences were observed with the Kruskal–Wallis. Since T-Hg concentrations in food items were normally distributed (Shapiro–Wilks; $p < 0.05$), differences between the two sites were analyzed using a Student's t-test. Significance was equal to $\alpha = 0.05$ for all tests performed.

3. Results

3.1. Total Hg in organs, tissues and food items of green turtles

All green turtles ($n = 41$) sampled in the two foraging sites were classified as juveniles, according to Almeida et al. (2011). No significant differences were observed between size (CCL_{min}) of green turtles from the two areas (Mann–Whitney $U = 212$; $p = 0.75$). Biometric data, sex identification and sample ID of specimens from Bahia and Ceará are shown in Table 1.

T-Hg concentrations in tissues and organs of juvenile green turtles from Bahia and Ceará are presented in Table 2. One green turtle from Bahia presented extremely high T-Hg concentrations in all tissues (ID 280 IL), however, because this animal did not interfere the statistical results, it was not removed from statistical analyses. For green turtles from Bahia's foraging grounds, T-Hg concentrations were higher in liver, with a median of 681.8 ng g^{-1}, and significantly different from T-Hg concentrations in scute (Dunn's test $z = 3.14$; $p = 0.047$) and muscle (Dunn's test $z = 5.97$; $p < 0.001$), but no different than kidney (Dunn's test $z = 2.64$; $p = 0.23$). On the other hand, T-Hg concentrations were lower in muscle, with a median value of 138.9 ng g^{-1}, and significantly different from T-Hg concentrations in kidney (Dunn's test $z = 3.33$; $p = 0.025$), but no different than scute (Dunn's test $z = 2.83$; $p = 0.13$). No significant differences were found between T-Hg concentrations in kidney and scute (Dunn's test $z = 0.498$; $p = 1.0$), with median concentrations of 365.6 ng g^{-1} and 380.7 ng g^{-1}, respectively. Similar T-Hg concentration patterns were observed in the green turtles from Ceará's foraging grounds, though no significant differences were observed among tissues, except for liver and muscle (Dunn's test $z = 3.72$; $p = 0.006$). T-Hg concentration was higher in liver (median of 468.6 ng g^{-1}) and lower in muscle tissue (median of 167.4 ng g^{-1}); kidney and scute showed median values of 287.0 ng g^{-1} and 425.5 ng g^{-1}, respectively.

Comparing the two areas, liver Hg concentrations were significantly higher in green turtles from Bahia compared to Ceará (Mann–Whitney $U = 95.0$; $p = 0.02$). However, we did not observe significant differences of mean T-Hg concentrations in the others organs and tissues (Fig. 3).

The T-Hg concentrations in tissues presented no correlation with size (CCL_{min}) or weight (Spearman's correlation $p > 0.05$), except for kidney from green turtles in Ceará which presented a weak correlation with CCL_{min} (Spearman's correlation $n = 16$; $r_s = 0.52$; $p = 0.04$), but not with weight (Spearman's correlation $n = 16$; $r_s = 0.48$; $p = 0.06$). Considering that no tendencies were detected, size and weight was not considered a covariate for T-Hg concentrations for our data set. Spearman's correlation tests revealed a positive correlation between scute Hg concentration in organs and tissues of green turtles from both foraging sites (Fig. 4). For Bahia's green turtles, T-Hg concentrations in scute were significantly correlated with T-Hg in liver ($r_s = 0.59$, $p = 0.002$), kidney ($r_s = 0.78$, $p < 0.001$) and muscle ($r_s = 0.84$, $p < 0.001$). On

Table 2
Sample ID and mean ± standard deviation of T-Hg concentration (ng g^{-1}) in muscle, kidney, liver and scute of juvenile *C. mydas* collected in two foraging grounds from northeastern Brazil.

Site	Sample ID	T-Hg (ng.g^{-1} dry weight)			
		Muscle	Kidney	Liver	Scute
Bahia	89 SA	285.8 ± 30.5	725.0 ± 106.8	1426.2 ± 104.7	815.4*
	221 IL	438.3 ± 54.3	1276.7 ± 34.2	1788.1 ± 57.3	397.8 ± 104.5
	92 SA	169.0 ± 23.3	408.1 ± 17.1	642.3 ± 21.6	385.3 ± 14.1
	147 IL	190.0 ± 26.5	418.0 ± 60.9	1086.2 ± 48.7	500.8 ± 47.2
	232 IL	249.2 ± 15.9	429.2 ± 37.5	1501.9 ± 61.0	456.0 ± 39.1
	90 SA	460.4 ± 15.1	616.8 ± 75.9	1547.3 ± 143.0	982.0*
	226 PF	45.1 ± 2.0	331.1 ± 10.5	1905.2 ± 101.7	28.2*
	235 PF	33.8 ± 1.7	199.9 ± 23.6	812.1 ± 38.8	20.1*
	125 IL	83.7 ± 6.9	260.6 ± 21.7	524.3 ± 26.7	335.1 ± 27.3
	74 SA	177.5 ± 17.9	361.6 ± 7.9	649.3 ± 37.5	214.0*
	250 PF	323.1 ± 7.8	455.8 ± 11.5	1612.7 ± 119.0	501.0 ± 37.3
	187 IL	35.4 ± 2.8	261.7 ± 15.8	622.5 ± 15.3	304.8*
	198 IL	104.2 ± 18.7	247.2 ± 8.4	231.3 ± 11.8	206.5*
	290 PF	38.2 ± 3.2	197.6 ± 17.3	119.2 ± 9.7	148.8*
	118 IL	13.5 ± 2.3	51.3 ± 2.5	272.0 ± 21.7	44.3 ± 5.0
	280 IL	856.9 ± 144.4	1064.9 ± 44.8	3135.0 ± 228.4	1244.6 ± 76.0
	270 IL	62.7 ± 7.7	591.9 ± 65.0	1005.7 ± 35.7	380.7 ± 27.4
	304 PF	31.6 ± 3.5	161.1 ± 19.6	343.7 ± 19.0	28.8*
	295 PF	14.2 ± 0.8	294.5 ± 14.3	399.9 ± 28.6	13.8*
	106 SA	255.4 ± 17.8	365.6 ± 49.2	681.8 ± 26.2	864.2*
	317 IL	138.9 ± 22.3	401.0 ± 6.6	641.3 ± 25.9	471.7 ± 13.4
	316 PF	255.9 ± 21.4	376.7 ± 5.9	1266.4 ± 59.0	375.5 ± 12.0
	103 PF	10.1 ± 0.2	171.2 ± 26.5	81.0 ± 11.1	19.3 ± 2.8
	273 PF	52.1 ± 5.7	303.4 ± 13.3	620.9 ± 40.6	659.1 ± 39.5
	115 SA	283.6 ± 44.0	767.9 ± 41.3	1645.1 ± 48.3	713.3 ± 68.5
Ceará	38/10	83.1 ± 9.5	61.4 ± 1.3	NA	NA
	35/10	152.8 ± 8.4	387.9 ± 38.7	660.3 ± 176.2	313.9 ± 10.8
	12/10	152.7 ± 8.1	254.9 ± 34.6	576.9 ± 12.1	72.3*
	34/10	217.2 ± 9.4	505.2 ± 35.8	NA	509.5 ± 27.7
	05/10	79.6 ± 14.6	175.7 ± 60.0	142.8 ± 18.4	47.5 ± 26.6
	470/09	141.2 ± 43.4	347.4 ± 155.6	259.2 ± 16.3	NA
	455/09	110.9 ± 45.8	115.8 ± 7.0	366.3 ± 70.5	NA
	458/09	183.9 ± 28.2	233.6*	335.8*	NA
	425/09	101.3 ± 19.4	206.1 ± 1.1	484.8 ± 29.7	NA
	406/09	199.4 ± 20.3	273.9 ± 9.3	452.3*	493.8 ± 138.5
	454/09	334.8 ± 111.0	236.0 ± 71.5	776.0 ± 262.1	NA
	391/09	253.0 ± 9.2	300.1 ± 10.5	505.5 ± 262.4	856.6 ± 27.9
	383/09	232.5 ± 71.8	581.3 ± 146.3	869.0 ± 128.5	428.4 ± 19.3
	24/10	182.0 ± 35.5	359.9 ± 35.7	529.5 ± 148.2	457.8 ± 15.3
	456/09	BDL	946.5 ± 50.7	344.1 ± 34.8	NA
	389/09	BDL	1204.7 ± 33.1	361.0 ± 61.4	7.3 ± 1.7

*Concentration from a single analysis; BDL – Below the Detection Limit; NA – Not Analyzed.

green turtles from Ceará, T-Hg concentrations in muscle were significantly correlated with T-Hg in scute ($r_s = 0.86$; $p = 0.006$), liver ($r_s = 0.59$; $p = 0.041$) and kidney ($r_s = 0.61$; $p = 0.021$).

T-Hg concentrations in marine algae from Bahia ranged from 9.4 to 30.5 ng g^{-1} with a mean of 16.4 ± 7.7 ng g^{-1}. Whereas, Hg levels in marine algae from Ceará ranged from 1.7 to 15.3 ng g^{-1} with a mean of 9.5 ± 5.4 ng g^{-1}. T-Hg concentrations observed in marine algae from the Bahia foraging ground were significantly higher compared with marine algae from Ceará foraging ground (Student's t; $F = 5.94$; $p = 0.02$) (Fig. 5).

3.2. Feeding assessment of *C. mydas* using C and N stable isotopes

Stable isotopic signatures of muscle and scute were obtained for 31 specimens of *Chelonia mydas* from both areas, and significantly higher values of $\delta^{13}C$ and $\delta^{15}N$ were observed for the muscle samples (Mann–Whitney $U = 60.0$ for $\delta^{13}C$ and $U = 138.0$ for $\delta^{15}N$; $p < 0.01$ for both elements). Considering the foraging grounds and isotope signature variations among tissues, higher mean $\delta^{13}C$ values for muscle in Ceará and scute in Bahia were observed. In addition, specimens caught in Ceará showed higher mean $\delta^{15}N$ for both tissues (Table 3). Nevertheless, mean values did not differ significantly between the two foraging grounds (Kruskal–Wallis, $p > 0.05$).

Nitrogen signatures of *C. mydas* varied from 7.76 to 12.41‰ and from 7.78 to 11.19‰ for muscle and scute, respectively. These values were higher than the $\delta^{15}N$ found in all potential food items, except for gastropods which suggests that this prey item may not be important for the feeding habits of green turtles from this study. The red algae genus *Cryptonemia* was responsible for the lowest $\delta^{15}N$ mean value presented herein. Carbon signatures of the Brown, Green and Red Algae compartments were closer to the values found for *C. mydas*, indicating the relevant contribution of these preys to the feeding habits of the green turtle (Fig. 6).

Mean $\Delta^{13}_{dt}C$ found were 0.91‰ ± 0.44 and −1.37‰ ± 0.58 for muscle and scute, respectively. Both tissues were ^{15}N enriched related to the diet, and a higher $\Delta^{15}_{dt}N$ was observed for muscle (4.73‰ ± 0.27) when compared to scute (3.98‰ ± 0.27). Results from the SIAR mixing models (based on the TEF proposed by Post, 2002), indicated that brown and green algae are the primary sources contributing to the isotopic values in muscle and scute of green turtles from Bahia (Fig. 7a and c), corresponding to more than 80% of total mean contribution. Isotopic signatures in muscle and scute of green turtles from Ceará (Fig. 7b and d), when compared to

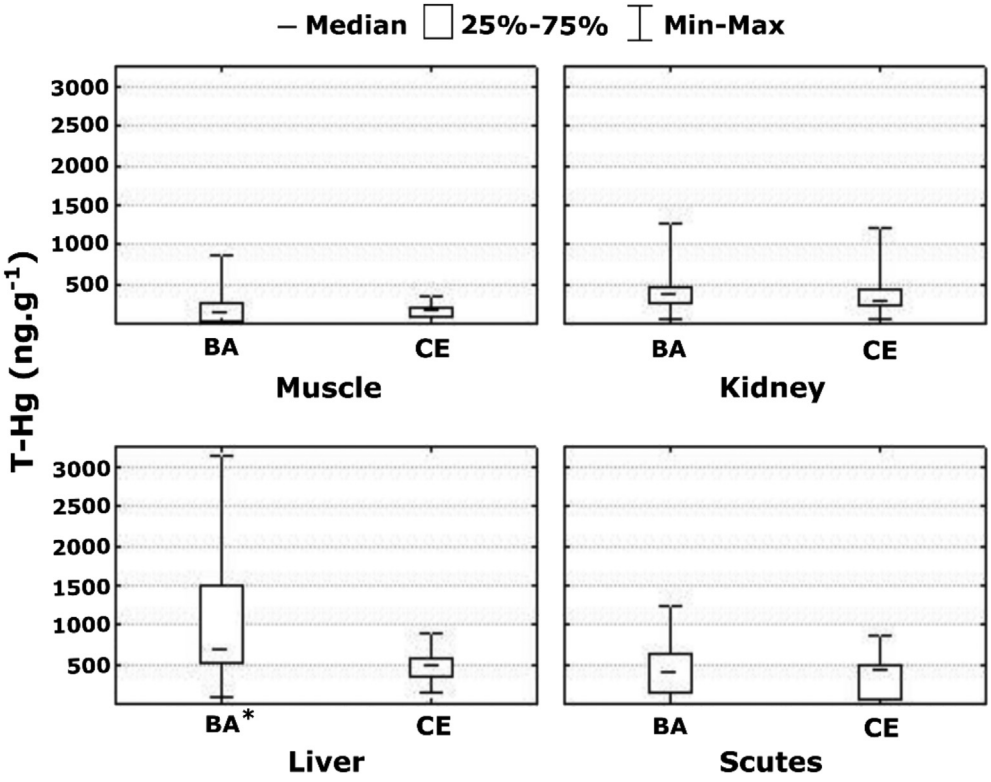

Fig. 3. Box plot of T-Hg concentrations grouped by organs and tissues of juvenile *C. mydas* from two foraging grounds (Bahia – BA; Ceará – CE). *Denotes significant difference between the two sites.

Bahia, showed a higher contribution of red algae to the feeding of *Chelonia mydas*, with brown algae as the main source when taking into account muscle signatures. The relative contribution of the red algae genus *Cryptonemia* was higher for scute-based models, with the opposite being observed for gastropods (Fig. 8). The same general pattern was observed in the second set of models, (developed with TEFs defined specifically for *C. mydas*), corroborating the importance of the brown and green algae to the specimens from Bahia and a more distributed assimilation of prey items from individuals from Ceará.

4. Discussion

The large range of T-Hg concentrations found in organs and tissues of juvenile green turtles from both foraging sites could be a result of different individual feeding strategies and an omnivorous feeding habit associated with this species (Vander Zanden et al., 2013). The correlation between T-Hg concentrations in scute and other organs, especially muscle, shows that carapace fragments could be used as a good monitoring matrix for Hg body burdens in *Chelonia mydas*. Similarly, previous studies have shown that T-Hg concentrations quantified in scute of green turtles correlated significantly with Hg content presented in muscle, blood and others internal tissues (Bezerra et al., 2013; Komoroske et al., 2011; Van de Merwe, 2008).

Hg bioaccumulation in marine animals results from a continuous Hg intake, mostly through feeding. This accumulation also results from a slow metabolic elimination rate, which can cause larger and/or older marine animals to exhibit an increased bodily Hg burden, especially in top predators (Morel et al., 1998). Paradoxically, green turtles could exhibit an inversed tendency with lower Hg body burdens in adults compared to juveniles, which is probably a response of the dietary shift from omnivorous/carnivorous juveniles to omnivorous/herbivorous adults (Bezerra et al., 2012). However, green turtles sampled in our study did not show any significant correlation between T-Hg concentrations and size, which was attributed to the great variability in T-Hg concentrations and the fact that all animals were of the same life stage class (i.e., juvenile).

Green turtles generally show strong fidelity to foraging areas (Aguirre and Lutz, 2004; Koch et al., 2007; Meylan et al., 2011). In this context, it is reasonable to attribute Hg body burdens of individuals to regional environmental levels. Considering the large distance from the Bahia foraging site to the Ceará foraging site (~1500 km), we predict few or no interactions between animals from the areas. Thus, T-Hg concentrations found in each population should reflect the Hg levels of the surrounding environment, especially those in the food items. The north coast of Bahia state has higher industrial development, with harbors, pigment industries and a petrochemical pole contributing to relatively high Hg background levels. On the other hand, the Ceará coast is characterized by low industrial development with no historical Hg contamination and low background Hg levels (Marins et al., 2004; Rocha et al., 2012; Souza et al., 2011; Wasserman and Queiroz, 2004). This is most likely why higher T-Hg concentrations were found in marine algae samples from Bahia when compared to Ceará. These differences may reflect Hg concentrations in green turtle tissues. In a case study, Bezerra et al. (2014) reported high T-Hg concentrations in a captive green turtle submitted to a predominantly carnivorous diet, highlighting the strong influence that food items may have on Hg incorporation in green turtles.

The Hg level comparisons between green turtle tissues around the world are shown in Fig. 9. The T-Hg concentrations found in kidney and muscle of green turtles from this study are in the same

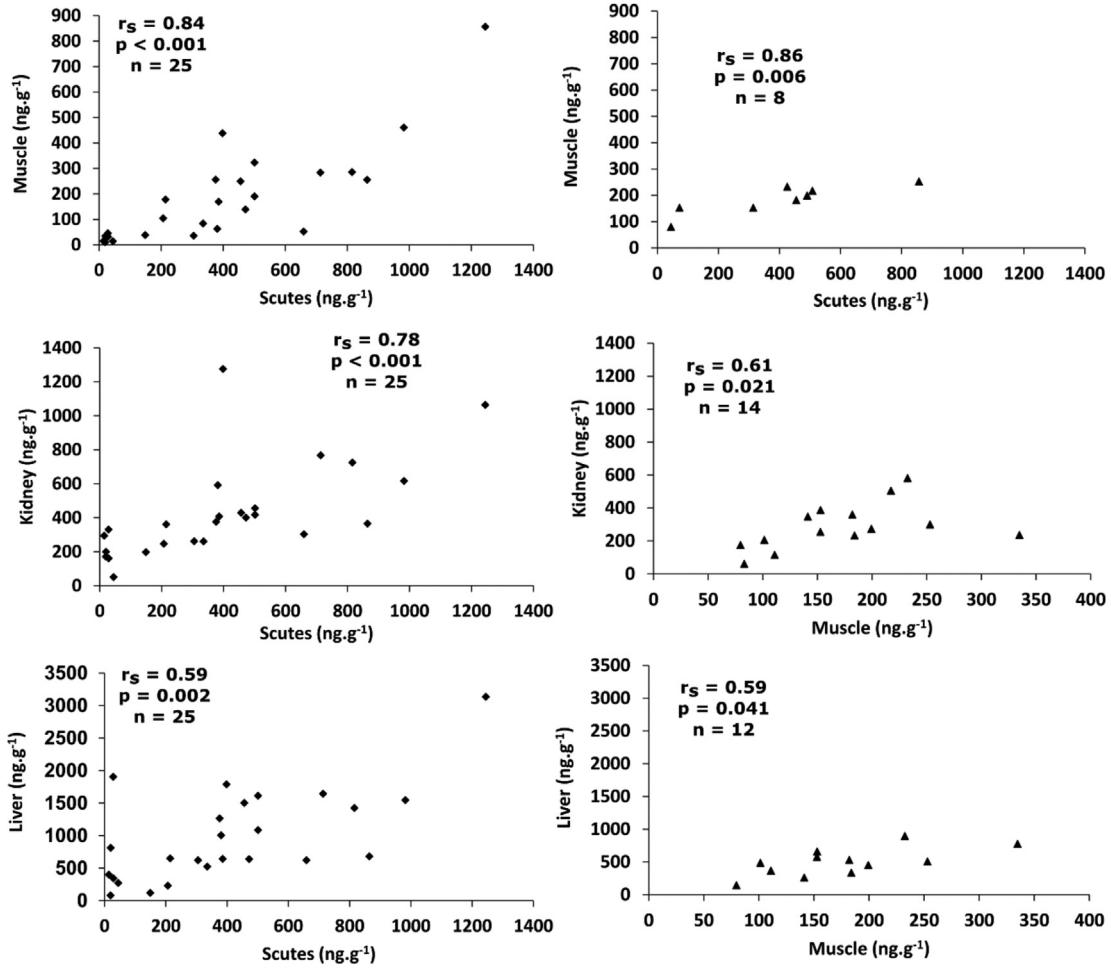

Fig. 4. Scatterplot graph of T-Hg concentrations (dry weight) between tissues of juvenile *C. mydas* from two foraging grounds (◆Bahia – BA; ▲Ceará – CE), showing significant correlations ($p < 0.041$) for all presented pairs of tissues.

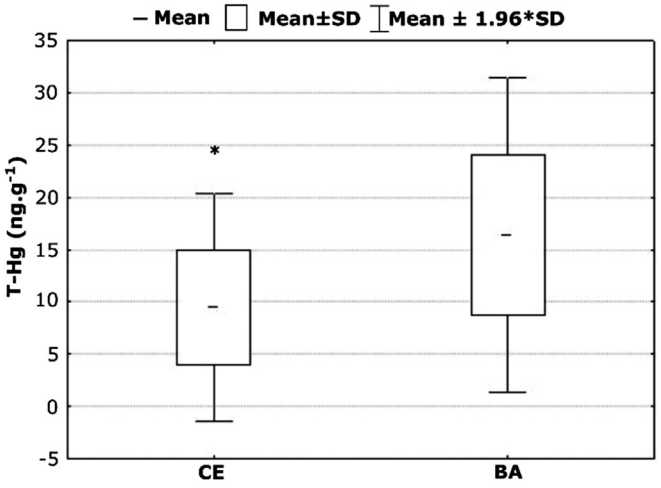

Fig. 5. Box plot of T-Hg concentrations in marine macro algae collected in two foraging grounds from northeastern Brazil. *Denotes significant difference between both areas.

Table 3
Stable isotope signatures of *C. mydas* and potential prey items divided by compartments. Values are means (±one standard deviation).

Compartments	n	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
C. mydas			
Muscle	31	10.36 (0.22)	−17.09 (0.93)
Scute	31	9.56 (0.22)	−18.87 (0.79)
C. mydas – Foraging Grounds			
Bahia			
Muscle	24	10.17 (0.25)	−17.21 (0.87)
Scute	24	9.41 (0.25)	−18.22 (1.31)
Ceará			
Muscle	7	10.98 (0.46)	−16.72 (1.06)
Scute	7	10.09 (0.46)	−18.54 (0.94)
Souces			
Brown algae	15	5.30 (0.03)	−16.00 (2.46)
Green algae	10	5.40 (1.37)	−18.70 (1.78)
Red algae	25	6.20 (0.86)	−17.80 (4.13)
Criptonemia sp.	5	6.11 (0.38)	−30.68 (2.96)
Gastropods	11	11.65 (0.21)	−16.20 (0.28)

range of Hg levels found in same tissues of juvenile green turtles from China, Australia and Japan, except for muscle in animals from China, which are much higher than our results (Lam et al., 2004; Sakai et al., 2000; van de Merwe et al., 2010). In addition, Green turtles from Mexico presented the lowest T-Hg concentrations for all tissues (i.e. muscle, kidney and liver) (Kampalath et al., 2006). The T-Hg concentrations in liver of green turtle from Ceará are in a lower range than animals from China and Australia, but higher than green turtles from Japan and Mexico (Kampalath et al., 2006; Lam et al., 2004; Sakai et al., 2000; van de Merwe et al., 2010).

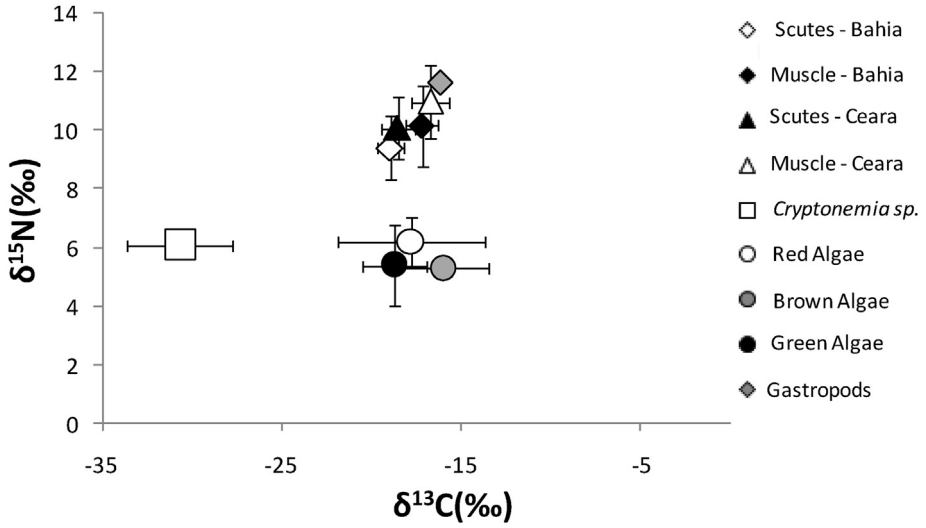

Fig. 6. Bivariate plots of isotopic signatures of green sea turtle *C. mydas* and potential prey items. Points are means and error bars are ±one standard deviation.

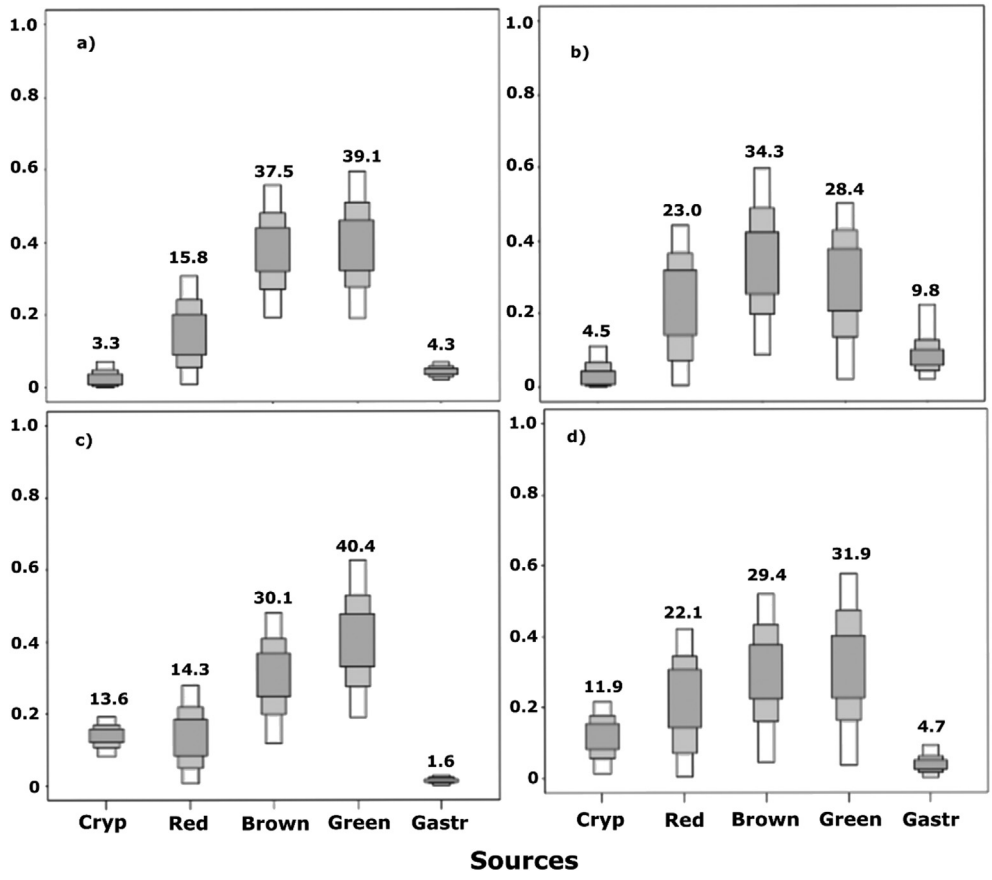

Fig. 7. Results of SIAR mixing models (50, 75 and 95% credibility intervals) showing the probable sources proportion (%) in the diet of *C. mydas*. Models based on muscle samples from Bahia (a) and Ceará (b), and scute from Bahia (c) and Ceará (d). Trophic Enrichment Factor (TEF) following Post (2002) along with group members presented in the methodology section. Values above bars represent mean contribution from each source.

Considering only juvenile green turtles from Bahia north coast, our results are among the highest ever reported for *C. mydas* and comparable with Hg levels found in strictly carnivorous sea turtle species (D'Ilio et al., 2011; Gordon et al., 1998; Kampalath et al., 2006; Perrault, 2012; Storelli and Marcotrigiano, 2003). A recent study on the same area (i.e. northern Bahia) also reported high T-Hg concentrations in kidney and liver of juvenile green turtles (Macêdo et al., 2015). These authors also reported high concentrations for other heavy metals (i.e. cadmium, nickel, lead and zinc) in liver and kidney of juvenile green turtles, as well as, juvenile hawksbill turtles (*Eretmochelys imbricata*, Linnaeus 1766) suggesting that an important source for several heavy metals exists in this region.

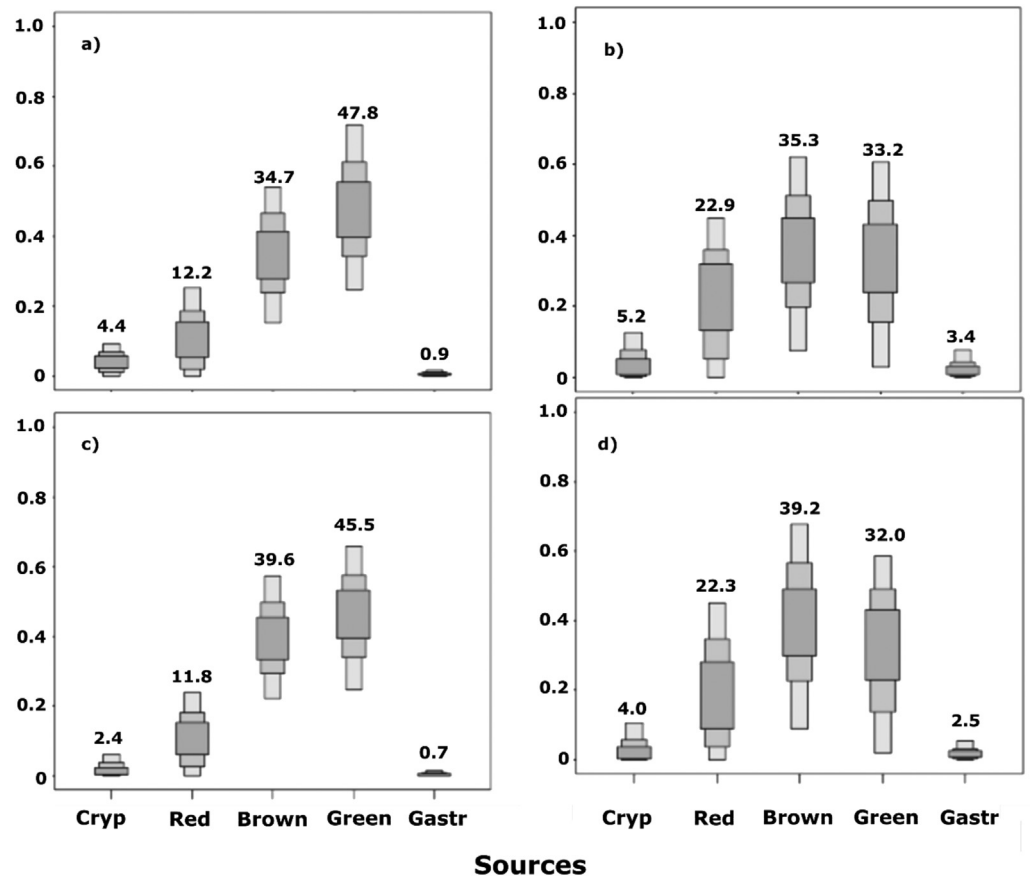

Fig. 8. Results of SIAR mixing models (50, 75 and 95% credibility intervals) showing the probable sources proportion (%) in the diet of *C. mydas*. Models based on muscle samples from Bahia (a) and Ceará (b), and scute from Bahia (c) and Ceará (d). Trophic Enrichment Factors (TEFs) for muscle ($\Delta^{15}N = 4.73‰ \pm 0.27$; $\Delta^{13}C = 0.91 \pm 0.41$) and scute ($\Delta^{15}N = 3.98‰ \pm 0.27$; $\Delta^{13}C = -1.37 \pm 0.58$) based on mean values of main sources described in the methodology section. Values above bars represent mean contribution from each source.

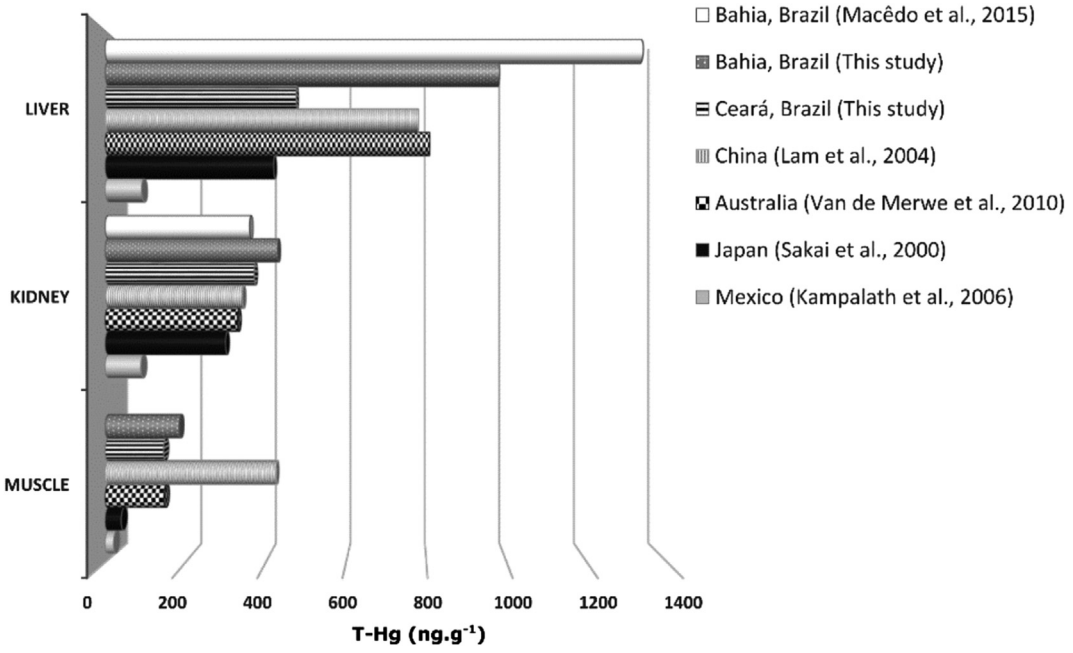

Fig. 9. Mean T-Hg concentrations (ng g^{-1} dry weight) in tissues of *C. mydas* from different regions of the world.

The isotope results seem to corroborate the possibility of environmental pollution exposure aforementioned. Marine algae were the main contributor for green turtles from both foraging areas, thus excluding a difference of T-Hg concentration due to distinct trophic levels. In fact, although not significant, mean nitrogen signatures of green turtles' tissues were lower in Bahia, contradicting a possible increase in T-Hg due to a higher trophic level of the species in this area.

Our results of the isotope composition of green turtles' food items were based on algae and animal prey from both areas. However, we did not consider the relative contribution of individual items, and therefore, should be understood as a general comparison. A greater evaluation regarding feeding habits in both areas is necessary to determine what proportions of each food items are ingested (Amorocho and Reina, 2007; Arthur et al., 2009; Cardona et al., 2009; Ferreira, 1968). This information is crucial to assess the extent of potential Hg contamination in these two important foraging areas for green turtles.

Until the present date, there is only one published study of green turtle feeding behavior in the Ceará coast, which showed a majority of marine algae, mostly Rhodophyta, in the stomach contents of juvenile and adult green turtles (Ferreira, 1968). The isotope results presented herein confirmed a diet based on marine algae, although green and brown algae, instead red algae, appear as main contributors for green turtles from Bahia and Ceará, respectively. This difference observed between isotope and stomach content data may be a consequence of the emphasis given by each methodology, which could indicate that the green turtle feeds mostly on red algae but assimilates mainly green and brown algae. Also, limitations of stomach content analysis are well known (Baker et al., 2014; Hyslop, 1980). It is important to highlight the lack of papers dealing with feeding behavior of the green turtles from northeastern Brazil.

5. Conclusions

Marine macro algae was confirmed as the major items responsible for isotopic signatures in muscle and scute of *Chelonia mydas* from two Brazilian foraging grounds. Isotope signatures were able to relate T-Hg concentrations in green turtles from a large extent of the tropical Brazilian coast with their diet. We observed a great variability of T-Hg concentrations in juvenile green turtles from the northeastern Brazil. In general, these concentrations are comparable or even higher than Hg levels reported from others areas around the world. Liver concentrations measured in juvenile green turtles from northern coast of Bahia are particularly high and may be associated with the legacy of Hg pollution in the region. Since this area is an important feeding ground for *C. mydas* and one of the largest units of the Brazilian Sea Turtle Protection Network, we suggest further and urgent investigations in this area to assess how these Hg levels are impacting the green turtles as well as others marine organisms. The assessment of T-Hg concentration in scutes provided an accurate estimation of internal Hg burdens, highlighting this tissue as an important non-lethal sampling technique for futures studies on Hg contamination monitoring in green turtles. We recommend that scute sampling should be included on the regular juvenile green turtle monitoring protocols for both areas, as a way to monitor Hg contamination at a regional level.

Acknowledgements

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