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Mercury and methylmercury in carapace of the marine turtle *Caretta caretta*, in northeastern Brazil and its potential for environmental monitoring

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Manuscript received on July 2, 2018; accepted for publication on October 30, 2018

How to cite: RODRIGUEZ CAB, BEZERRA MF, REZENDE CE, BASTOS WR AND 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: e20180672. DOI 10.1590/0001-3765201920180672.

Abstract: The present work tests the use of carapace fragments of the marine turtle *Caretta caretta* as a tool for environmental biomonitoring of mercury (Hg) and to evaluate the influence of biological and ecological factors in Hg concentrations. Samples of carapace fragments were obtained during the nesting season of 2012 and 2016 and were analyzed for their total-Hg and methyl-Hg concentrations and the isotopic composition of carbon and nitrogen (δⁱ⁵N and δ¹³C). Seventy-six females were sampled, with an average size of 87.1 to 107 cm of curved carapace length (CCL). The results showed a wide variation in total Hg concentrations (3.3 – 1,672 ng g⁻¹) and low concentrations of methyl-Hg, not showing any pattern of accumulation among the individuals. The isotopic composition of δ¹⁵N and δ¹³C suggests that the individuals sampled belong to a high trophic level but did not present any relationship with the Hg concentrations. It suggests that, at least with the existing results, and unlike other turtle species, carapace fragments of *C. caretta* cannot yet be used in environmental monitoring.

Key words: *Caretta caretta*, mercury, carapace, methylmercury, stable isotopes.

INTRODUCTION

The long residence time of mercury (Hg) in the oceans (approximately 30 years) and the high toxicity of its organic form (i.e. methyl-Hg) make particularly important to study Hg distribution in marine species (UNEP 2013).
Methyl-Hg is highly poisonous and the toxicity varies according to the intake path, exposure amount, and individual susceptibility (Hong et al. 2012). The main target for methyl-Hg toxicity is the central nervous system, where it alters the biochemical and ultrastructural machinery of both astrocytes and neurons (Shanker et al. 2003). However, Hg accumulation and concentration patterns may vary among species depending on trophic level, habitat and the life span (Kidd et al. 1995). Mercury can undergo biomagnification through the food chain, resulting in higher concentrations and exposure in species at higher trophic levels (Camacho et al. 2013), with exposure effects modulated by demographic traits (e.g. age, sex, habitat), metabolic responses and availability (Pugh and Becker 2001).

Marine organisms present some mechanisms of metal removal. Aquatic reptiles are known to reduce contaminant burdens by the maternal transfer of essential and non-essential elements to eggs (Ehsanpour et al. 2014). This mechanism is thought to explain in part the wide range of Hg contamination observed in hawksbill turtles, *Eretmochelys imbricata* (Ehsanpour et al. 2014) and differences in Hg accumulation between males and females specimens of the same species (Meyers-Schöne and Walton 1994). However, as the biochemistry of Hg in marine turtles is not fully understood, it makes more challenging their use in environmental monitoring efforts (Sakai et al. 1995, Guirlet et al. 2008).

All sea turtle species are listed in different risk categories within the IUCN Red List (2018), therefore it is important to develop non-invasive monitoring methods for toxic metals, such as Hg (Hopkins et al. 2013). One of these methods is the use of keratinized structures composing the turtle’s carapace, skin and nails. The high amino acid content in keratin increases Hg binding capacity and results in its immobilization in the metabolic stable keratin layers, which can further be assessed for Hg exposure over time (Toni et al. 2007, Schneider et al. 2015).

Among the seven species of marine turtles, loggerhead turtles (*Caretta caretta*) and green turtle (*Chelonia mydas*) are the most used species for this monitoring approach. In *C. caretta*, carapace fragments (i.e. scutes) have been shown as reliable tissues to monitor Hg exposure either in the whole body burden (Sakai et al. 2000) and in internal tissues (Day et al. 2005). Recently, Casini et al. (2018), not found a statistically significant correlation between Hg concentrations in carapace fragments and body size in individuals of *C. caretta* from the Mediterranean Sea, probably due to the low number (n = 3) of specimens. In contrast, scutes of the green turtle *C. mydas*, were found to reflect Hg concentrations of internal organs (Bezerra et al. 2013) and varied with size class and habitat-specific Hg backgrounds which was associated to Hg content in the turtle’s diet (Bezerra et al. 2012, 2015).

Since no data existed, so far, on Hg concentrations in the South Atlantic population of the loggerhead turtle *C. caretta*, we sampled a large number of individuals to quantify the total-Hg and methyl-Hg contents in carapace fragments and the influence of biological and ecological factors in Hg concentrations, as a first step to evaluate scutes as a tool for environmental Hg monitoring and individual exposure to this pollutant.

**MATERIALS AND METHODS**

All procedures and analyzes of this work were carried out within the current norms of Brazilian environmental legislation, carried out under the authorization of the System of Authorization and Information in Biodiversity - SISBIO, License No. 21693-9 (2016).

This study was conducted along the coastal zone of Bahia state, the largest nesting area of loggerhead turtles in Brazil (Marcovealdi et al.
Scutes samples were obtained from mature females specimens found nesting at the beach of Praia do Forte, Sauípe and Busca Vida, Bahia localities (Fig. 1) during nesting seasons of 2012 and 2016. Carapace fragments were collected from 76 adult females, which were classified as adult (n = 72) and subadult (n = 4), according to their size categories following Dodd (1988) categories. Nesting females were momentarily restrained in situ after laying and covering the eggs in order to measure (e.g. curved carapace length (CCL) and curved carapace width (CCW)) and to collect carapace fragments that were loose or falling off due to barnacle incrustations. The Brazilian Marine Turtle Conservation Program (TAMAR) keeps a detailed inventory of the females that come to nest in Praia do Forte and nearby areas by tagging all nesting individuals, which makes possible to classify them as first-time nester or remigrants.

**TOTAL AND METHYL Hg QUANTIFICATION**

Quantification procedure of total-Hg in biological samples followed Bezerra et al. (2012). In summary, samples of 0.5 g in dry mass were placed in duplicate in Teflon tubes containing 10 mL of concentrated nitric acid (HNO₃ 65%) for one-hour pre-digestion. Digestion was carried out in a microwave oven, 800 W at 200 °C for 30 min. After cooling, 1 mL of hydrogen peroxide (H₂O₂) was added. The final extract was transferred and diluted in volumetric flasks to 100 mL. Hg concentrations were quantified by cold vapor atomic absorption spectrophotometer CV-ASS), in a NIC RA-3 (NIPPON ®) spectrophotometer. Instrument calibration was performed using solutions with known Hg concentrations varying from 0.0 to 5.0 ng g⁻¹. The average detection limit (LD) of the method was 0.08 ± 0.08 ng g⁻¹ calculated as three times the standard deviation of reagent blanks.

**Figure 1** - Study area on the coast of the state of Bahia (Praia do Forte) in NE Brazil. Point black represent the location of female turtles spotted nesting.
divided by the inclination of the calibration curve (US EPA 2000). The validation of the methodology was obtained by using certified reference materials (SRM), mussel tissue (ERM-CE278K) and lobster hepatopancreas (TORT-2), with recovery values of 93% and 100% respectively.

For methyl-Hg quantifications, approximately 200 mg of carapace fragments was weighed in PTFE tube and 5.0 mL of 25% KOH methanolic solution was used to extract methyl-Hg 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 methyl-Hg. Subsequently, the ethylation process was done with 300 µL of 2 mol/L acetate buffer (pH 4.5) followed by the addition of 30 µL of sample and 50 µL of tetraethyl 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 MEX-TM automated methyl mercury system from Brooks Rand Labs (Seattle, USA) coupled an atomic fluorescence spectrophotometer. Precision and accuracy of methyl-Hg determinations were ensured by the use of duplicate analyses of samples and certified reference materials, Tuna Fish - BCR-463 and Dorm-2 - NRC, was run with each batch of samples with a mean recovery of 96% ± 85% for methyl-Hg determination, respectively. Limits of detection (LD) and quantification (LQ) for methyl-Hg determinations were 0.003 mg kg⁻¹ and 0.009 mg kg⁻¹, respectively (CITAC 2002).

NITROGEN AND CARBON STABLE ISOTOPES (δ¹⁵N AND δ¹³C)

Approximately 1 mg of dry sample which was weighed in tin capsules and analyzed on an elemental analyzer (Flash 2000) coupled to a continuous flow mass spectrometer (Isotope Ratio Mass Spectrometry – IRMS, Delta V Advantage, Thermo Scientific, Germany). All results are expressed as delta value (δ), Pee Dee Belemnite notation for δ¹³C in parts per thousand (‰) and atmospheric N₂ for δ¹⁵N, 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) and the results showed 97±1%.

STATISTICS

The Shapiro Wilk test was used to evaluate the normality of the data. Non-parametric Spearman’s correlation was used to evaluate the relationship between biometric data and concentrations of total-Hg and methyl-Hg and Mann-Whitney test was used to compare total-Hg between remigrant turtles and those nesting for the first time. Pearson’s correlation was applied to evaluate the relationship between CCL and CCW. Student’s t-test was used to compare size between the same turtle’s groups. The relationships between the δ¹³C and δ¹⁵N values and the concentrations of total-Hg and methyl-Hg were determined from linear regressions. Outliers, which totaled three (3) in our samples were removed for a better interpretation of the graphics. The significance level used for the tests was 95% (p <0.05). Statistical tests and graphing were performed using RStudio software (version 0.98.976 - © RStudio, Inc. 2009-2013 and Microsoft Office 365).

RESULTS

Carapace fragments were collected from 76 adult females ranging from 87.1 to 107 cm of CCL (mean of 98.5 ± 5 cm) and 79.6 to 98.9 cm of CCW (mean of 88.9 ± 4 cm). Thirty-nine females were identified as remigrants while 29 females were nesting at the location for the first time (Table I). Information were missing for eight specimens. A significant correlation was observed (Pearson’s r = 0.76, p< 0.05) between CCL and CCW (Fig. 2). When comparing the CCL of the groups of remigrant
turtles and those arriving for the first time, no significant difference was found (Student’s t-test; \( p > 0.05 \)). The first-time nesting turtles showed an average of 98 ± 4 cm of CCL, while remigrant turtles presented an average of 98 ± 5 cm of CCL. Average concentrations of total-Hg, methyl-Hg, \( \delta^{13}C \) and \( \delta^{15}N \) isotopes and nesting status are also presented in Table I. Individual results for each animal are detailed in the supplementary material (Table SI).

The concentrations of total-Hg in the carapace fragments of the 76 animals showed a wide variability, even though the individuals studied showed similar morphological, physiological and migratory traits. The observed concentrations varied from 3.3 ng g\(^{-1}\) to 2,169 ng g\(^{-1}\), with a mean of 183.6 ± 326 ng g\(^{-1}\) (Table I). Methyl-Hg concentrations in carapace fragments of 15 adult females, varying in size from of 89.6 to 107 cm; average of 99 ± 4 cm, being 12 categorized as remigrants and 3 as first-time arrivals, varied from 0.2 ng g\(^{-1}\) to 55.2 ng g\(^{-1}\), with a mean of 11.9 ± 15 ng g\(^{-1}\) (Table I). The percentage of methyl-Hg relative to total-Hg varied from 0.5 to 32.6%, with an average of 10.7 ± 10%.

No correlation was found (Spearman’s \( r = 0.28, p > 0.05 \)) between total-Hg levels and CCL (Fig. 3). According to the nesting status, the remigrant individuals showed a concentration varying from 3.3 ng g\(^{-1}\) to 1,672 ng g\(^{-1}\), with a mean of 142 ± 270 ng g\(^{-1}\), while the turtles that arrived for the first time had Hg concentrations ranging from 3.8 ng g\(^{-1}\) to 835 ng g\(^{-1}\), with a median of 165 ± 188 ng g\(^{-1}\). The comparison between the total-Hg content in the remigrants populations and those that arrived for the first time showed no significant difference (Mann Whitney’s \( U = 681.5; p > 0.05 \)).

The correlation between methyl-Hg concentrations with CCL, and % methyl-Hg with CCL, were not significant (\( r = 0.27, r = -0.14 \)). The concentrations of methyl-Hg in the migratory turtles (\( n = 8 \)) ranged from 0.19 to 8.3 ng g\(^{-1}\), with a mean of 4.0 ± 3.1 ng g\(^{-1}\), whereas in the first-time turtles (\( n = 3 \)), the concentrations ranged from 2.9 to 18.2 ng g\(^{-1}\), with a mean of 9.9 ± 7.3 ng g\(^{-1}\). There was no significant difference between the methyl-Hg concentrations between remigrants turtles and those that arrived for the first time (Mann Whitney’s \( U = 21; p > 0.05 \)).

Isotope determination was performed in nine individuals, all of whom had their measured total-Hg concentrations, but only 4 had quantified methyl-Hg concentrations (Table I). The \( \delta^{13}C \) and \( \delta^{15}N \) presented a mean of -16.7 ± 1.0‰ (-18.8 to -15.6‰) and of 7.4 ± 2.1‰ (5.7 to 11.9‰), respectively. The size of the individuals used in these analyzes varied from 94 to 104 cm with an average of 99 ± 3.12 cm. The relationship between \( \delta^{13}C \) and \( \delta^{15}N \) showed that the nine turtles had similarities in their diet. Seven turtles showed values of \( \delta^{13}C \) and \( \delta^{15}N \) in the range of -17.0 to -15.5‰, and 5.7 to 7.6‰ respectively; while the other two turtles presented a higher enrichment of \( \delta^{15}N \), with values of 9.8‰ to 11.9‰ and values of \( \delta^{13}C \) from -18.8 to -17.6‰. No correlation was observed between \( \delta^{15}N \) and total Hg (Spearman’s \( r = 0.66; p > 0.05 \)).
We present here the first account of total-Hg and methyl-Hg distribution in a *C. caretta* population from the South Atlantic. With our dataset composed exclusively of nesting females categorized as remigrants female and first-time nesting females, it was possible to evaluate the effect of maternal metal offloading between these two groups. However, the comparison of the Hg concentration for the two groups did not show a significant difference, as it is mentioned by different works, which have shown a low maternal transfer rate of toxic metals like Hg, in comparison with essential metals like Zn, Cu or Se (Guirlet et al. 2008, Bergeron et al. 2010, Hopkins et al. 2013).

One of the main routes of Hg exposure in marine organisms is the diet (Mackay and Fraser 2000, Gray 2002), which often varies with life-stage. Therefore, organisms’ ecological niche and feeding habitats are two major factors controlling Hg concentrations. Our results with loggerhead turtles are an example of that *C. caretta*, feeds opportunistically and consumes a wide variety of food items (Tomas et al. 2001, Frick et al. 2009). Such variable diet and the feeding frequency directly affect Hg incorporation.

In the early and juvenile stages, loggerhead turtles are epipelagic inhabiting oceanic zones and feeding most of the time in the first five meters of the water column. As sub-adults and adults they become neritic feeding mainly on the bottom (Bolten 2003). The change in feeding habitat and diet may lead to differences in the exposure level to Hg as shown in juvenile and adult specimens of *C. mydas* (Bezerra et al. 2012). However, the sample presented here is composed mostly of adults and, thus, with similar diets. Loggerhead turtles larger than 50 cm change their distribution from oceanic zones to coastal zones, where they feed mainly on benthic animals (Bjorndal 1997). In this context, assuming no significant differences in diet for these adults, and with feeding being the major form of Hg intake, one would expect a smaller range of Hg concentrations than that found in this study. However, despite their comparable size as adults, the concentrations varied from 3.8 up to 1,672 ng g⁻¹.

There are few studies reporting on total-Hg concentrations in carapace fragments of *C. caretta*. Day et al. (2005) reported similar concentrations

### Table I

| Size (n = 76), total-Hg (n = 76), Methyl-Hg (n = 15), %Methyl-Hg (n = 15), δ¹³C (n = 9) and δ¹⁵N (n = 9) concentrations of nesting loggerhead turtles from Sauipe, Busca Vida and Praia do Forte. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| CCL (cm)       | CCW (cm)        | Total-Hg (ng g⁻¹) | Methyl-Hg (ng g⁻¹) | %Methyl-Hg | δ¹³C | δ¹⁵N |
| Mean            | 98.5            | 88.9            | 183.6            | 11.9        | 10.7 | -16.7 | 7.4 |
| SD              | 5               | 4               | 326              | 8           | 6    | 5     | 2   |
| Min             | 87.1            | 79.6            | 3.3              | 0.2         | 0.5  | 18.8  | 5.7 |
| Max             | 107             | 98.9            | 2,169            | 55.2        | 32.6 | 15.6  | 11.9 |

SD: Standard deviation.
to the Bahia turtles in 34 individuals of loggerhead turtle, with similar sizes (50-95 cm) to those used in the present work, in the southeastern coast of the United States, where Hg concentrations varying from 62 – 2,837 ng g⁻¹. Casini et al. (2018) reported Hg concentrations in the range of 210 – 3,220 ng g⁻¹ in scutes of loggerhead turtle, with CCL in the range of 20 – 78 cm, in the Mediterranean Sea. Results from species, such as *Lepidochelys kempii*, that exhibit a similar diet to *C. caretta*, also showed a large variability of Hg concentrations in the carapace. *L. kempii* from the Gulf of California studied by Presti et al. (1999) and from the Massachusetts coast studied by Innis et al. (2008), showed total-Hg concentration varying from 41.3 to 7,486 ng g⁻¹ and 48 to 1,058 ng g⁻¹, respectively.

Unfortunately, there is no reported measured concentration of methyl-Hg in the carapace of *C. caretta*, making impossible comparison with other areas. Day et al. (2005), however, estimated methyl-Hg based on total Hg content suggesting concentrations from 5 to 220 ng g⁻¹. Compared to other species, however, the average methyl-Hg concentrations found in *C. caretta* scutes from Bahia were much lower than those found in carapace fragments of 86 individuals of *C. mydas* from South China by Ng et al. (2018) (10 to 570 ng g⁻¹; average 90 ng g⁻¹).

The variability of Hg concentrations found in *C. mydas*, differently of *C. caretta*, is related to a major shift in diet of that species from pelagic carnivore to benthic herbivore, which is also strongly correlated with size. Thus, studies with green turtles (*C. mydas*) found negative relationships between size and Hg in kidney and muscle tissues (Sakai et al. 1995, Godley et al. 1999, McKenzie et al. 1999) as well as in scutes (Bezerra et al. 2012), with juveniles exhibiting higher Hg concentrations than adults, reflecting the carnivorous diet of juveniles.

The δ¹⁵N and δ¹³C signatures of scutes (7.4 ± 2.5‰ and -16.7 ± 5.4‰, n = 9; for δ¹⁵N and δ¹³C respectively) suggests a high trophic level of the loggerhead turtles from Bahia, agreeing with the results found in carapace fragments of this species by Revelles et al. (2007) in the Mediterranean and Zanden et al. (2014) in the Florida coast, USA. Unfortunately, the stable isotope composition of carapaces are not enough for a proper characterization of food web position, since the isotopic turnover rate for different type of tissue may influence the isotopic concentrations that these tissues may reflect, such as suggested by Vander Zanden et al. (2015).

A very interesting finding is that the Hg concentrations of these nine individuals ranged from 10.1 to 1,672 ng g⁻¹, with no statistically significant correlation with δ¹⁵N, showing that although allocated at the same trophic level, Hg concentrations varied by at least 3 orders of magnitude and that, at least in the carapace, the concentrations of total-Hg or methyl-Hg are not influenced by the trophic level. Thus, for a species that is considered carnivorous, the concentrations of Hg in carapace fragments did not agree with its type of feeding. The lack of a sample of individuals with a smaller size did not allow to determine if there is some type of change in the trophic level of *C. caretta*.

Organisms of higher trophic levels have large distribution areas, and thus have larger feeding ranges than organisms at lower trophic levels (Atwell et al. 1998). Consequently, in the higher trophic levels, individuals of the same species may present fairly large ranges of Hg concentration, due to consumption of preys with different Hg content as shown for other large live, wide distributed species such as tunas (Lacerda et al. 2017). In addition, there are morphological factors and keratin composition of the carapace that may affect the degree of Hg deposition (Day et al. 2005). Mattei et al. (2015), found that the concentrations of some metals can be influenced by the ossification process, showing differences in concentrations that are found in the loggerhead turtle carapace. Reptile scutes are subdivided into various patterns, shapes, thickness
and degree of overlap, factors that could influence the concentrations of total-Hg and methyl-Hg (Toni et al. 2007).

CONCLUSIONS

The Hg found in carapace fragments in adult females of loggerhead turtle, showed a wide variation, as previously found in the same species in different areas of the world. In the case of methyl-Hg, the concentrations were lower than those found in the few studies using carapace fragments. The isotopic composition of $\delta^{15}$N and $\delta^{13}$C suggests that the individuals sampled belong to a similar and high trophic level, but isotope concentrations do not present any type of relation with the Hg content. Diet, morphology and chemical composition of the carapace would be factors to be evaluated in future studies, to determine the degree of influence they exert on the accumulation of this element. However, with the results found in the present study, it is still not possible to suggest the use of carapace fragments of *C. caretta* as a tool for environmental biomonitor of Hg.

ACKNOWLEDGMENTS

The author would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support for the development of this project. Special thanks to the team of the Coastal Biogeochemistry Laboratory for the support in handling the equipment, processing and analysis of the samples, and to the team of the Marine Turtle Conservation Project (TAMAR Project) for the support in the field work. Moises F. Bezerra is funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Brasil) doctoral scholarship (proc. 202788/2014-8). Carlos Eduardo de Rezende is a member of the FEC initiative (https://www.futureearthcoasts.org/regional-engagement-partners/).

AUTHOR CONTRIBUTIONS

CABR, MFB and LDL conceived the study and were responsible for the sampling, analysis, and writing of the manuscript. CER performed the nitrogen and carbon stable isotopes analyses and the discussion of the results, WRB performed the methyl-Hg analyses and the discussion of the results.

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SUPPLEMENTARY MATERIAL

Table SI: Size, total-Hg, Methyl-Hg, %Methyl-Hg, δ^{13}C and δ^{15}N concentrations of nesting loggerhead turtles from Praia do Forte, northern Bahia.