Environmental Science and Pollution Research (2020) 27:20527–20537
https://doi.org/10.1007/s11356-020-08623-5

RESEARCH ARTICLE

Influence of size on total mercury (THg), methyl mercury (MeHg), and stable isotopes of N and C in green turtles (*Chelonia mydas*) from NE Brazil

César Augusto Barrios Rodriguez[1] · Luiz Drude de Lacerda[1] · Moises Fernandes Bezerra[2] · Victor Lacerda Moura[1] · Carlos Eduardo de Rezende[3] · Wanderley Rodrigues Bastos[4]

Received: 20 November 2019 / Accepted: 26 March 2020 / Published online: 3 April 2020

Abstract

The green turtle (*Chelonia mydas*) is known to present an herbivorous diet as an adult; however, juveniles may have an omnivore habit, and these changes in food preference may affect the uptake and accumulation of pollutants, such as mercury (Hg). In order to better understand the influence of this ontogenetic shift on Hg accumulation, this study evaluates the concentrations of total mercury (THg), methyl mercury (MeHg), and stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in a group of juveniles of the green turtle. Tissue samples (liver, kidney, muscle, and scutes) were sampled from 47 turtles stranded dead on the coast of Bahia, NE, Brazil, between 2009 and 2013. The turtles analyzed showed a size range of 24.9–62.0 cm and an average of 36.4 ± 7.2 cm of curved carapace length. The scutes showed to be a viable method for Hg monitoring in the green turtles. The concentrations of THg and MeHg decreased with increasing size. The isotope values of $\delta^{15}N$ and $\delta^{13}C$ did not show a clear relationship with the size, suggesting that the green turtles used in our work would be occupying similar trophic levels, and foraging habitat.

Keywords *Chelonia mydas* · Mercury · MeHg · Carbon isotope · Nitrogen isotope · Green turtle · SIA

Introduction

Pollution from anthropogenic activities exerts a strong pressure on the marine ecosystems, negatively affecting the health of the species inhabiting contaminated areas (Hamann et al. 2010). In addition to illegal fishing, and degradation of breeding and feeding habitats, pollution is another major factor affecting the stability of sea turtle populations worldwide (Miguel and de Deus Santos 2019). Adverse effects of pollution can include compromised physiology, chronic stress, impaired immune function, and an increase in disease susceptibility, like fibropapillomatosis (Miguel and de Deus Santos 2019).

With millions of years of existence, sea turtles can be regarded as sentinel species of the oceans (Aguirre and Lutz 2004) due to their characteristic long lifespan, feeding at different trophic levels and wide distribution, which make them excellent monitors of contamination by toxic metals, such as mercury (Hg) (Barbieri 2009). This metal occurs naturally in the environment, but anthropogenic activities such as goldmining, coal, and solid waste burning have increased Hg mobilization in the environment, raising its emissions to the atmosphere, soils, rivers, and oceans (Driscoll et al. 2013; UNEP 2013).

The abundance of Hg chemical species varies among the different environmental compartments: more than 95% of the

Responsible editor: Philippe Garrigues

✉ César Augusto Barrios Rodriguez
hpbarrios15@gmail.com

[1] Laboratório de Biogeoquímica Costeira, Instituto de Ciências do Mar, Universidade Federal do Ceará, Av. Abolição, 3207, Fortaleza, CE 60165-081, Brazil

[2] Department of Biology, San Diego State University, San Diego, CA 92182-4614, USA

[3] Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade estadual do Norte Fluminense, Darcy Ribeiro, Av. Alberto Lamego, 2000, Parque California, Campos dos Goytacazes, RJ 28013-602, Brazil

[4] Regional Development and Environment Postgraduate Program, Environmental Biogeochemistry Laboratory Wolfgang C. Pfeiffer, Rondônia Federal University, Av. Pres. Dutra, 2967, Olaria, Porto Velho, RO 76801-059, Brazil

Hg found in the atmosphere is in elementary form. Most of the Hg in the water, soils, and sediments is in the inorganic form (Hg^{2+}), while the (mono) methyl mercury (MeHg) is dominant in the animals (Beckers and Rinklebe 2017). MeHg has the capacity to accumulate in organisms (bioaccumulation) and biomagnify along the food chain (Nica et al. 2017), presenting lower concentrations in herbivores and higher in carnivores (Jakimska et al. 2011). Because MeHg is lipid soluble while inorganic Hg is not, their concentration ratios in animal tissues can vary with trophic status, tissue, animal size, and metabolic ability to convert MeHg into inorganic forms (Storelli et al. 1998).

The physiological functions performed by the liver, kidney, muscle, and the carapace in the metabolism of non-essential metals exert influences on Hg content stored in them. The kidney and liver are the main organs involved in the metabolism of trace metals, acting in the detoxification process, and presenting higher concentrations of Hg, mostly in its inorganic form (Chételat et al. 2020). The THg concentrations in the muscle are relatively lower, but as muscle comprises more biomass than the other tissues, it can have the largest contribution to internal mercury burden. Most of the mercury in muscle tissue is MeHg, making it a useful indicator of both MeHg exposure to the organism itself as well as potential consumers (Lescord et al. 2018; Chételat et al. 2020). Whereas scutes, also metabolically inactive, store Hg strongly bound to constituents of the carapace, keratin in particular, rendering low mobility and toxicity (Schneider et al. 2013; Chételat et al. 2020). Thus, this element may be used as a marker of diet type and help to distinguish between trophic positions and feeding preferences of organisms when employed in combination with stable isotope analysis (SIA) (Di Beneditto et al. 2013).

The use of the stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) can provide important data on feeding ecology of different organisms (Haywood et al. 2019). In the case of $\delta^{13}C$, variations in value reflect changes of carbon source at the base of food web, while $\delta^{15}N$ is considered an indicator of trophic levels (Peterson and Fry 1987). However, the stable isotope composition of different tissues of the same organism can differ due to factors such as differential fractionation during assimilation and metabolic processing, macronutrient routing, and the biochemical composition (Vander Zanden et al. 2014). Several studies have reported tissue-specific differences (e.g., internal organs and blood plasma tend to have high rates of isotopic incorporation compared with muscle tissue and blood cells) (Vander Zanden et al. 2015). Since tissues integrate consumer diets at different time scales, examination of multiple tissues can potentially provide information about the temporal dynamics of resource use (Vander Zanden et al. 2015).

In sea turtles, the use of SIA has illuminated facets of sea turtle ecology that are otherwise difficult to study, such as the habitat used during cryptic juvenile life stages and foraging area origin of nesting females (Vander Zanden et al. 2014). The stable isotope values $\delta^{15}N$ and $\delta^{13}C$ in sea turtles show that the green turtle (*Chelonia mydas*) occupies the lowest trophic level, while other species, such as the loggerhead turtle (*Caretta caretta*), occupies the highest trophic level (Haywood et al. 2019). However, with complex life histories, often with multiple ontogenetic changes in habitat, in diet, and migrations of considerable distances between foraging and nesting areas (Haywood et al. 2019), the green sea turtle can present variations in trophic level, and therefore, also in the Hg concentrations found in juvenile, subadult, and adult individuals.

The green sea turtle has a cosmopolitan distribution, from the tropics to the temperate zones, being the species of sea turtle that presents more coastal habitats, including using estuaries of rivers and lakes. In Brazil, the spawning occurs mainly in the oceanic islands, Trindade Island (Espirito Santo state), Rocas Atoll (Rio Grande do Norte state), and Fernando de Noronha island (Pernambuco state) (Almeida et al. 2011). Non-reproductive occurrences are recorded throughout the coast of Brazil and also in the islands (Almeida et al. 2011). Juvenile green turtles are frequently found along the northern coast of Bahia, which is an important feeding ground for this species (Jardim et al. 2016). In Brazil, feeding habitat has been linked to Hg concentrations in juvenile green turtles, and considering that juvenile green turtles are mainly omnivorous and that Hg tends to accumulate through several food web levels, individuals at this life stage can show higher risk of Hg exposure than adults (Bezerra et al. 2012; Bezerra et al. 2015).

Of the seven existing sea turtle species, the green turtle is the only species known to present a significant shift in diet from omnivore juveniles to predominantly herbivore adults (Bolten 2003). This shift, according to Vélez-Rubio et al. (2016) and Burgett et al. (2018), starts between 40 and 45 cm, where individuals under 40 cm are predominantly carnivorous. Moreover, individuals larger than 40 cm present higher consumption of macroalgae. This development pattern results in different accumulation of metals such as Zn, Cu, Cd (Sakai et al. 2000a), and Hg (Komoroske et al. 2011; Bezerra et al. 2013), with juveniles often presenting higher concentrations than adults. The objective of the present study is to evaluate the influence of the size in juvenile green turtles on the concentrations of total mercury (THg), methyl mercury (MeHg), and the isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ in different tissues and organs (kidney, liver, muscle, and carapace).

Materials and methods

All procedures and analyses were carried out within the current norms of the Brazilian environmental legislation, under

the authorization of the System of Authorization and Information in Biodiversity - SISBIO, License No. 21693-9 (2016) from the Ministry of the Environment.

Forty-seven individuals of green turtles were sampled and measured (e.g., curved carapace length (CCL) and curved carapace width (CCW)). Sampled individuals were found dead on the beach between 2009 and 2013 by the team of the Brazilian Marine Turtle Conservation Program (TAMAR) in three localities (Praia do Forte, Sauípe, and Camaçari) in the northern coastal zone of Bahia State, Northeastern Brazil. Analyzed tissues included the kidney, liver, muscle, and scutes. Soft tissue samples weighted approximately 10 g, whereas scute fragments were collected randomly in different areas of the carapace.

For Hg quantifications, tissue subsamples (0.5 g d.w.) were placed, in duplicate, in Teflon tubes containing 10 mL of concentrated nitric acid (HNO_3 65%) for 1 h pre-digestion. Total sample digestion was carried out in a microwave furnace for 30 min at 200 °C. After cooling, 1 mL of hydrogen peroxide (H_2O_2) was added. The final extract was transferred and diluted in volumetric flasks to 100 mL. THg concentrations were quantified by cold vapor atomic absorption spectrophotometer (CV-ASS), in a NIC RA-3 (NIPPON ®) spectrophotometer. The average detection limit (LOD) of the method was 0.08 ± 0.02 ng g^{-1}. The validation of the methodology was obtained by using certified reference material (mussel tissue ERM-CE278K), with recovery of 84%.

For MeHg quantifications, approximately 200 mg of samples was weighed in PTFE tube, and 5.0 mL of 25% KOH methanolic solution was used to extract MeHg in an oven at 70 °C for 6 h with gentle stirring every hour; the samples were then kept in the dark to avoid possible degradation of MeHg. Subsequently, the ethylation process was done with 300 μL of 2 mol L^{-1} acetate buffer (pH 4.5) followed by the addition of 30 μL of sample and 50 μL of tetra ethyl sodium borate (1%) according to Taylor et al. (2011). The final volume was brought to 40 mL with ultra-pure water (milli-Q, Millipore, Cambridge, MA, USA) and analyzed on a gas chromatograph coupled to atomic fluorescence spectrometer (GC-AFS - MERX-TM automated methyl mercury system from Brooks Rand Labs., Seattle, USA). Precision and accuracy of MeHg determinations were ensured using duplicate analyses of samples and certified reference material (Tuna Fish - BCR-463), run with each batch of samples, with a mean recovery of 96%. Detection limits (LOD) and quantification limit (LOQ) for MeHg determinations were 0.003 mg kg^{-1} and 0.009 mg kg^{-1}, respectively.

For stable isotope quantifications, dry subsamples of tissues (~ 1 mg), weighed in tin capsules, were analyzed in a Flash 2000 elemental analyzer coupled to a continuous flow mass spectrometer (Isotope Ratio Mass Spectrometry – IRMS, Delta V Advantage, Thermo Scientific, Germany) to quantify the stable isotopes. All results are expressed as delta value (δ), relative to Pee Dee Belemnite notation for $\delta^{13}C$ in parts per thousand (‰) and atmospheric N_2 for $\delta^{15}N$, according to Peterson and Fry (1987).The analytical replicates showed variations lower than 5%, and the accuracy was determined from a certified standard of protein (B2155) with average recovery of 97 ± 1%.

The Shapiro Wilk test was used to assess the normality of the data. Non-parametric Spearman's correlation was used to evaluate the relationship between THg concentrations in scutes with THg concentrations in the liver, muscle, and kidney. The relationship between THg, MeHg, $\delta^{15}N$, and $\delta^{13}C$ concentration values and turtle size (curved carapace length, CCL) was modeled using generalized additive models (GAM) using the "*mgcv*" package in R 1.2.5033. This method is based on the use of non-parametric smoothing functions that allow flexible description about the effect of size, in the values of $\delta^{15}N$ and $\delta^{13}C$, and THg and MeHg concentrations in the tissues (liver, kidney, muscle, and scutes) of the sea turtle *C. mydas*. Kruskal-Wallis tests were used to evaluate potential differences between THg and MeHg concentrations among tissues. Mann-Whitney's post hoc tests were performed to identify the tissues in which Hg differed significantly. The significance value used for the tests was 95% ($p < 0.05$). Statistical tests and graphing were performed using RStudio software (version 1.1.423 – © 2009–2018 RStudio, Inc.).

Results

THg concentrations

Size (CCL) of sampled individuals ($n = 47$) ranged from 24.9 to 62 cm (average of 36.4 ± 7.2 cm), and classified as juveniles according to Almeida et al. (2011) and Jensen et al. (2016).

THg concentrations were higher in the liver with a median of 650.9 ± 90.3 ng g^{-1}, and the lowest concentration was observed in the muscle, with a median of 113.3 ± 24.8 ng g^{-1} (Table 1) (Fig. 1). A strong positive correlation was found between THg concentrations in scutes with liver (Spearman's $r = 0.78$, $p < 0.05$), kidney (Spearman's $r = 0.88$, $p < 0.05$), and muscle (Spearman's $r = 0.83$, $p < 0.05$) (Fig. 2).

According to the generalized additive model (GAM), concentrations of THg were inversely correlated with turtle size in liver ($p < 0.05$), kidney ($p < 0.05$), muscle ($p < 0.05$), and scutes ($p < 0.05$) (Fig. 3). However, the *R*-squared of this model was very low for each tissue, liver (*R*-squared = 0.21), kidney (*R*-squared = 0.39), muscle (*R*-squared = 0.16), and scutes (*R*-squared = 0.41).

MeHg concentrations

MeHg concentrations were higher in muscle with a median of 63.9 ± 28.4 ng g^{-1}, and the lowest concentration was observed

Table 1 THg, MeHg, $\delta^{13}C$, and $\delta^{15}N$ concentration in liver, kidney, muscle, and scutes of juvenile's green turtle. *SE*, standard error

	THg (ng g^{-1})		MeHg (ng g^{-1})		%MeHg		$\delta^{13}C$		$\delta^{15}N$
Tissue	*n*	Median ± SE (range)	*n*	Median ± SE (range)	*n*	Median ± SE (range)	*n*	Median ± SE (range)	*n*	Median ± SE (range)
Liver	47	650.9 ± 90.3 (19.3–3135)	22	55.0 ± 27.7 (0.77–416.3)	22	8.8 ± 3.5 (0.1–58.7)	22	18.1 ± 0.29 (− 22.2–16.5)	22	9.6 ± 0.3 (7.1–12.5)
Kidney	47	303.4 ± 36.3 (44.8–1276.7)	22	56.7 ± 13.6 (0.77–261.8)	22	16.7 ± 6.7 (0.8–151.5)	22	17.5 ± 0.2 (− 19.9–15.9)	22	9.8 ± 0.3 (7.2–12.9)
Muscle	47	113.3 ± 24.8 (7.0–856.9)	22	63.9 ± 28.4 (2.03–430.3)	22	70.5 ± 7.1 (5.7–115.9)	22	17.5 ± 0.1 (− 19.5–16.3)	22	9.1 ± 0.2 (7.6–12.1)
Scutes	41	365.4 ± 51.3 (13.8–1244.6)	19	16.7 ± 14.2 (0.05–226.8)	14	4.0 ± 1.5 (0.2–17.6)	21	17.1 ± 0.2 (− 18.9–15.8)	21	9.0 ± 0.3 (6.3–11.5)

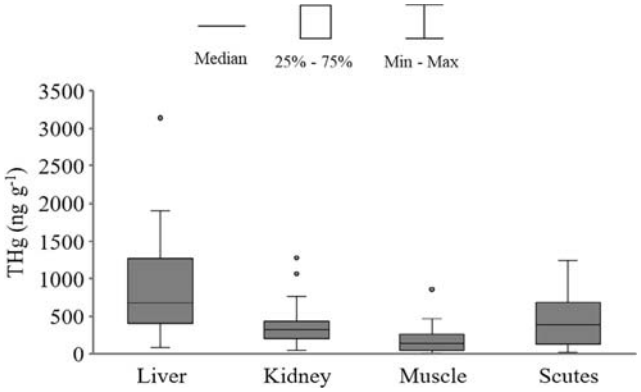

Fig. 1 Concentrations of THg in tissue samples of green turtles (*C. mydas*) from NE Brazil. Gray points represent outliers

in the scutes, with a median of 16.7 ± 14.2 ng g^{-1} (Table 1). There were no significant differences between MeHg concentrations in any tissue (Kruskal-Wallis $H = 5.1$; $p > 0.05$) (Fig. 4).

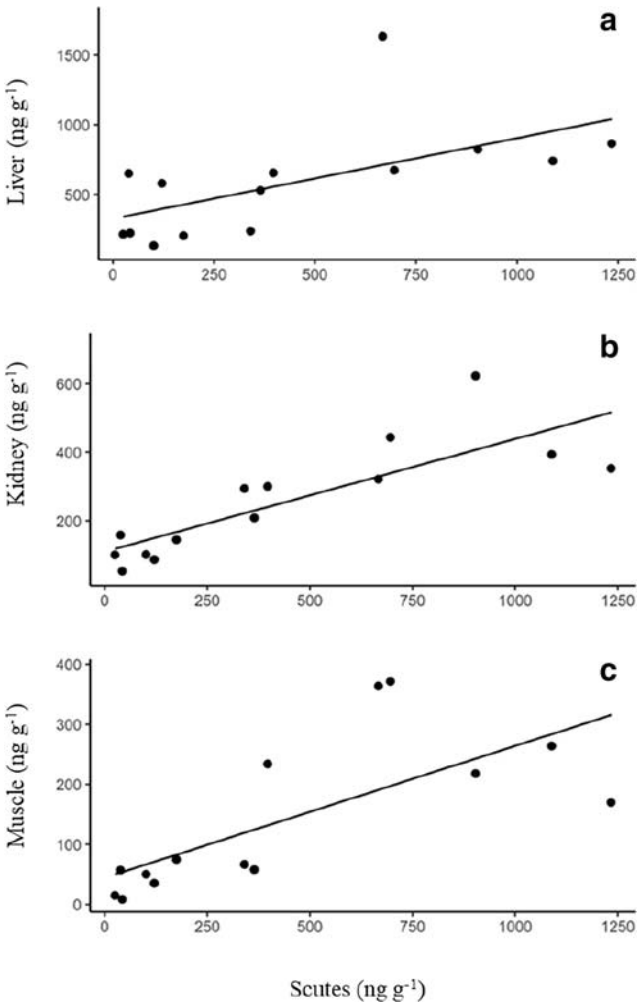

Fig. 2 Relationship between THg concentrations in scutes and liver (**a**), kidney (**b**), and muscle (**c**) from green turtles

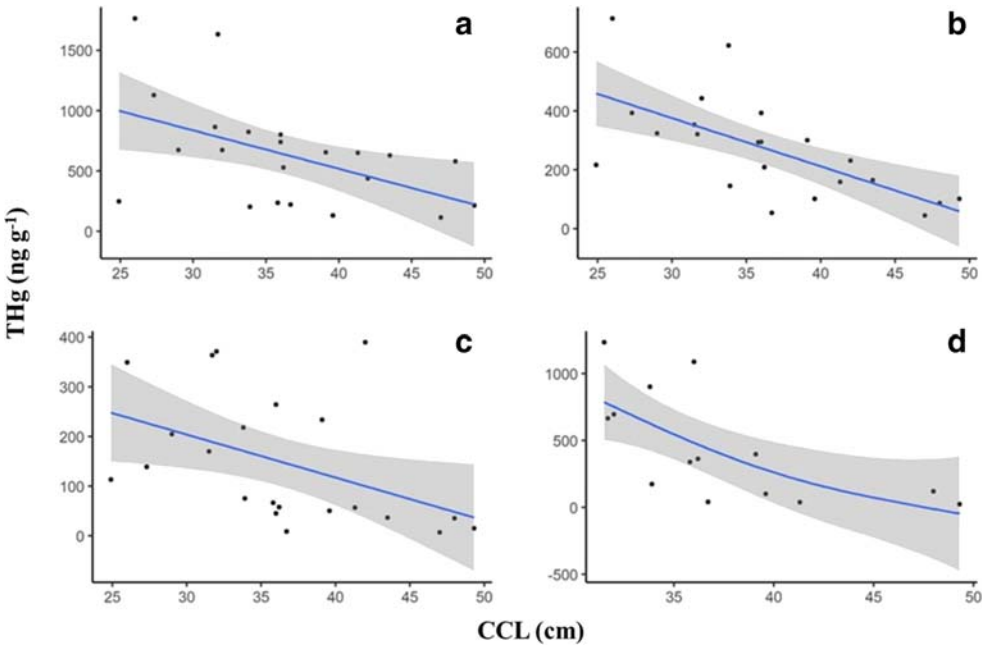

Fig. 3 Graphical representation of a generalized additive model (GAM) of the variation in THg concentrations in green turtle concerning curved carapace length (CCL). Estimated smooth functions (solid lines). (**a**) Liver, (**b**) kidney, (**c**) muscle, and (**d**) scutes

The relative contribution of MeHg to the THg burden in the organisms was also higher in the muscle (70.5%), followed by the kidney (16.7%), liver (8.8%), and scutes (4.0%) (Kruskal-Wallis $H = 37.6$; $p < 0.05$) (Table 1).

Similar to the results observed for THg using GAM, significant inverse relationship of MeHg with size was observed in the liver ($p < 0.05$), kidney ($p < 0.05$), muscle ($p < 0.05$), and scutes ($p < 0.05$) (Fig. 5). In contrast, size was better in explaining variation in MeHg compared with THg for all tissues, liver (R-squared = 0.44), kidney (R-squared = 0.27), muscle (R-squared = 0.54), and scutes (R-squared = 0.62).

Stable isotope values

No difference in stable nitrogen isotope values was observed among green turtle tissues (Kruskal-Wallis $H = 3.9$; $p > 0.05$). On the other hand, the carbon showed difference between the liver, scutes, and muscle (Mann-Whitney's $U = 29$; $p < 0.05$) (Fig. 6). The kidney presented a median of 9.8‰, whereas the liver presented a median of 9.6‰ and the muscle and scutes of 9.1‰ and 9.0‰, respectively (Fig. 6). Interesting to note is that the highest $\delta^{15}N$ values were observed in scutes (11.50‰) and muscle (12.11‰) in the smallest individuals, with 26 cm in size.

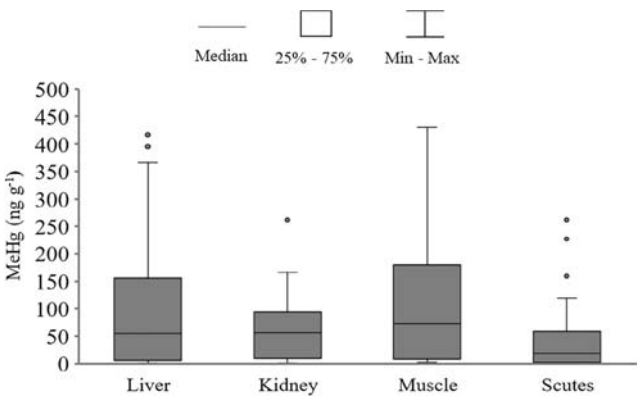

Fig. 4 Concentrations of MeHg in tissue samples of green turtles (*C. mydas*) from NE Brazil. Gray points represent outliers

The results for $\delta^{13}C$ showed difference between tissues (Kruskal-Wallis $H = 3.9$; $p < 0.05$). The highest value in the scutes with a median of − 17.1‰, kidney and liver showed similar results (median − 17.5‰), whereas the liver showed the lowest value, with a median of − 18.1‰ (Fig. 6). The scutes (− 10‰) showed the highest value in one individual with size of 47 cm. The liver showed significative difference with muscle (Mann-Whitney's $U = 40$; $p < 0.05$), and with the scutes (Mann-Whitney's $U = 35$; $p < 0.05$).

The GAM showed a significant relationship between size and stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) for all tissues (Figs. 7 and 8). However, considering R-squared coefficient, size could only explain a very small portion of stable isotope variation in green turtle tissues. $\delta^{15}N$ in liver ($p < 0.05$; R-squared = 0.04), kidney ($p < 0.05$; R-squared = 0.04), muscle ($p < 0.05$; R-squared = 0.02), and scutes ($p < 0.05$; R-squared = 0.07). $\delta^{13}C$ in liver ($p < 0.05$; R-squared = 0.01), kidney ($p < 0.05$; R-squared = 0.02), muscle ($p < 0.05$; R-squared = 0.04), and scutes ($p < 0.05$; R-squared = 0.07).

Discussion

The present study showed a significant negative relationship of THg and MeHg concentrations with size. This kind of

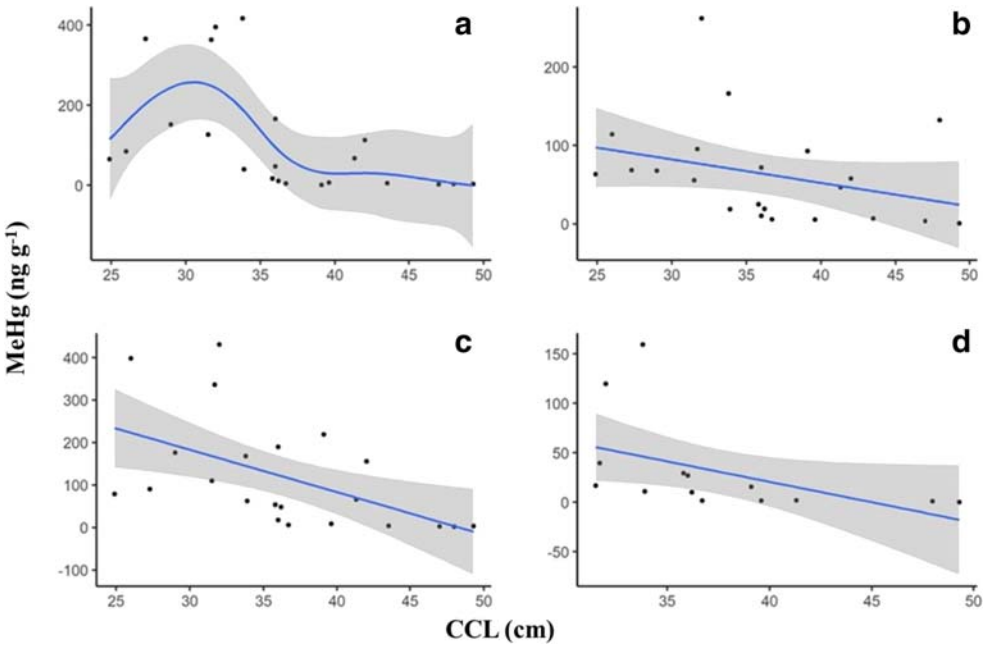

Fig. 5 Graphical representation of a generalized additive model (GAM) of the variation in MeHg concentrations in green turtle concerning curved carapace length (CCL). Estimated smooth functions (solid lines). (**a**) Liver, (**b**) kidney, (**c**) muscle, and (**d**) scutes

relationship has already been reported for others green turtle populations in Baja California (Kampalath et al. 2006), San Diego Bay in the USA (Komoroske et al. 2011), and Ceará coast in Brazil (Bezerra et al. 2012 and 2013). These patterns of Hg distribution have been associated with the known shift in feeding habitat, from omnivorous to predominantly herbivorous occurring in green turtles (Vander Zanden et al. 2013). Vélez-Rubio et al. (2016) detected this shift by analyzing the esophagus and stomach content of 74 *C. mydas* ranging from 45 to 52 cm. These authors reported a gradual shift from a diet mainly composed of gelatinous macrozooplankton in smaller juveniles to predominantly macroalgae in larger juveniles. Similar patterns were also reported by Cardona et al. (2010), Di Beneditto et al. (2017), and Burgett et al. (2018) in populations from Mediterranean, Rio de Janeiro coast, in Brazil, and Bermuda coast, respectively.

Our studied population presented a range of 24.9 to 62 cm of CCL and were all classified as juveniles, according to Almeida et al. (2011). Therefore, the decreasing trend in THg and MeHg concentrations found in the present study suggests the onset of ontogenetic shifts in diet and habitat. The inverse relationship of Hg concentrations with size and the larger variation of Hg concentrations in smaller individuals indicate that these animals might be exposed to more varied sources of Hg in the oceanic habitat compared with the neritic/coastal feeding grounds. Changes in diet of green turtles have been previously reported for other *C. mydas* populations (González Carman et al. 2012; Morais et al. 2014; Vélez-Rubio et al. 2016).

The diet is the main routes of Hg exposure in marine organisms (Gray 2002; Mackay and Fraser 2000). In herbivorous animals, Hg is mostly present as inorganic species, which

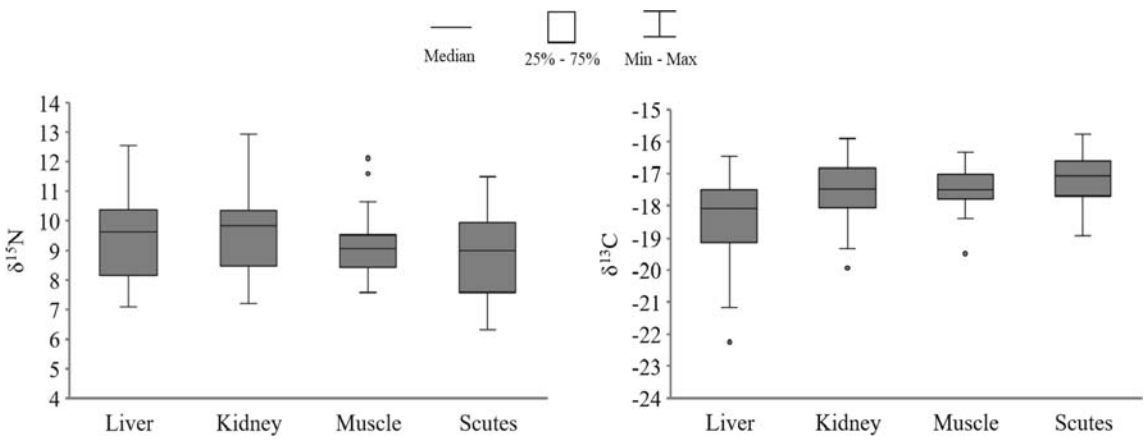

Fig. 6 Boxplot of $\delta^{15}N$ and $\delta^{13}C$ concentrations in tissue samples of green turtle (*C. mydas*) from NE Brazil. Gray points represent outliers

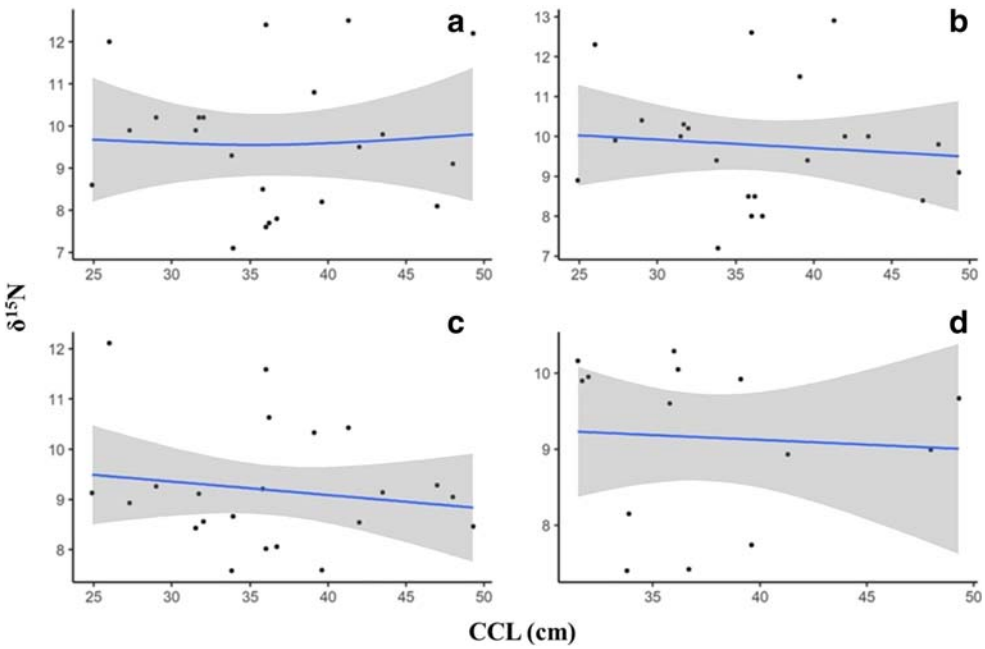

Fig. 7 Relationship between isotope values of $\delta^{15}N$ and CCL in tissues of juvenile green turtles (*C. mydas*). Estimated smooth functions (solid lines)

are the dominant Hg chemical species found in macroalgae, whereas MeHg concentrations are generally very small (May et al. 1987); thus, different diet would also change the relative importance of THg and MeHg in *C. mydas*, as observed in the present study.

In contrast, variation patterns of carbon and nitrogen isotopes were not as clear as expected. Although a significant relationship between the size and the isotopic values of $\delta^{15}N$ and $\delta^{13}C$, the determination coefficient (R^2) recorded with the GAM was very low so that the model cannot explain the observed values.

Distribution of $\delta^{13}C$ in muscle of the studied *C. mydas* population, although with small variability, suggests that the major diet of the animals derives from marine algae (− 16.3 to − 19.5‰), in agreement with previous results found by Bezerra et al. (2015). There was no significant relationship between the size and $\delta^{15}N$, as reported for this species in other geographical areas (Cardona et al. 2009; Vélez-Rubio et al. 2016; Di Beneditto et al. 2017; Monzón-Argüello et al. 2018), but the average $\delta^{15}N$ of the smallest individuals (CCL of 26 cm to 31.8 cm) was the highest (12.11 to 11.59‰, respectively), a result also suggesting omnivory more frequent in

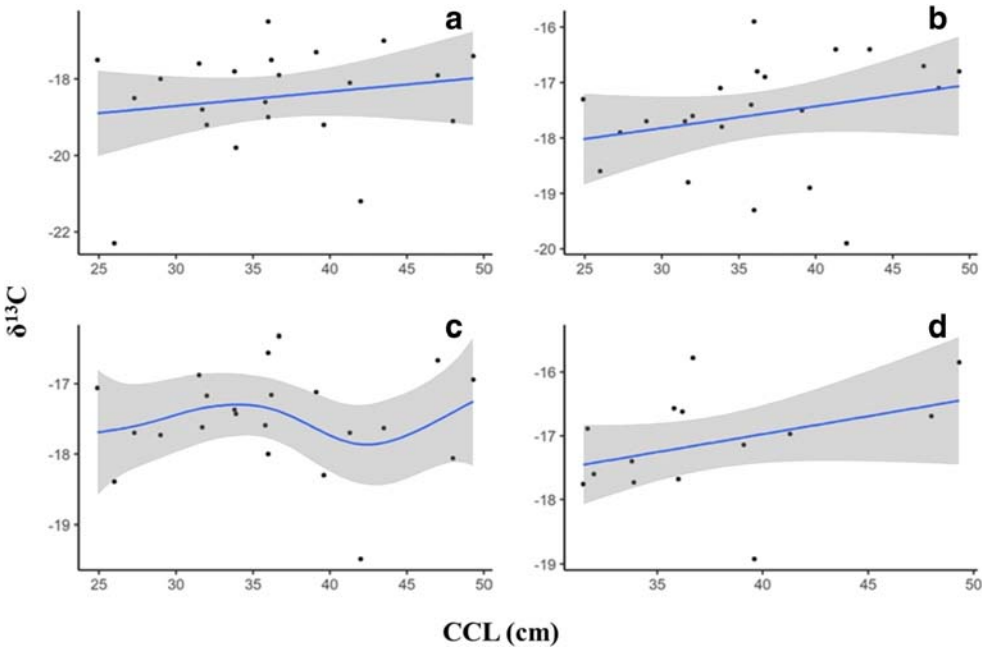

Fig. 8 Relationship between isotope values of $\delta^{13}C$ and CCL in tissues of juvenile green turtles (*C. mydas*). Estimated smooth functions (solid lines)

smaller animals. This result may indicate high level of individual variability in the consumption of animal prey or be an artifact caused by the wide variability in the $\delta^{15}N$ of primary producers (Cardona et al. 2009). However, other factors rather than diet may affect $\delta^{15}N$ and $\delta^{13}C$ in tissues of *C. mydas*. According to Haywood et al. (2019), intra-species variations between ocean basins are due to differences in local and ocean basin nutrient cycling regimes that influence isotope ratios at the base of the food web, which in turn influence the ratios in higher trophic level consumers. It is essential that when comparing isotope values from multiple regions, researchers quantify the local isotope ratios or obtain a proxy for the baseline ratios.

Studies in different geographical areas show this variation. The observed average of $\delta^{15}N$ content in the studied population ($9.0 \pm 0.8‰$) is higher than those found in African and mid-ocean populations by Cardona et al. (2009), $8.6 \pm 1.9‰$ and Burgett et al. (2018), $7.3 \pm 1.6‰$, respectively, but lower than those reported for other green turtle populations in the Atlantic:$10.8 \pm 2.3‰$ (Arthur et al. 2008); $10.2 \pm 0.25‰$ (Bezerra et al. 2015); and $9.9 \pm 1.8‰$ (Di Beneditto et al. 2017). Lemons et al. (2011) reported one of the highest isotopes $\delta^{15}N$ value for *C. mydas* ($17.1 \pm 1.33‰$), from San Diego Bay, USA, and associated it to the large anthropogenic load of nitrogen in this bay.

Omnivorous individuals with a more diversified diet showed a greater variability in isotope values of $\delta^{15}N$ and $\delta^{13}C$, produced by different ecological reasons (Arthur et al. 2008). It is possible that the different incorporation of $\delta^{13}C$ and $\delta^{15}N$ in pelagic juvenile green turtles can be result from a transitional stage during which they complement their diet with a higher proportion of macroalgae, showed a relatively large variability in their isotope contents (Arthur et al. 2008). Vander Zanden et al. (2013) quantified $\delta^{13}C$ and $\delta^{15}N$ in three life stages of *C. mydas*: oceanic juveniles, neritic juveniles, and adults, and showed large variation in isotopes contents in neritic juveniles, because of adaptation to a new environment and feeding strategy. In addition, the existence of large individual variability in the consumption of animal preys and the high variability in $\delta^{15}N$ found in primary producers (Cardona et al. 2009) may also contribute to a weak or non-existent relationship between size and $\delta^{15}N$.

The level of anthropogenic development in addition to affecting nitrogen concentrations as observed by Lemnons et al. (2011) could also influence Hg concentrations found in a region. 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 (Bezerra et al. 2015; Marins et al. 2004). Bezerra et al. (2015) showed that the level of exposure to environmental contamination of *C. mydas* is an important factor to be evaluated in areas impacted by anthropogenic activities, such as the Bahia coast. In this coast, although algae may be the main food resource for juvenile green turtles, Hg concentrations can be elevated due to environmental contamination relative to other sectors of the NE Brazilian coast, thus excluding a difference in concentration of Hg due to distinct trophic levels. In the present study, there was no reason to expect differences in Hg contamination background among specimens as all turtles came from the same site in Bahia coast, and thus, they are likely exposed to similar environmental Hg levels. We hypothesize that differences in Hg concentrations with size are, due to a more generalist diet in smaller juveniles, consuming both plant and animal items, whereas larger juvenile feeds more on macroalgae.

On the other hand, our study showed that the liver was the tissue that presented the highest concentrations of THg, similar to results obtained for other species in different studies (Table 2), confirming the metabolic role of this organ, while scutes proved to be a reliable non-invasive method for monitoring the internal Hg concentrations in green turtles such as Bezerra et al. (2013) and Sakai et al. (2000b).

Concentration of MeHg was found higher in the muscle as expected, a result also observed in other species, such as *C. caretta*, *Chelonia mydas agassizii*, and *Lepidochelys olivacea* (Storelli et al. 1998; Kampalath et al. 2006).

Unlike the muscle, the scutes showed the lowest MeHg concentrations, only 4%, of the THg concentration, showing to be a structure with small capacity to store Hg in organic form. Metabolically, inter-keratinaceous tissues, like scutes, offer a less invasive sampling matrix than internal tissues, and form an inert complex with MeHg during growth, preserving an index of MeHg bioaccumulation over discrete periods. However, the seeming simplicity associated with using keratinaceous tissues to asses MeHg bioaccumulation can result in misleading interpretations, making these tissues inappropriate for some uses (Chételat et al. 2020).

One of the few studies evaluating the concentrations of MeHg in the carapace was performed by Ng et al. (2018), for large individuals of *C. mydas* with sizes from 67 to 84 cm in southern China. The authors found MeHg concentrations in scutes from 10 to 570 ng g^{-1} and with a median of 60 ng g^{-1}, significantly exceeding our median of 16.7 ng g^{-1} and concentrations ranging from 0.05 to 226.8 ng g^{-1}. Rodriguez et al. (2019) reported much lower MeHg concentrations in the carapace of 15 adult individuals of *C. caretta* (0.2 to 55.2 ng g^{-1}, with a median of 6.64 ng g^{-1}) compared with *C. mydas*, notwithstanding the more carnivorous diet of this species. There is a lack of studies evaluating MeHg concentrations in scutes of sea turtles, which is why we were unable to make a comparison with studies using individuals in the same size ranges, and of the same species. However, it is important to mention that although the studies shown are on individuals of different size and of different species, there are two factors controlling these variations in the MeHg concentrations. The ecological factors such as trophic position,

Table 2 Hg concentrations in tissues of green turtles *C.mydas*. [(a)]Adult. [(j)]Juveniles. *Hg concentration in wet weight. **Median values. *NA*, not analyze

Species	*n*	CCL (cm)	Hg concentration mean ± SD (ng g^{-1})				Source
		Mean ± SD	Liver	Kidney	Muscle	Scutes	
C. mydas	50	51	287 ± 156	132 ± 77	19 ± 30	NA	*Sakai et al. 2000a
C. mydas	2	95 ± 2.8	188.5	68.9	4.5	2.41	*Sakai et al. 2000b
C. mydas (a)	4	-	125.7 ± 84.8	NA	52.52 ± 35.9	NA	Lam et al. 2006
C. mydas (j)	2	-	780.6 ± 192.7	341.7 ± 37.64	425.6 ± 215.1	NA	
C. mydas	16	33–82	190	60	30	NA	*Van de Merwe et al. 2010
C, mydas	10	35.6 ± 3.1	1340 ± 610	360 ± 140	NA	NA	De Macedo et al. 2015
C. mydas	26	36.4	982.4	429.5	184.3	354.1	Bezerra et al. 2015
C. mydas	47	36.4 ± 7.2	650.9	303.4	113.3	365.4	**Present study

ontogenetic changes in diet, migration and type of diet, and physiological processes, include elimination of MeHg, internal circulation of MeHg, excretion, maternal transfer, among others (Chételat et al. 2020).

Stable isotopes and Hg concentrations were not able to show differences in the trophic position of the individuals considered to be omnivorous and herbivorous in our study. Di Benedito et al. (2013) mentions that the ability of $\delta^{13}C$ and $\delta^{15}N$ tracers to reflect diet depends on the characteristics of the environment, the characteristics of each species, and the complementation with other types of proxies. Cardona et al. (2010) points out that although the use of stable isotopes as diet markers is a powerful technique, interpreting the results is not always simple, because the method is reliable only when there are large differences between the isotopic value of the sources considered and when the isotopic value varies consistently between the habitats and trophic levels. In the case of the green turtle, the availability of food can change the omnivorous (juvenile) and herbivorous (adult) behavior (Nagaoka et al. 2012). Studies using SIA show that herbivory behavior in adults can be optional and does not happen strictly (Hatase et al. 2006; Reich et al. 2007; González Carman et al. 2012; Velez-Rubio et al., 2016; Di Beneditto et al. 2017). Thus, as a direct result of this factor, the relationship between Hg and the stable isotopes of $\delta^{15}N$ and $\delta^{13}C$ with juvenile and adult food preferences in *C. mydas* could be altered, whereby the ontogenetic change as such would not be able to be determined by variables previously exposed, being important the integration of this type of study with stomach contents.

Conclusions

The progressive and gradual change in the diet experienced by green turtles during growth can be considered as one of the main factors responsible for the variations in the concentrations of THg and MeHg. Our results showed a decrease of THg and MeHg concentrations, during the growth of the green turtle. This variation is possibly related to a response of a more diverse diet in small individuals, and a diet composed of a higher proportion of algae in larger individuals. The relationship between Hg concentrations in the scutes with the other tissues proved that the scutes can be a reliable non-invasive method for monitoring the internal Hg concentrations in green turtles. However, the low concentrations of MeHg in the scutes showed that this type of tissue may be inappropriate for the evaluation of MeHg in the green turtle. The isotope values of $\delta^{15}N$ and $\delta^{13}C$ did not show a clear relationship with the size, suggesting that the green turtles used in our work occupied similar trophic levels, and/or foraging habitats. It is important that individuals in the future studies be sampled in different life stages (juvenile, subadult, and adult), and analyze the influence of this factor in the Hg, MeHg, and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) concentrations.

Acknowledgments The author would like to thank the team of the Coastal Biogeochemistry Laboratory for the support in processing and analysis of samples, and to the team of the Marine Turtle Conservation Project (TAMAR Project) for the support in the field work.

Funding information This study was funded by Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq (Proc. No. 405.244/2018-5).

References

Aguirre AA, Lutz PL (2004) Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator? EcoHealth 1(3):275–283. https://doi.org/10.1007/s10393-004-0097-3

Almeida AP, Moreira LMP, Bruno SC, Thomé 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. https://doi.org/10.3354/esr00357

Arthur KE, Boyle MC, Limpus CJ (2008) Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. https://doi.org/10.3354/meps07440

Barbieri E (2009) Concentration of heavy metals in tissues of green turtles (*Chelonia mydas*) sampled in the Cananéia estuary, Brazil. Braz J Oceanogr 57(3):243–248. https://doi.org/10.1590/S1679-87592009000300007

Beckers F, Rinklebe J (2017) Cycling of mercury in the environment: sources, fate, and human health implications: a review. Crit Rev Environ Sci Technol 47:693–794. https://doi.org/10.1080/10643389.2017.1326277

Bezerra MF, Lacerda LD, Costa BGB, Lima EHSM (2012) Mercury in the sea turtle *Chelonia mydas* (Linnaeus, 1958) from Céara coast, NE Brazil. An Acad Bras Cienc 84:123–128

Bezerra MF, Lacerda LD, Lima EHSM, Melo MTD (2013) Monitoring mercury in green sea turtles using keratinized carapace fragments (scutes). Mar Pollut Bull 77:424–427. https://doi.org/10.1016/j.marpolbul.2013.09.020

Bezerra MF, Lacerda LD, Rezende CE, Franco ML, Almeida MG, Macêdo GR, Pires TT, Rostán G, Lopez GG (2015) Food preferences and Hg distribution in *Chelonia mydas* assessed by stable isotopes. Environ Pollut 206:236–246. https://doi.org/10.1016/j.envpol.2015.07.011

Bolten A (2003) Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. Biol. Sea turtles 2:243–257. https://doi.org/10.1201/9781420040807.ch9

Burgett CM, Burkholder DA, Coates KA, Fourqurean VL, Kenworthy WJ, Manuel SA, Outerbridge ME, Fourqurean JW (2018) Ontogenetic diet shifts of green sea turtles (*Chelonia mydas*) in a mid-ocean developmental habitat. Mar Biol 165:1–12. https://doi.org/10.1007/s00227-018-3290-6

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. https://doi.org/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 BioEcol 393:83–89. https://doi.org/10.1016/j.jembe.2010.07.004

Chételat J, Ackerman JT, Eagles-Smith CA, Hebert CE (2020) Methylmercury exposure in wildlife: a review of the ecological and physiological processes affecting contaminant concentrations and their interpretation. Sci Total Environ 135117. https://doi.org/10.1016/j.scitotenv.2019.135117

De Macedo GR, Tarantino TB, Barbosa IS, Pires TT, Rostan G, Goldberg DW, Pinto LFB, Korn MGA, Franke CR (2015) Trace elements distribution in hawksbill turtle (*Eretmochelys imbricata*) and green turtle (*Chelonia mydas*) tissues on the northern coast of Bahia. Brazil Mar Pollut Bull 94:284–289. https://doi.org/10.1016/j.marpolbul.2015.02.033

Di Beneditto APMD, Bittar VT, Rezende CED, Camargo PB, Kehrig HA (2013) Mercury and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) as tracers during the ontogeny of *Trichiurus lepturus*. Neotropical Ichthyology 11(1):211–216. https://doi.org/10.1590/S1679-62252013000100024

Di Beneditto APM, Siciliano S, Monteiro LR (2017) Herbivory level and niche breadth of juvenile green turtles (*Chelonia mydas*) in a tropical coastal area: insights from stable isotopes. Mar Biol 164:1–12. https://doi.org/10.1007/s00227-016-3044-2

Driscoll CT, Mason RP, Chan HM, Jacob DJ, Pirrone N (2013) Mercury as a global pollutant: sources, pathways, and effects. Environ Sci Technol 47:4967–4983. https://doi.org/10.1021/es305071v

González 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. Bio. Ecol. 429:64–72. https://doi.org/10.1016/j.jembe.2012.06.007

Gray JS (2002) Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 45:46–52. https://doi.org/10.1016/S0025-326X(01)00323-X

Hamann M, Godfrey MH, Seminoff JA, Arthur K, Barata PCR, Bjorndal KA, Casale P (2010) Global research priorities for sea turtles: informing management and conservation in the 21st century. Endanger Species Res 11(3):245–269. https://doi.org/10.3354/esr00279

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(1):52–64. https://doi.org/10.1007/s00442-006-0431-2

Haywood J, Fuller W, Godley B, Shutler J, Widdicombe S, Broderick A (2019) Global review and inventory: how stable isotopes are helping us understand ecology and inform conservation of marine turtles. Mar Ecol Prog Ser 613:217–245. https://doi.org/10.3354/meps12889

Jakimska A, Konieczka P, Skóra K, Namieśnik J (2011) Bioaccumulation of metals in tissues of marine animals, part II: metal concentrations in animal tissues. Polish J Environ Stud 20:1127–1146

Jardim A, López-Mendilaharsu M, Barros F (2016) Demography and foraging ecology of *Chelonia mydas* on tropical shallow reefs in Bahia, Brazil. J Mar Biol Assoc U K 96(6):1295–1304. https://doi.org/10.1017/S0025315415001629

Jensen MP, Bell I, Limpus CJ, Hamann M, Ambar S, Whap T, FitzSimmons NN (2016) Spatial and temporal genetic variation among size classes of green turtles (*Chelonia mydas*) provides information on oceanic dispersal and population dynamics. Mar Ecol Prog Ser 543:241–256. https://doi.org/10.3354/meps11521

Kampalath R, Gardner SC, Méndez-Rodríguez L, Jay JA (2006) Total and methylmercury in three species of sea turtles of Baja California Sur. Mar Pollut Bull 52(12):1816–1823. https://doi.org/10.1016/j.marpolbul.2006.09.019

Komoroske LM, Lewison RL, Seminoff JA, Deheyn DD, Dutton PH (2011) Pollutants and the health of green sea turtle's resident to an urbanized estuary in San Diego, CA. Chemosphere 84:544–552. https://doi.org/10.1016/j.chemosphere.2011.04.023

Lam JCW, Tanabe S, Chan SKF, Lam MHW, Martin M, Lam PKS (2006) Levels of trace elements in green turtle eggs collected from Hong Kong: evidence of risks due to selenium and nickel. Environ Pollut 144:790–801. https://doi.org/10.1016/j.envpol.2006.02.016

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 Bio Ecol 405:25–32. https://doi.org/10.1016/j.jembe.2011.05.012

Lescord GL, Johnston TA, Branfireun BA, Gunn JM (2018) Percentage of methylmercury in the muscle tissue of freshwater fish varies with body size and age and among species. Environ Toxicol Chem 37(10):2682–2691. https://doi.org/10.1002/etc.4233

Mackay D, Fraser A (2000) Bioaccumulation of persistent organic chemicals: mechanisms and models 110, 375–391. https://doi.org/10.1016/S0269-7491(00)00162-7

Marins RV, de Paula Filho FJ, Maia SRR, de Lacerda LD, Marques WS (2004) Distribuição de mercúrio total como indicador de poluição urbana e industrial na costa brasileira. Química Nova 27:763–770. https://doi.org/10.1590/S0100-40422004000500016

May K, Stoeppler M, Reisinger K (1987) Studies in the ratio total mercury/methylmercury in the aquatic food chain. Toxicol Environ Chem 13:153–159. https://doi.org/10.1080/02772248709357178

Miguel C, de Deus Santos MR (2019). Ecotoxicological studies of metal pollution in sea turtles of Latin America. In Pollution of water bodies

in Latin America (pp. 129–156). Springer, Cham. https://doi.org/10.1007/978-3-030-27296-8_9

Monzón-Argüello C, Cardona L, Calabuig P, Camacho M, Crespo-Picazo JL, García-Párraga D, Mayans S, Luzardo OP, Orós J, Varo-Cruz N (2018) Supplemental feeding and other anthropogenic threats to green turtles (*Chelonia mydas*) in the Canary Islands. Sci Total Environ 621:1000–1011. https://doi.org/10.1016/j.scitotenv.2017.10.126

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 Conservation and Biology 13(2):260–266. https://doi.org/10.2744/CCB-1058.1

Nagaoka SM, Martins AS, Dos Santos RG, Tognella MMP, de Oliveira Filho EC, Seminoff JA (2012) Diet of juvenile green turtles (*Chelonia mydas*) associating with artisanal fishing traps in a subtropical estuary in Brazil. Mar Biol 159(3):573–581. https://doi.org/10.1007/s00227-011-1836-y

Ng CKY, Lam JCW, Zhang XH, Gu HX, Li TH, Ye M, Bin Xia ZR, Zhang FY, Duan JX, Wang WX, Lam IKS, Balazs GH, Lam PKS, Murphy MB (2018) Levels of trace elements, methylmercury and polybrominateddiphenyl ethers in foraging green turtles in the South China region and their conservation implications. Environ Pollut 234:735–742. https://doi.org/10.1016/j.envpol.2017.11.100

Nica A, Popescu A, Ibănescu DC (2017) A current problem: mercury pollution. Current Trends in Natural Sciences 6(12):165–169

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18(1):293–320. https://doi.org/10.1146/annurev.ecolsys.18.1.293

Rodriguez CAB, Bezerra MF, Rezende CE, Bastos WR, Lacerda LD (2019) Mercury and methylmercury in carapace of the marine turtle *Caretta caretta*, in northeastern Brazil and its potential for environmental monitoring. An Acad Bras Cienc 91(2):e20180672. **Epub July 10, 2019**. https://doi.org/10.1590/0001-3765201920180672

Sakai H, Saeki K, Ichihashi H, Kamezaki N, Tanabe S, Tatsukawa R (2000a) Growth-related changes in heavy metal accumulation in green turtle (*Chelonia mydas*) from Yaeyama Islands, Okinawa, Japan. Arch Environ Contam Toxicol 39:378–385. https://doi.org/10.1007/s002440010118

Sakai H, Saeki K, Ichihashi H, Suganuma H, Tanabe S, Tatsukawa R (2000b) Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. Mar Pollut Bull 40:701–709. https://doi.org/10.1016/S0025-326X(00)00008-4

Schneider L, Maher W, Green A, Vogt RC (2013) Mercury contamination in reptiles: an emerging problem with consequences for wildlife and human health. Mercury: Sources, Applications and Health Impacts. Nova Science Publishers, Inc., Hauppauge, New York, USA, 173–232

Storelli MM, Ceci E, Marcotrigiano GO (1998) Distribution of heavy metal residues in some tissues of *Caretta caretta* ({ILinnaeus}) specimen beached along the Adriatic Sea (Italy). Bull Environ Contam Toxicol 60:546–552. https://doi.org/10.1007/s001289900660

Taylor VF, Carter A, Davies C, Jackson BP (2011) Trace-level automated mercury speciation analysis. Anal Methods 3(5):1143–1148. https://doi.org/10.1039/C0AY00528B

Unep (2013) Global mercury assessment 2013: sources, emissions, releases, and environmental transport. Unep 42. https://doi.org/DTI/1636/GE

Van de Merwe JP, Hodge M, Olszowy HA, Whittier JM, Lee SY (2010) Using blood samples to estimate persistent organic pollutants and metals in green sea turtles (*Chelonia mydas*). Mar Pollut Bull 60:579–588. https://doi.org/10.1016/j.marpolbul.2009.11.006

Vander Zanden HB, Bjorndal KA, Bolten AB (2013) Temporal consistency and individual specialization in resource use by green turtles in successive life stages. Oecologia 173:767–777. https://doi.org/10.1007/s00442-013-2655-2

Vander Zanden HB, Tucker AD, Bolten AB, Reich KJ, Bjorndal KA (2014) Stable isotopic comparison between loggerhead sea turtle tissues. Rapid Commun Mass Spectrom 28:2059e2064. https://doi.org/10.1002/rcm.6995

Vander Zanden MJ, Clayton MK, Moody EK, Solomon CT, Weidel BC (2015) Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLoS One 10(1). https://doi.org/10.1371/journal.pone.0116182

Vélez-Rubio GM, Cardona L, Mendilaharsu LCML, Souza GM, Carranza A, González-Paredes D, Tomás J (2016) Ontogenetic dietary changes of green turtles (*Chelonia mydas*) in the temperate southwestern Atlantic. Mar Biol 163:1–16. https://doi.org/10.1007/s00227-016-2827-9