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Biological and ecological traits rather than geography control mercury (Hg) in scutes of marine turtles from the Southwest Atlantic

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ABSTRACT

The use of sentinel species in monitoring programs for toxic metals such as mercury (Hg) is essential to understand these pollutants' impact on the environment. For this purpose, it is essential to use organisms that have a lifespan compatible with the residence time of Hg in the oceans, and preferably with a wide geographical distribution, such as sea turtles. Here, we assess the regional variability of Hg concentrations using carapace scutes of four sea turtle species along the foraging and spawning area in the northeast coastline of Brazil. Mercury concentrations in samples showed no relationship with the environmental Hg levels (obtained from literature). Rather, Hg concentrations varied according to species-specific biological, and ecological traits. Characteristics such as the ontogenetic shift in the diet of *Chelonia mydas*, capital breeding in females, depth of foraging in oceanic waters, and selectivity of food items, such as in *Eretmochelys imbricata*, significantly influenced Hg concentrations.

1. Introduction

Mercury (Hg) is a widely distributed element on the planet from natural sources, but anthropogenic activities have altered Hg fluxes and concentrations in diverse environmental compartments, producing major contamination events (Ali et al., 2019) which require mitigating measures and monitoring. Biomonitoring is a well-established procedure to assess the health of organisms and ecosystems by tracking changes in Hg concentrations and biota exposure over time (Evers et al., 2018). The Minamata Convention suggests Hg measurements be conducted on key taxa (e.g., fish, sea turtles, birds, marine mammals, etc.) to track changes in environmental loads and provide important information about the impacts of Hg pollution and the potential risks to human health and wildlife (Gustin et al., 2016; Evers et al., 2018; UNEP, 2019).

Chelonians have important characteristics that support their use in biomonitoring. They have a heterogeneous diet, wide distribution (Schneider et al., 2013), and a long-life cycle allowing them to accumulate contaminants through long periods, thus favoring their use for biomonitoring purposes (Dos Santos et al., 2021). Because of their slow metabolism, recovery from contamination effects can be slow and potentially making them more sensitive to xenobiotics (Schaumburg et al., 2012). Despite this, the number of studies on Hg contamination in sea turtles is limited compared to other marine taxa, which may be related to their status as endangered species, and thus protected worldwide, making their capture illegal in most countries (Rodriguez et al., 2022). As an alternative, the use of non-invasive methods (e.g., blood and carapace) has been proposed to monitor Hg in these chelonians, and consequently monitor Hg distribution in oceanic and coastal environments (Villa et al., 2019; Rodriguez et al., 2022).

The carapace is an inert structure composed of keratin, where some of the main components are amino acids such as cystine (Achrai and Wagner, 2013). This amino acid is formed by thiol groups, and its side chain is the main binding site for the organic and inorganic forms of Hg, in which this element will be immobilized and eventually expelled when the carapace scutes are shed. Thus, this process may serve as a detoxification mechanism in reptiles such as sea turtles (Schneider et al., 2013; Warner and Jalilehvand, 2016; Escobedo-Mondragón et al., 2023). As a non-invasive method, carapace scutes have proven to be a viable tool to

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estimate Hg concentrations in chelonians, since Hg contents measured in this tissue correlate with those found in internal organs (Sakai et al., 2000; Bezerra et al., 2013; Rodriguez et al., 2020). Furthermore, carapace scutes can retain records of ontogenetic changes in diet and habitat, including the accumulation of Hg throughout the animal's life. Despite this, the number of studies using scute samples is still low, compared to other tissues (Rodriguez et al., 2022) which hampers definitive conclusions on their use in biomonitoring programs. Studies such as Day et al. (2005), Bezerra et al. (2015), Rodriguez et al. (2018), Villa et al. (2019), and Barraza et al. (2019) have proposed the use of chelonians for biomonitoring Hg availability in near-shore habitats, where terrestrial influences and anthropogenic impacts are substantial. Furthermore, the seasonal fidelity to foraging sites and the regional variability in environmental Hg levels have been proposed to explain the observed intraspecific variation of Hg concentrations in sea turtles (Day et al., 2005; Bezerra et al., 2015).

In a recent review on Hg concentrations in carapace scutes of sea turtles, Rodriguez et al. (2022), showed that even for the two most studied species *Chelonia mydas* and *Caretta caretta*, there are still major knowledge gaps, including poor representation of some age groups depending on species, low confidence on feeding and distribution of most species during oceanic life stages, and limited understanding of ethology in females on reproductive ages. As an example, foraging depth has been shown to strongly influence Hg concentrations in oceanic fishes, including tuna (Lacerda et al., 2017), but it is poorly understood for sea turtle species. Resolving these knowledge gaps would advance the use of sea turtles as a Hg biomonitor, especially in key areas for developing these organisms, including many coastal areas that are often of great economic importance.

To help fill some of these gaps, the main objective of this study is to assess Hg concentrations, using carapace scutes, of four species of sea turtles (*Caretta caretta*, *Chelonia mydas*, *Lepidochelys olivacea*, and *Eretmochelys imbricata*) along a latitudinal gradient (from approximately 2°S to 13°S). This area, c.a. 1600 km of coastline, is one of the most important foraging sites for sea turtles in the South Atlantic Ocean. Variability in Hg concentrations is discussed in relation to the biology and ecology of each species, as well as the Hg environmental levels to evaluate the feasibility of using carapace scutes as a tool for the environmental monitoring of Hg in the tropical Southwest Atlantic.

2. Materials and methods

All procedures and analyses were carried out within the current norms of Brazilian environmental legislation, under the authorization of the System of Authorization and Information in Biodiversity – SISBIO, License No. 66837 and 66,088 (2022) from the Ministry of the Environment.

Scute samples from the carapace of four sea turtle species were collected in four regions of the Brazilian coast: Maranhão State (2016) (*E. imbricata*, dead hatchlings (n = 8)), Ceará State (2013–2019) (*C. mydas*, juveniles found dead on the beach (n = 19); *C. caretta*, subadult and adults (n = 8), found dead on the beach), Pernambuco State (2019) (*E. imbricata*, nesting females (n = 41); *L. olivacea*, adults found dead on the beach (n = 4); *C. mydas*, juveniles, and subadult found dead on the beach (n = 28)), and Bahia State (2019 2020) (*C. mydas*, juveniles found dead on the beach (n = 40); *C. caretta*, nesting females (n = 78); *L. olivacea*, nesting females (n = 63)) (Fig. 1).

Individuals were classified as hatchlings, juvenile, sub-adult, and

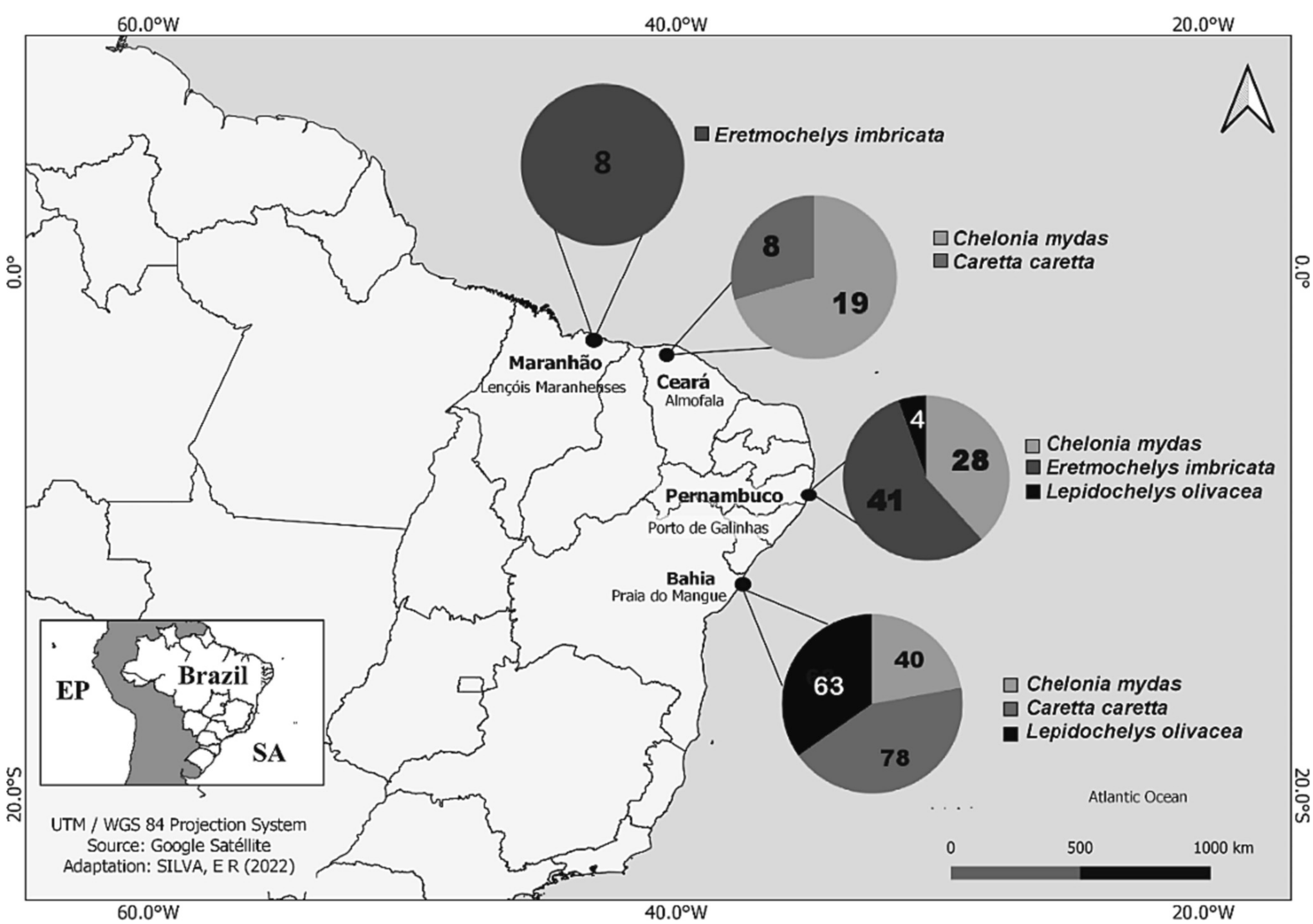

Fig. 1. Sampling areas and distribution of *C. mydas*, *C. caretta*, *E. imbricata*, and L. *olivacea* along the northeast coast of Brazil (Maranhão, Ceará, Pernambuco, and Bahia). SA: South Atlantic. EP: East Pacific. The number on the wheels is the sample number for each species in each region.

adult according to Dodd (1988) for *C. caretta*; Jensen et al. (2016) for *C. mydas*; Reichart (1993) for L. *olivacea*; Márquez (1994) and Witzell (1983) for *E. imbricata*. Samples (1.0 to 1.5 g) were collected by carefully scraping the superficial keratinized layer of the carapace with a dissection knife, avoiding the skin and dermal tissues. Regarding the carapace region of sampling, the samples were collected from the lateral scutes in *C. mydas*, *C. caretta*, and *E. imbricata*. In L. *olivacea*, samples were only collected on the marginal scutes, as they were thicker than the vertebral and lateral scutes. Mapping of Hg concentrations in different scute positions in the carapace of these four species showed no significant difference between lateral scutes, but differences were found between vertebral scutes and lateral, and marginal scutes (Barrios-Rodríguez et al., 2023), which were then avoided in this sampling. Curved carapace length (CCL) was recorded for each individual.

For total Hg quantification, 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 one hour of 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, 35 %) was added. The final extract was transferred and diluted in volumetric flasks to 100 mL with Milli-Q® water (Bezerra et al., 2012). All materials were washed with acid, and duplicate procedural blanks were included in each digestion bath. Total Hg was quantified by cold vapor generation atomic absorption spectrophotometry (CV-ASS) in a NIC RA-3 (NIPPON®) spectrophotometer. The average linearity coefficient of the calibration curves (R^2) obtained was 0.9995 ± 0.0002. The method's mean limit of detection (LOD) was 0.04 ± 0.13 ng g^{-1}, calculated as three times the standard deviation of the reagent blanks divided by the slope of the calibration curve. The methodology was validated using certified reference material (fish muscle ERM-BB422), with a mean recovery of 96.4 ± 9.4 % ($n = 26$).

Statistical analyses were performed using R 4.1.2 (R Development Core Team 2021). Data normality was tested using the Shapiro-Wilk test. To improve the comparability of Hg concentrations among sea turtles of different sizes and sites, we normalized Hg levels (Hg_{norm}) by dividing the reported Hg levels by the average animal size (CCL) for the respective sample pool (Scudder-Eikenberry et al., 2015). Normalized Hg levels remove the effect of size on variation in Hg among sites, improving the interpretation of observed differences. Parametric ANOVAs and Kruskal Wallis non-parametric test were used to test if Hg concentrations differed among sites. We tested for differences in size (e.g., CCL) and Hg concentrations among species and sampling sites. When differences were found, a post hoc pairwise Wilcoxon rank test with Holm corrections was performed. All tests were conducted assuming a significance level of 95 % (values were considered significant at $p < 0.05$). All the outliers were included because they did not interfere with the statistical test.

3. Results

Sampled individuals were of different age groups, including hatchlings, juveniles, sub-adults, and adults. *C. mydas* included juvenile and sub-adult individuals (CCL: 27 to 76.9 cm), *C. caretta* included sub-adult and adult individuals (CCL: 79 to 107 cm), *E. imbricata* included hatchling and adult individuals (CCL: 5.1 to 100 cm), and L. *olivacea* included adults' individuals only (CCL: 63 to 79.2 cm). A description of sampled individuals by species, life stage, and region is presented in Table 1. A complete description of the data including, locality, size, and Hg concentrations is listed in the supplementary material (Table S1 to S5).

3.1. Size (CCL) comparison between regions

Animal size differed among sampling sites for *C. mydas* and *E. imbricata* (post hoc pairwise Wilcoxon rank test, $p < 0.05$). *C. mydas* showed differences in CCL between Bahia (36.9 ± 6.8 cm) and

Table 1
Mean and standard deviation (SD), minimum and maximum size (CCL in cm), and Hg_{norm} concentrations (ng g^{-1}/cm) in scutes of *C. mydas* (*Cm*), *C. caretta* (*Cc*), *E. imbricata* (*Ei*) and *L. olivacea* (*Lo*) from Maranhão, Ceará, Pernambuco and Bahia in NE Brazil.

		Maranhão		Ceará		Pernambuco		Bahia	
Sp.	Life stage	Size	Hg	Size	Hg	Size	Hg	Size	Hg
Cm	Juvenile	–	–	41 ± 9.5 27–59 *n* = 19	6.5 ± 6.0 0.1–19.9 Median = 7.3 *n* = 19	50 ± 8.5 33.2–77 *n* = 24	2.44 ± 4.3 0.1–16.5 Median = 0.3 n = 24	37 ± 7 27–62 *n* = 40	9.7 ± 8.2 0.3–29 Median = 8.8 n = 40
	Subadult	–	–	–	–	71 ± 4.5 66–77 n = 4	2.7 ± 2.7 0.13–6.3 Median = 2.3 n = 4	–	–
	Adult	–	–	–	–	–	–	–	–
Cc	Juvenile	–	–	–	–	–	–	–	–
	Subadult	–	–	79 n = 1	3.9 n = 1	–	–	–	–
	Adult	–	–	95.2 ± 10 80–107 *n* = 7	2.2 ± 2 0.3–4.4 Median = 2.5 n = 7	–	–	99 ± 5 87–107 *n* = 78	1.3 ± 1.3 0.03–5.3 Median = 0.9 n = 78
Ei	Juvenile/hatchling	5.5 ± 0.4 5.1–6.1 n = 8	0.8 ± 0.1 0.6–0.9 Median = 0.8 n = 8	–	–	–	–	–	–
	Subadult	–	–	–	–	–	–	–	–
	Adult	–	–	–	–	91 ± 5 82–100 *n* = 41	0.2 ± 0.2 0.04–1.2 Median = 0.2 n = 41	–	–
Lo	Juvenile	–	–	–	–	–	–	–	–
	Subadult	–	–	–	–	–	–	–	–
	Adult	–	–	–	–	70.5 ± 4.5 70–74.5 n = 4	9.0 ± 6.7 0.9–14.6 Median = 10.3 n = 4	72 ± 3.3 63–79.2 *n* = 63	10.3 ± 8.0 0.9–39.9 Median = 7.7 n = 63

Pernambuco (52.8 ± 11 cm), and Ceará (40.8 ± 9.5 cm) and Pernambuco (52.8 ± 11 cm) however, no differences were found between Bahia and Ceará (post hoc pairwise Wilcoxon rank test, $p > 0.05$). *E. imbricata* showed significant differences between Maranhão (5.5 ± 0.4) and Pernambuco (90.7 ± 5) (Kruskal-Wallis test, Chi-square = 19.7, $p < 0.05$). The *C. caretta* and L. *olivacea* species did not differ in CCL between sites.

3.2. Mercury (Hg_{norm}) distribution in regions by species

The CCL values of *C. mydas* showed significant differences between the sampling areas; due to this fact, their Hg concentrations were normalized and compared to avoid biases due to size effects. The comparison between Hg concentrations normalized by size showed statistically significant higher Hg concentrations in *C. mydas* individuals from Bahia (median = 8.8 ng g^{-1}/cm) compared to *C. mydas* individuals from Pernambuco (median = 0.5 ng g^{-1}/cm). Significantly higher Hg concentrations were found in *C. mydas* individuals from Ceará (median = 7.3 ng g^{-1}/cm) compared to *C. mydas* individuals from Pernambuco (median = 0.5 ng g^{-1}/cm) (post hoc pairwise Wilcoxon rank test, $p < 0.05$). However, there was no significant difference between Hg concentrations in *C. mydas* from Ceará and Bahia (post hoc pairwise Wilcoxon rank test, $p = 0.142$) (Fig. 2). Similarly, there were no significant differences between Hg concentrations in *C. caretta* from Ceará (median = 2.8 ng g^{-1}/cm) and Bahia (median = 0.9 ng g^{-1}/cm) (Kruskal-Wallis test, $p = 0.12$), as well as between *L. olivacea* sampled in Bahia (median = 7.7 ng g^{-1}/cm) and Pernambuco (median = 10.3 ng g^{-1}/cm) (Kruskal-Wallis test, $p = 0.93$) (Fig. 2), while Hg concentrations in hatchlings of *E. imbricata* from Maranhão (median = 0.8 ng g^{-1}/cm) were significantly higher (ANOVA, F = 24.12, $p < 0.05$) than *E. imbricata* adults from Pernambuco (median = 0.2 ng g^{-1}/cm) (Fig. 2).

3.3. Comparison of Hg_{norm} concentration in scutes between sea turtle species

The Hg_{norm} concentration in the four species of sea turtles showed a general trend between species as follows: *L. olivacea* > *C. mydas* > *C. caretta* > *E. imbricata*. The highest concentration was found in L. *olivacea* (median: 7.7 ng g^{-1}/cm) and was significantly higher than in the other three species, *C. mydas* (median: 4.7 ng g^{-1}/cm), *C. caretta* (median: 0.9 ng g^{-1}/cm), and *E. imbricata* (median: 0.2 ng g^{-1}/cm) (post hoc pairwise Wilcoxon rank test, $p < 0.05$). The second highest concentrations were found in *C. mydas*, and also significantly higher than those found in *C. caretta* (post hoc pairwise Wilcoxon rank test, $p < 0.05$), and *E. imbricata* (post hoc pairwise Wilcoxon rank test, $p < 0.05$). *E. imbricata* was the species with the lowest Hg concentrations (Fig. 3).

4. Discussion

Sea turtles present important changes in habitat and diet as they grow (Bolten et al., 2003). Species such as *C. caretta*, *C. mydas*, and *E. imbricata* present an early development in the oceanic zone followed by later development in the neritic zone (Bolten et al., 2003; Figgener et al., 2019). On the other hand, *L. olivacea* tends to have a more oceanic distribution throughout its life, coming to coastal areas only for nesting (Figgener et al., 2019). The shift from the oceanic environment to the neritic zone implies the use of different food resources and greater interaction with estuarine and coastal environments that are more vulnerable to anthropogenic contamination by Hg compared to open ocean waters (Gworek et al., 2016). Therefore, Hg concentrations in each species are strongly controlled by their biological and ecological traits and by the environmental characteristics of their foraging areas.

4.1. *C. mydas*

According to Marins et al. (2004), Hg regional geoaccumulation indexes suggest higher Hg contamination in Pernambuco and Bahia relative to the nearly pristine coastal environment in Ceará, but in the present study, the Hg concentrations in *C. mydas*, that occurred in these three areas, were not related to regional differences in Hg concentrations (Fig. 2). It is important to highlight that 62.1 % of the individuals sampled in the three regions were in the coastal recruitment size range, 25–44 cm (Bjorndal, 1980) or 30–40 cm (Lenz et al., 2017), and 37.9 %

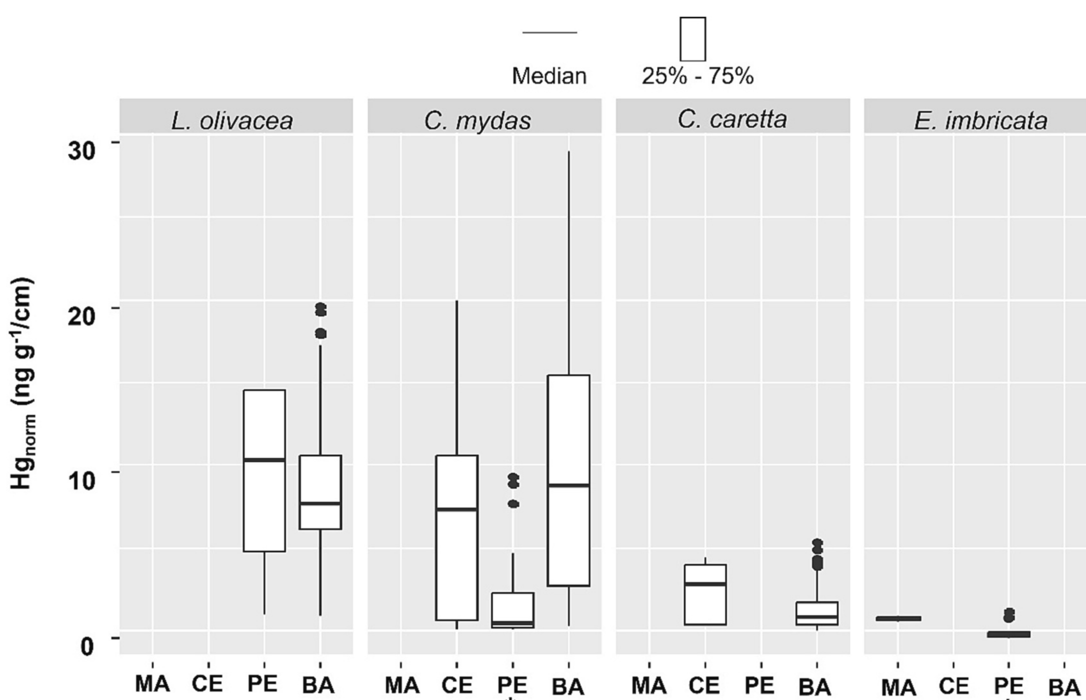

Fig. 2. Regional comparison of Hg_{norm} concentrations in scutes between the species L. *olivacea*, *C. mydas*, *C. caretta*, and *E. imbricata* in Maranhão (MA), Ceará (CE), Pernambuco (PE) and Bahia (BA). *Statistically significant difference from other sites ($p < 0.05$). The boxplot shows the outliers because it did not interfere with the statistical analysis.

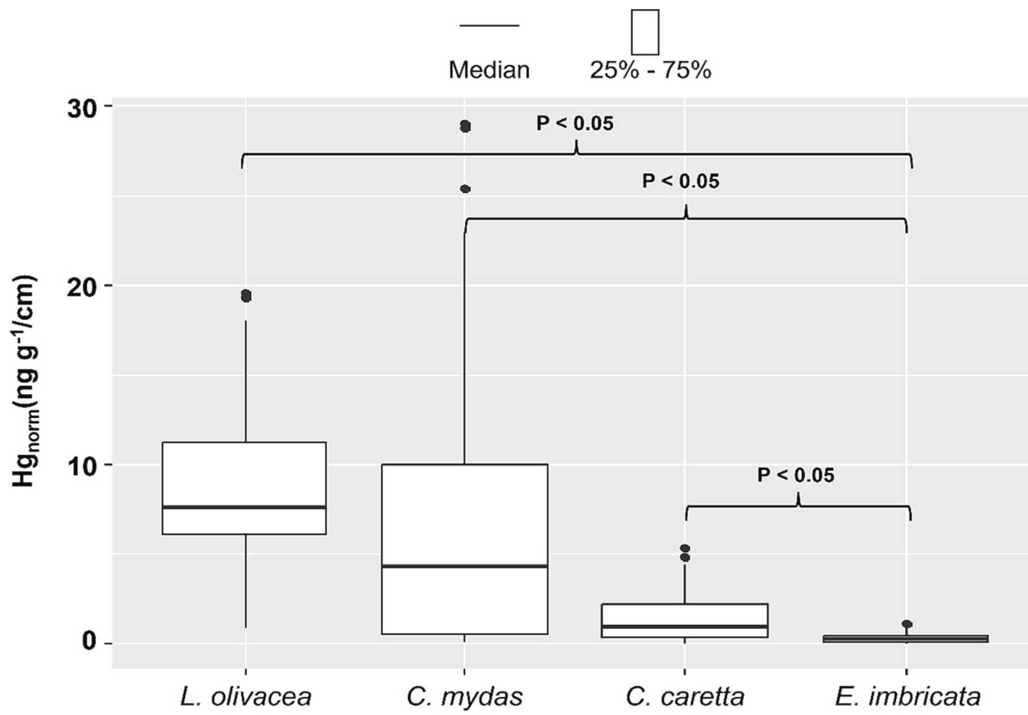

Fig. 3. Comparison of Hg_{norm} concentrations in scutes between species, *L. olivacea*, *C. mydas*, *C. caretta*, *E. imbricata*. The boxplot shows the outliers because it did not interfere with the statistical analysis.

had a neritic distribution. This information is especially important since juvenile green turtles with <50 cm of CCL found in neritic environments of the southern coast of Brazil, and in Uruguay and Argentina are omnivores in transition between an omnivorous and herbivorous diet (Bugoni et al., 2003; Carman et al., 2012; Vélez-Rubio et al., 2016; Lenz et al., 2017).

The ontogenetic change in diet occurring in *C. mydas* is characterized by a shift from an omnivorous behavior (oceanic stage) to an herbivorous diet, mostly of macroalgae, as adults (neritic stage) (Bjorndal, 1985, 1996). This change has been associated with an inverse relationship between size and Hg concentrations in *C. mydas* (Bezerra et al., 2012, 2015; Rodriguez et al., 2020). It is precisely this process that would explain the higher Hg observed in *C. mydas* from Ceará and Bahia, compared to Pernambuco, which presented larger individuals. For Ceará and Bahia, the percentage of individuals in the recruitment process was 73.6 % and 87.5 %, respectively, while for Pernambuco, considerably lower with only 25 %. Although in this size range, occasional ingestion of cnidarians and ctenophores still occurs (Bjorndal, 1996), in the South Atlantic, green turtles larger than 45 cm CCL exhibit a gradual increase in the occurrence of macroalgae in their diet with increasing size (Vélez-Rubio et al., 2016; Lenz et al., 2017), explaining the significantly lower Hg concentration in the *C. mydas* individuals from Pernambuco.

The interpretation of this result will also depend on the evaluation of environmental factors, as well as the type of tissue used. For example, in Brazil, Bezerra et al. (2015) showed that higher Hg concentrations in the liver of *C. mydas* sampled in Bahia could be associated with the higher level of contamination by Hg in macroalgae and invertebrates found in Bahia, which are components of *C. mydas* diet. However, these authors did not find these differences in carapace scute, muscle, and kidney. An explanation is the function of these organs, where the liver is responsible for storing and redistributing recently ingested Hg, while tissues such as muscle and scutes represent a sink for accumulated Hg with a longer half-life (Schneider et al., 2013; Day et al., 2005). Also, although sea turtles lose scutes during growth, the exact scute growth and shedding rates are unknown, but the persistence of commensal barnacles (*Chelonibia testudinaria*) on the carapace suggests that the scutes may represent growth over several years (Day et al., 2005). The individuals studied by Bezerra et al. (2015) similarly to those in the present work from Ceará and Bahia, were mostly juveniles recently recruited to coastal environments, so measured Hg levels in the carapace could have reflected diet from the oceanic stage rather than that associated with the coastal region.

4.2. *C. caretta*

Mercury concentrations in the carapace of *C. caretta* sampled in Bahia and Ceará were reported for sub-adult and adult individuals (Table 1), in addition, they were very variable for both populations and did not show significant differences between regions (Fig. 2). This variability and the presence of individuals with low concentrations of Hg may be related to their feeding areas and the organisms consumed by *C. caretta*, which include a variety of food items, such as mollusks, crustaceans, and gelatinous macroplankton (Bjorndal, 1985; Dodd, 1988). Large variability of Hg concentrations in the carapace for *C. caretta* was also reported by Day et al. (2005), Casini et al. (2018), and Rodriguez et al. (2019) and was related to diet and environmental Hg concentrations in their foraging areas.

In addition to evaluating diet and foraging areas as factors influencing Hg concentrations, it is important to consider the unique characteristics of *C. caretta* populations in Brazil. Genetic studies based on mitochondrial DNA show that the Brazilian population of *C. caretta* is different from other known populations in the world and is divided into two subpopulations: the northeast, with priority spawning areas on the beaches of Bahia and Sergipe, and the southeast coast, with priority spawning areas on the beaches of Rio de Janeiro and Espírito Santo. Thus, for the subpopulation of Bahia, it is possible to find a migratory corridor along the entire Northeast coast of Brazil and resting and feeding areas on the North Coast, especially in Ceará (Marcovaldi et al., 2009, 2010). Based on this information and considering that the samples used for this work were the carapace, we can assume that the spawning females sampled in Bahia reflected the Hg concentrations of their feeding areas in Ceará and not their spawning areas in Bahia. This hypothesis could be reinforced by the strategy known as capital breeding, where female sea turtles consume little or no food during the reproductive period, allowing the organisms to spatially and temporally separate suitable areas for foraging and reproduction (Bonnet et al., 1998). This strategy has been reported in different species of sea turtles (*C. caretta*: Perrault and Stacy, 2018; *C. mydas*: Page-Karjian et al., 2020; *E. imbricata*: Goldberg et al., 2013; *D. coriacea*: Perrault et al., 2016) and could help to understand the origin of the Hg concentrations in the *C. caretta* individuals analyzed in this work.

4.3. L. olivacea

The only two studies reporting Hg concentrations in L. *olivacea* were carried out by Kampalath et al. (2006) and Páez-Osuna et al. (2011) in Mexico. They analyzed the kidney, liver, muscle, blood, and eggs (yolk, albumen, and eggshell) but not the carapace. There are more studies reporting concentrations of other metals and metalloids (e.g., Pb, Cd, Cu, Zn, Mn, Se, Ni, As, Fe, etc.) but not Hg (Gordon et al., 1998; Gardner et al., 2006; Páez-Osuna et al., 2010; Cortés-Gómez et al., 2014). Therefore, the present results are the first to report Hg concentrations in the carapace of L. *olivacea*.

Different from the other species of sea turtles studied, *L. olivacea* inhabits the oceanic zone in the adult stage (Bolten et al., 2003; Figgener et al., 2019). Despite this, there are reports of adult individuals of L. *olivacea* capable of using a wide variety of foraging areas, including pelagic and benthic environments (Plotkin, 2010; Da Silva et al., 2011). Satellite tracking studies showed behavioral plasticity among populations of L. *olivacea* (Rees et al., 2012), and adults can be found in oceanic environments, diving at depths of up to 400 m (Swimmer et al., 2006) or using coastal or continental shelf areas (McMahon et al., 2007; Whiting et al., 2007; Colman et al., 2014). Consequently, the Hg concentrations presented by this species would probably reflect the Hg concentrations of their diet and foraging areas in oceanic and shelf environments. *L. olivacea* shows flexibility in the use of pelagic and benthic environments, which, in connection with its omnivorous and opportunistic diet behavior (Kampalath et al., 2006), result in a wide variability of food items consumed and therefore in Hg concentrations.

Several food items have been reported in the L. *olivacea* diet in the Atlantic, including salps, fish, mollusks, crustaceans, algae, ascidians, sipunculids, fish eggs, etc. (Bjorndal, 1996; Colman et al., 2014; Di Beneditto et al., 2015). In the eastern Pacific region, studies of stomach contents also showed tunicates (Mortimer, 1982; Carpena-Catoira et al., 2022). Thus, both the foraging areas and the consumption of organisms from different trophic levels, especially with varying Hg concentrations, may be responsible for the wide variation of Hg concentrations found in this study for L. *olivacea*.

Notwithstanding, the occurrence of L. *olivacea* in deep oceanic waters is a characteristic that needs to be studied, as it would allow us to understand its influence on Hg concentrations, especially if we consider that this species can forage at great depths (Polovina et al., 2003). The depth at which many predators forage, such as tuna and other large oceanic fishes (e.g., *Thunnus obesus*, *T. albacares*, *Katsuwonus pelamis*, *Xiphias gladius*, *Lampris guttatus*, *Coryphaena hippurus*, *Taractichthys steindachneri*, *Tetrapturus audax*, and *Lepidocybium flavobrunneum*) directly influences their Hg concentrations. This is because deep oceanic waters with lower oxygen content present higher levels of bioavailable Hg and most prey items are also omnivorous or carnivorous with relatively higher Hg concentrations than species dwelling closer to the sea surface (Mason et al., 1998; Gill and Fitzgerald, 1988; Choy et al., 2009; Lacerda et al., 2017; Romero-Romero et al., 2022). Thus, vertical differences in foraging behaviors over the lifetime of a pelagic predator are likely to be directly responsible for total Hg loads.

4.4. E. imbricata

The Hg concentrations found in scutes of adults of *E. imbricata* averaged 19.9 ± 16.9 ng g^{-1} (Table S1), like those reported by Escobedo-Mondragón et al. (2021, 2023) on Holbox Island and Quinta Roo, Mexico (Table 2). Both studies showed Hg concentrations in the carapace of adult females, and the results were also significantly lower when compared to other species of sea turtles (e.g., *C. mydas*, Bezerra et al., 2015; Rodriguez et al., 2020; *C. caretta,* Rodriguez et al., 2019; *L. olivacea*, *C. mydas*, and *C. caretta* present work) (Table 3), which may be related to several factors such as diet, geographic distribution, age and physiological characteristics of each species as well as the morphological and chemical characteristics of the carapace of this species (Gardner et al., 2006; Kampalath et al., 2006; Escobedo-Mondragón et al., 2021).

There are a very limited number of studies determining Hg concentrations in *E. imbricata* individuals and even less reporting on the carapace. The main studies published so far are listed in Table 2, and it is possible to observe that scutes show the lowest concentration of Hg among all tissues. This result is quite interesting considering the concentrations of Hg in *E. imbricata* reported by Anan et al. (2001) and De Macêdo et al. (2015) in the liver and kidney which were much higher than the concentrations in the carapace and comparable to those reported for *C. caretta* and *C. mydas*, also in liver and kidney, which perhaps would make the use of this tissue in *E. imbricata* unfeasible for environmental monitoring.

E. imbricata, like *C. caretta* and L. *olivacea*, is an omnivorous species (Bjorndal, 1996) with its foraging areas associated with coral reefs, and its diet is composed of food items that may vary depending on the region. For example, Von Brandis et al. (2014) report the prevalence of *Stelleta* sp., *Spheciospongia* sp. (both demosponges), and *Zoanthus sansibaricus* (Anthozoa) in *E. imbricata* from the Indian Ocean. Proietti et al. (2012), reported the prevalence of sessile benthic organisms, mainly zoanthids (*Zoanthus sociatus* and *Palythoa caribaeorum*) and occasionally sponges in the diet of immature individuals of *E. imbricata* in the Brazilian islands of the São Pedro and São Paulo Archipelago, Equatorial Atlantic and the Abrolhos Marine Park and the Arvoredo Marine Reserve, in the SE coast. Rincon-Diaz et al. (2011) showed a preference for *Ricordea florida*, a species of coral and the alga *Lobophora variegata*, and a low preference for the sponge *Chondrilla nucula* in juveniles in the Culebra Archipelago in Puerto Rico. Other studies reported the presence of benthic species, including tunicates, bryozoans, mollusks, corals, and, to a lesser extent, algae in the diet of *E. imbricata* (Carr and Stancyk, 1975; Meylan, 1988; Bjorndal, 1996; Rincon-Diaz et al., 2011; Martínez-Estévez et al., 2022). In this work, the Hg concentrations of food items were not quantified, preventing a discussion of an eventual relationship between the Hg concentrations found in *E. imbricata* and its diet. Measurements of Hg concentrations in the food web of their foraging areas would be ideal for establishing their diet and trophic level. Thus, it would be possible to have an explanation for the low concentrations of Hg reported in the carapace of *E. imbricata*.

There is a clear difference in the age range of individuals sampled in Maranhão and Pernambuco (Fig. 2), which did not allow for a comparison between areas. Despite this, the difference between the Hg concentrations of these two groups of individuals is undeniable and interesting, especially because hatchlings showed higher Hg concentrations than adults. The samples collected in Maranhão were only composed of hatchlings, and it was not possible to collect samples from the females that spawned in that area, making any type of comparison and verification of the existence of maternal transfer impossible. However, essential (e.g., Zn, Cu, etc.) and non-essential (e.g., Pb, Hg, etc.) metals have been detected in the blood and eggs of *E. imbricata* (Ehsanpour et al., 2014), *Dermochelys coriacea* (Guirlet et al., 2008; Perrault et al., 2011), *C. mydas* (Sinaei and Bolouki, 2017), and other chelonian species (Nagle et al., 2001; Hopkins et al., 2013), suggesting maternal transfer to embryos. In this way, it is possible that the Hg found in the hatchlings is reflecting the Hg concentrations of the females that spawned in this region, and that consequently transferred the accumulated Hg to the eggs, as a way of reducing the Hg body load (Nagle et al.,

Table 2
Mercury (Hg) concentrations (ng g^{-1} dry weight) in tissues of hawksbill turtles (*E. imbricata*) and size (CCL) in cm.

Source	n	Country	CCL	Liver	Kidney	Muscle	Scutes	Blood
Gordon et al. (1998)[a,b]	2	Australia	NI	144–192	100–141	–	–	–
Anan et al. (2001)	22	Japan	46.4	870	1300	40	–	–
Ehsanpour et al. (2014)[a]	12	Iran	63.5	–	–	–	–	180
Camacho et al. (2014)[a]	13	Cape Verde	54.5	–	–	–	–	260
De Macêdo et al. (2015)	16	Brazil	33.6	1360	570	–	–	–
Escobedo-Mondragón et al. (2021)[a]	19	Mexico	Adult	–	–	–	14	33
Escobedo-Mondragón et al. (2023)[a]	19	Mexico	86.9	–	–	–	2	24
Present study	41	Brazil	90.7	–	–	–	20	–
Present study	8	Brazil	5.5	–	–	–	57	–

NI: not informed.
[a] Data originally presented as wet weight, values were converted for comparison purposes to dry weight using a moisture percentage of 84.6 % (blood) and 29.1 % (scutes) (Perrault et al., 2017).
[b] Study reporting minimum and maximum. Concentration values were rounded.

Table 3
- Mercury (Hg) concentrations (ng g^{-1} dry weight) in tissues of *C. caretta* (*Cc*), *C. mydas* (*Cm*), and size (CCL) in cm.

Reference	sp	N	Country	CCL	Liver	Kidney	Muscle	Scutes
Day et al. (2005)[a]	*Cc*	6	USA (South Carolina)	77.7	2376	629	775	1325
Bezerra et al. (2013)	*Cm*	10–17	Brazil (Ceará)	39.1	474	371	163	318
Bezerra et al. (2015)	*Cm*	16	Brazil (Ceará)	32.4	476	387	173	354
Bezerra et al. (2015)	*Cm*	25	Brazil (Bahia)	36.4	982	430	184	404
Rodriguez et al., 2020	*Cm*	41–47	Brazil (Bahia)	36.4	651	303	113	365
Present study	*Ei*	41	Brazil (Pernambuco)	90.7	–	–	–	20
Present study	*Ei*	8	Brazil (Maranhão)	5.5	–	–	–	57

[a] Data originally presented as wet weight, values were converted for comparison purposes to dry weight using a moisture percentage of 75 % (liver), 66 % (kidney), 80 % (muscle), and 29.1 % (scutes) (García-Fernández et al., 2009; Perrault et al., 2017). Concentration values were rounded.

2001; Sinaei and Bolouki, 2017). Furthermore, it is important to assess the exchange of water and gases between the egg and its environment during embryogenesis (Ackerman, 1997; Wallace et al., 2006), as both may be responsible for part of the metal load found in sea turtle hatchlings (Muñoz and Vermeiren, 2020).

4.5. Comparison of Hg_{norm} concentrations in scutes among species

In this work, comparisons of Hg_{norm} concentrations in scutes among species included adult females (*L. olivacea*, *C. caretta*, and *E. imbricata*), and juvenile/hatchling individuals (*C. mydas* and some *E. imbricata* individuals) Table 1. Therefore, it was not possible to carry out any type of comparison based on the sex of the individuals.

Mercury (Hg_{norm}) concentrations in scutes among species (*L. olivacea* > *C. mydas* > *C. caretta* > *E. imbricata*) showed significant differences and matched with the feeding behaviors of L. *olivacea*, *C. mydas*, and *C. caretta*, but not in the case of *E. imbricata* (Fig. 3). Few studies have compared Hg concentrations among species using different tissues, such as Kampalath et al. (2006) with liver, kidney, and muscle; Gordon et al., 1998 with liver and kidney; and Escobedo-Mondragón et al. (2023) with blood and scutes. However, Escobedo-Mondragón et al. (2023) and Gordon et al. (1998) did not present Hg concentration results for L. *olivacea*, and Gordon et al. (1998) had a small sample size for three of the four species: *L. olivacea* ($n = 1$), *C. caretta* ($n = 8$), and *E. imbricata* ($n = 3$).

In this study, it was found that L. *olivacea* had the highest Hg concentrations among the four species, consistent with Kampalath et al. (2006), who attributed this to the omnivorous diet of L. *olivacea* compared to the carnivorous *C. caretta* and the herbivorous *C. mydas*. However, in the present study, higher Hg concentrations were observed in *C. mydas* than in *C. caretta*, contrary to Kampalath et al. (2006). This discrepancy may result from the differences in the size and feeding behavior of *C. mydas* in both studies. Kampalath et al. (2006) reported an average size of 57 ± 11.7 cm for *C. mydas*, which likely indicates a longer residence in the neritic region and an herbivorous diet (Bjorndal, 1980). In contrast, in this study, *C. mydas* samples had an average size of 42.8 ± 11.3 cm, suggesting a predominance of pelagic individuals with omnivorous diets (Bjorndal, 1980; Lenz et al., 2017). This could explain why the *C. mydas* individuals in this study had higher Hg concentrations than *C. caretta* individuals.

E. imbricata had the lowest Hg concentrations among the four species, despite having an omnivorous diet similar to L. *olivacea* (Bjorndal, 1980). This is probably a result of foraging at pristine reef ecosystems (Marcovaldi et al., 2012) and on low trophic level prey presenting low Hg content. However, further research is needed to understand the relationship between diet composition and Hg accumulation in this species.

5. Conclusion

Sea turtle species change their diet and habitat throughout their life, consequently influencing their Hg accumulation. In the case of *C. mydas*, the shift from the oceanic to neritic environments is associated with a change in diet which explains in part the results obtained in the present study. The ontogenetic shift in diet during growth can produce an inverse relationship between Hg concentrations with animal size which was also observed in our results. This process proved to be one of the main controllers of Hg concentrations in *C. mydas*, increasing intraspecific variability and contributing to the absence of a direct relationship to local Hg background levels, as reported in the literature for, Ceará, Pernambuco, and Bahia.

Of the four species studied in this work, three are known to have an omnivorous diet; however, they show selectivity for certain types of food items that can influence Hg concentrations. In the case of L. *olivacea*, unlike the other species, there is no permanent interaction with coastal areas; there is an oscillation in its distribution between oceanic and shelf areas, so Hg concentrations observed in this species may have different origins and not be directly related to the areas where the samples were taken for this work. But the higher concentrations found in this species relative to the others, are most probably a reflection of its more oceanic distribution and deeper depth of foraging.

The use of the carapace as a non-invasive method for monitoring Hg in sea turtles has proven to be a viable tool as an indicator of Hg concentrations and changes in diet in *C. mydas*. In the case of *C. caretta*, the number of works has gradually increased; however, they are directed at adult individuals, making it necessary to increase the number of individuals in other age groups. In the case of *E. imbricata* and L. *olivacea*, the number of studies is very small. Thus, in the future, it is essential to produce more studies focused on less studied species and addressing characteristics such as foraging in deep waters and capital breeding. Furthermore, it would be important to evaluate the morphological and chemical characteristics of the carapace, especially in *E. imbricata*, as it could help to understand the low concentrations of Hg found in this species.

Based on our results, the use of sea turtle carapace as a tool for monitoring Hg in the oceans seems to be much more complex and perhaps only possible in foraging areas. A broad approach to biology, ecology, and environmental characteristics would allow for greater reliability in establishing the use of these chelonians as sentinels in the oceans.

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CRediT authorship contribution statement

César Augusto Barrios-Rodriguez: Conceptualization, Data curation, Funding acquisition, Methodology, Writing – original draft. **Moises Fernandes Bezerra:** Writing – original draft, Writing – review & editing. **Nathali Ristau:** Investigation, Writing – review & editing. **Débora Melo Mendonça:** Investigation, Writing – review & editing. **Thaís Torres Pires:** Investigation, Writing – review & editing. **Luana Rocha de Souza Paulino:** Investigation, Resources, Writing – review & editing. **Luiz Drude de Lacerda:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest regarding the present paper.

Data availability

Data will be made available on request.

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