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Baseline

Monitoring mercury in green sea turtles using keratinized carapace fragments (scutes)

M.F. Bezerra [a,*], L.D. Lacerda [a], E.H.S.M. Lima [b], M.T.D. Melo [b]

[a] Laboratório de Biogeoquímica Costeira, Instituto de Ciências do Mar, Universidade Federal do Ceará, Av. Abolição 3207, Fortaleza, CE 60165-081, Brazil
[b] Fundação Centro Brasileiro de Proteção e Pesquisa das Tartarugas Marinhas, Acesso Projeto TAMAR 151, Alto Alegre, Itarema, CE 62592-000, Brazil

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ABSTRACT

The green sea turtles are facing a very high risk of extinction in the wild and the impacts of heavy metals contamination contributes with the decline of their populations. It is very important to assess noninvasive and nonlethal methods for monitoring Hg contamination in sea turtles. Thus, Hg concentrations were measured in keratinized fragments (scutes) and internal tissues of green sea turtles from the Ceará coast to test the usefulness of scutes as a monitoring subject for sea turtles. A significantly positive correlation was found between Hg concentrations in muscle and scutes, which demonstrate that scutes can be used as a predictive matrix of Hg concentration in muscle tissue of green sea turtles.

Trace metals contamination in wild animals is the object of several studies worldwide, especially in organisms considered as biomonitor of environmental quality. In this context, sea turtles are considered truly sentinels of the oceans because they are long living animals, have a global distribution and, therefore, liable to anthropogenic impacts and marine ecosystem degradation (Aguirre and Lutz, 2004). The green turtle (*Chelonia mydas*) is the most tropical turtle species and the only with a predominantly herbivore diet when adult (Bjorndal, 1980; Marquez, 1990). This species is classified as endangered by the IUCN, meaning a very high risk of extinction in the wild. The main factors responsible for the reduction of *C. mydas* wild populations is coastal environments degradation; accidental capture; harvest and consumption of meat and eggs; climate changes; and metals and organic compounds contamination (Anan et al., 2001; Hamann et al., 2010; Lam et al., 2004). Since sea turtles have a large migratory behavior, it is crucial to understand the trace metals fate in these animals, once their body loads of contaminants may accumulate from all areas where these animals use along their life cycle.

Since the 1970s, scientists have been concerned about trace metal contamination in sea turtles. A literature overview showed that between 1974 and 2012 about 80 papers have been published on trace metal concentrations in sea turtles. About half of these have dealt with mercury, a global pollutant, highly toxic to aquatic organisms. Unfortunately, many of these studies were opportunistic, depending on the occasional sampling of debilitated and stranded animals, generally resulting in a small number of specimens samples; for a review on this subject see D'Ilio et al. (2011), Kampalath et al. (2006) and Storelli and Marcotrigiano (2003).

In this context, non-invasive and non-lethal methods of monitoring are crucial to allow a large number of samples, including live and healthy animals. Some authors have aimed assessing the use of blood, scutes and/or eggs as a predictive matrix for the pollutants metabolized by the organism. Among these, scutes have been reported as reliable tissues because they can be collected non-invasively and non-lethally, either in juvenile or adult animals (Bezerra et al., 2012; Day et al., 2005; Presti et al., 1999; Sakai et al., 1995, 2000; van de Merwe et al., 2010). However, to assess scutes as a predictive matrix for monitoring Hg in internal tissues of living sea turtles, it is necessary to measure both keratinized fragments and internal tissues in the same animal.

A few studies have addressed the Hg content in keratinized fragments from some species of sea turtles: *C. mydas* (Bezerra et al., 2012; Komoroske et al., 2011; Presti et al., 1999; Sakai et al., 2000), *Caretta caretta* (Day et al., 2005; Sakai et al., 2000) and *Lepidochelys kempii* (Innis et al., 2008; Presti et al., 1999). Studies simultaneously analyzing scutes and internal organs, unfortunately, are still scarce in the literature (Day et al., 2005; Innis et al., 2008; Sakai et al., 2000). Furthermore, there is no information to date on *C. mydas* from the Atlantic Ocean. To fill this gap we aim to assess the keratinized fragments, scutes, as a non-lethal and non-invasive predictive matrix for monitoring total Hg concentrations in *C. mydas*, found at an important feeding ground in northeastern Brazil, by simultaneously analyzing Hg concentrations in internal organs (muscle, kidney and liver).

The Ceará coast, northeastern Brazil, extends along 573 km in the Equatorial South Atlantic Ocean (Fig. 1). This coastal region is characterized by low industrial development with no impact of Hg

* Corresponding author. Tel.: +55 85 3366 7038.
E-mail address: mmoisesfb@hotmail.com (M.F. Bezerra).

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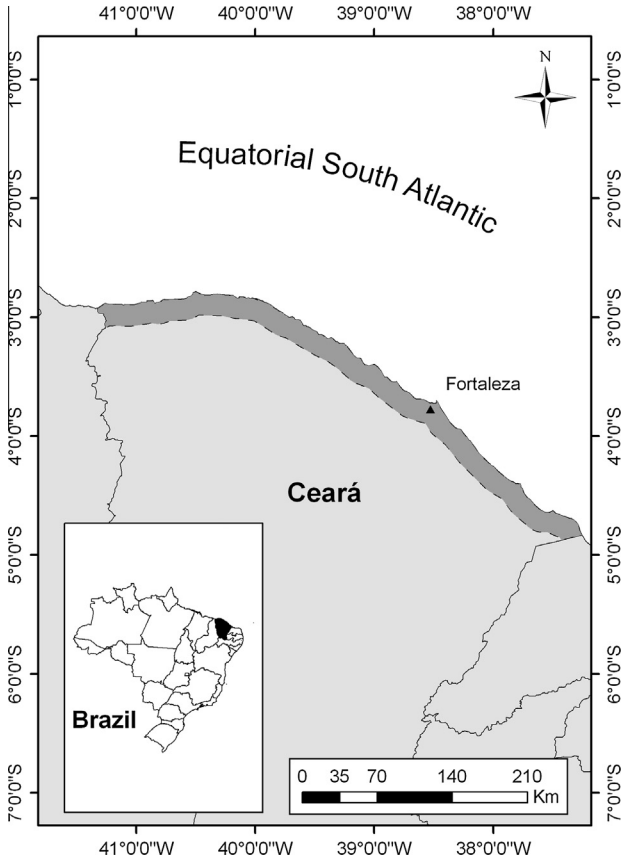

Fig. 1. Location where green sea turtles (*C. mydas*) were sampled along the Ceará coast, northeastern Brazil.

contamination ever reported in the literature (Marins et al., 2004; Monteiro-Neto et al., 2003). Also, it is an important feeding ground for green sea turtles, and registers the occurrence of four other species of sea turtles (*C. caretta*, *Eretmochelys imbricata*, *Lepidochelys olivacea* and *Dermochelys coriacea*). Previous studies have shown that animals tagged in this feeding ground were recaptured in other foraging grounds in Nicaragua, suggesting an interaction between the populations from a large oceanic area (Lima et al., 2003). Although major threat to sea turtles in this region is the high incidental capture of juveniles and adults by fish weirs, gill nets and trawl nets (Lima et al., 1999, 2013; Marcovaldi and dei Marcovaldi, 1999), the knowledge on Hg concentrations levels in this species is still poorly known (Bezerra et al., 2012), hampering an assessment of the potential risks of Hg exposure to this species.

This research followed all protocols for wildlife handling and sampling accepted and authorized by the Ministry of the Environment and Natural Resources (IBAMA/ICMBio) of Brazil (Licenses numbers: 21693-1; 21693-2; 21693-3). Animals found stranded and/or debilitated along de coast between November 2009 and August 2012 were collected for recuperation purposes by a technical staff of the Brazilian Sea Turtles Conservation Program (Projeto TAMAR/ICMBio). Only those animals that died a few days from collection were used in this study. Although one animal that died after remaining a few years in captivity was also included for comparison. The specimens were classified in juveniles ($n = 17$) and subadults ($n = 1$) based on Heppell et al. (2003).

Internal tissues samples were obtained by subsampling of the respective organs and moisture tissue content was obtained by lyophilization and varied from 74.1%, in liver; 77.7% in muscle and 80.9% in kidney. Scutes were collected randomly from different areas of the carapace, cleaned with ultrapure water and washed in an ultrasonic bath to remove algae and other residues. Total Hg was determined by cold vapor atomic absorption spectrophotometry following an acid digestion procedure described in detail in Bezerra et al. (2012). All materials used in the preparation of samples were previously acid washed with HCl 10%. Analyses were performed in duplicate, except for four samples that contained insufficient tissue mass (Table 1). Two samples of NIST 2976 (mussel tissue) standard reference material (SRM) were digested and analyzed with along each bath of sea turtle tissue sample and showed a recovery of reference concentrations ranging from 82.5% to 95.9%. Because the most data did not meet the assumptions of normality, non-parametric test was used; Spearman Rank Correlations (R_s). Linear regression between Hg concentrations in different tissues and those in scutes were performed. Statistical analysis used the software Statistica 8.0 (StatSoft, Inc. 1984–2007) and an α value of 0.05 was used to asses statistical significance.

Biometry, sex and total Hg concentrations in internal tissues and scutes are shown in Table 1. Considering only the wild

Table 1
Biometric data, sex and total Hg concentration ($\mu g\ g^{-1}$, dry weight), for green turtles (*C. mydas*) sampled in the Ceará coast, northeastern Brazil.

Sample ID	CCL (cm)	Sex	Mean ± standard deviation of total Hg concentration			
			Muscle	Kidney	Liver	Scutes
38/10	25.4	F	0.08 ± 0.01	0.06 ± 0.001	NA	NA
35/10	27.0	F	0.15 ± 0.01	0.39 ± 0.04	0.66 ± 0.18	0.31 ± 0.01
12/10	27.5	F	0.15 ± 0.01	0.25 ± 0.04	0.58 ± 0.01	0.07*
34/10	28.5	F	0.22 ± 0.01	0.50 ± 0.04	NA	0.51 ± 0.03
05/10	30.0	F	0.08 ± 0.01	0.18 ± 0.06	0.14 ± 0.02	0.04 ± 0.03
470/09	32.9	F	0.14 ± 0.04	0.35 ± 0.16	0.26 ± 0.02	NA
455/09	33.0	F	0.11 ± 0.05	0.11 ± 0.01	0.37 ± 0.07	NA
458/09	34.1	M	0.18 ± 0.03	0.23*	0.34*	NA
425/09	35.1	F	0.10 ± 0.02	0.21 ± 0.001	0.48 ± 0.03	NA
406/09	35.5	M	0.20 ± 0.02	0.27 ± 0.01	0.45*	0.49 ± 0.14
391/09	36.4	F	0.25 ± 0.01	0.30 ± 0.01	0.50 ± 0.26	0.86 ± 0.03
454/09	36.4	F	0.33 ± 0.11	0.24 ± 0.08	0.78 ± 0.26	NA
383/09	39.7	F	0.23 ± 0.07	0.58 ± 0.15	0.87 ± 0.13	0.42 ± 0.02
24/10	41.0	F	0.18 ± 0.04	0.36 ± 0.04	0.53 ± 0.15	0.46 ± 0.02
456/09	46.0	F	BDL	0.95 ± 0.05	0.34 ± 0.04	NA
389/09	55.4	F	BDL	1.20 ± 0.03	0.36 ± 0.06	0.01 ± 0.001
473/09	88.5	F	0.04 ± 0.02	0.13 ± 0.03	0.45 ± 0.01	0.005 ± 0.001
54/12[a]	52.0	F	0.82 ± 0.08	1.22 ± 0.02	4.32 ± 0.50	1.16 ± 0.07

* Concentration from a single sample; CCL – curve carapace length; NA – tissue sample not available; BDL – below the limit of detection (LOD) calculated according to US EPA (2000) (LOD = 0.003 $\mu g\ g^{-1}$).
[a] Captivity animal.

Table 2
Comparison of total Hg concentrations in three species of sea turtles from studies where correlations between scutes and internal tissues (μg g^{-1}) and blood (μg L^{-1}) were tested.

Species	Mean ± standard deviation[a] or standard error[b] of total Hg concentration (wet weight)					Study
	Muscle (n)	Kidney (n)	Liver (n)	Blood (n)	Scutes (n)	
C. caretta	0.155 ± 0.070[b] (6)	0.214 ± 0.046[b] (6)	0.594 ± 0.155[b] (6)	0.099 ± 0.042[b] (6)	0.941 ± 0.299[b] (6)	Day et al. (2005)
L. kempii	N.A.	N.A.	0.067 ± 0.070[a] (6)	N.A.	0.389 ± 0.239[a] (29)	Innis et al. (2008)
C. mydas	N.A.	N.A.	N.A.	0.001 ± 0.0001[b] (30)	0.048 ± 0.015[b] (31)	Komoroske et al. (2011)
C. mydas	0.03 ± 0.004[b] (16)	0.06 ± 0.02[b] (16)	0.19 ± 0.04[b] (16)	2.51 ± 0.05[b] (16)	N.A.	Van de Merwe et al. (2010)
C. mydas[*]	0.04 ± 0.02 (15)	0.07 ± 0.05 (17)	0.12 ± 0.05 (15)	N.A.	0.32 ± 0.27 (10)	Present study

[*] Concentration converted from dry weight basis (Table 1) to wet weight for comparison. N.A. – not analyzed.

animals, Hg concentrations were higher in liver and kidneys, ranging from 0.14 to 0.87 μg g^{-1} (median 0.47 μg g^{-1}) and from 0.06 to 1.20 μg g^{-1} (median 0.26 μg g^{-1}), respectively, and were not different between juveniles and sub-adult. Lower concentrations were found in scutes and muscle varying from <0.003 to 0.857 μg g^{-1} (median 0.42 μg g^{-1}) and from 0.04 to 0.33 μg g^{-1} (median 0.15 μg g^{-1}), respectively. Concentrations of Hg in these two matrices were much lower in sub-adult than in juveniles. Bezerra et al. (2012) also found this difference and associated it with different diets between juveniles (omnivorous) and sub-adults/adults (predominantly herbivorous). *C. mydas* from northeastern Brazil showed concentrations of Hg in internal organs comparable to those reported for *C. mydas* from Australia (Van de Merwe et al., 2010) and lower than those reported for *C. caretta*, which have a different feeding habit, from Japan (Sakai et al., 2000) and USA (Day et al., 2005) as showed in Table 2. Hg concentrations found in scutes of green turtles from Brazil was higher than those reported for green turtles from USA (Komoroske et al., 2011) although the size range of the animals of the studies are different. Much higher concentration in all tissues sampled were found in the adult individual living a few years in captivity, and this probably reflects its diet being based on fish, compared to mostly algae and invertebrates typical of the wild animals diet. Therefore, the results from specimen 54/12 (Table 1) were not considered in the statistical tests.

A significant positive correlation ($R_s = 0.900$; $p < 0.001$) was observed between the Hg concentrations in muscle and scutes (Fig. 2), supporting the suggestion from some authors to use keratinized fragments for monitoring Hg in sea turtles (Bezerra et al., 2012; Day et al., 2005; Sakai et al., 2000). Scutes Hg concentrations showed no significantly correlation with any other internal tissue. However, Hg concentrations in muscle also showed significant correlation with Hg concentrations in liver ($R_s = 0.590$; $p < 0.032$) and kidney ($R_s = 0.660$; $p < 0.007$) tissues (Fig. 2). Mercury is generally higher in liver because in this organ occurs the detoxification process and thus representing the majority of the recent ingested Hg. On the other hand, muscle represents a sink for the metabolized Hg ingested along the life of the animal. The linkage between muscle tissue and carapace tissue is closer than with other organs, like liver and kidney, which reflects in a stronger correlation. It is important to highlight that although the non-living keratin layers are metabolically unavailable for the stored Hg, this concentration could be useful for monitoring long term exposure (Day et al., 2005; Golet and Haines, 2001; Komoroske et al., 2011; Schneider et al., 2013).

Previous studies which resulted in correlation analysis of Hg concentrations in scutes and other tissues of sea turtles are compared in Table 2. To our knowledge the results presented here are the first published assessing correlations between Hg concentrations in keratinized fragments and internal organs of *C. mydas* for the Atlantic Ocean. Nevertheless, Van de Merwe (2008) showed a significant correlation between Hg concentrations in scutes and internal tissues of *C. mydas* from a rehabilitation center in Australia, but highlighted that carapace Hg concentration may be subject

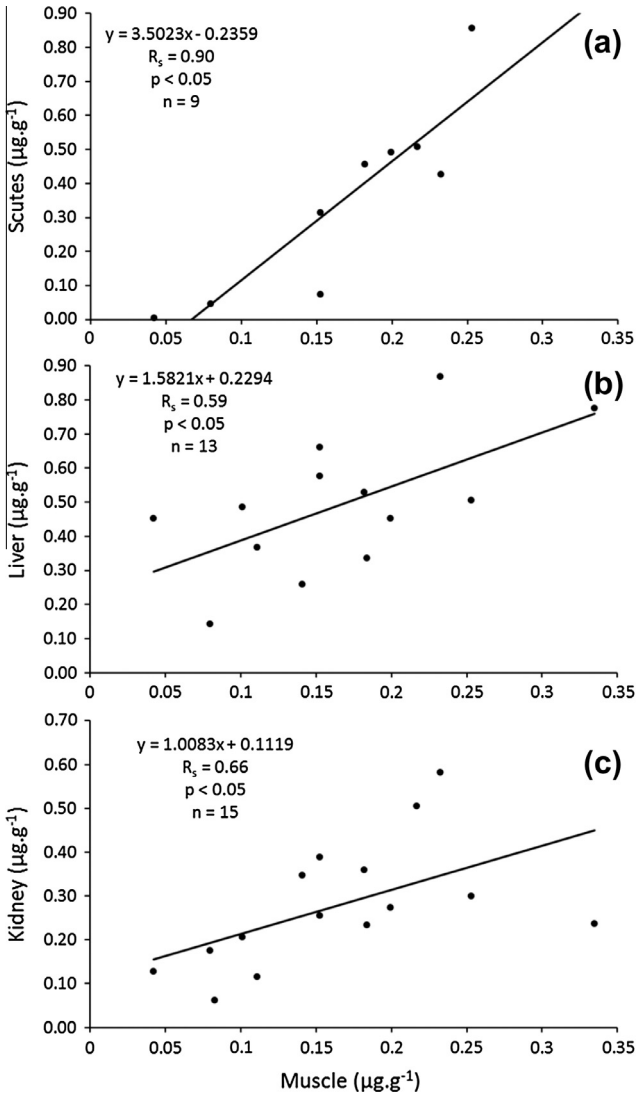

Fig. 2. Linear regressions between total Hg concentrations in muscle tissue and scutes (a), liver (b) and kidney (c) from green turtles (*C. mydas*) sampled in the Ceará coast, northeastern Brazil.

to great variability due to different sampling methods and change in the animal's diet. Komoroske et al. (2011) evaluated the total Hg content in scutes and blood from green sea turtles and found a significant positive correlation between them. Some authors have shown similar results in *C. caretta* but no correlation was found in *L. kempii*.

Sakai et al. (2000) evaluated Hg concentrations in six loggerhead sea turtles (*C. caretta*) from Japan and found a significant increase ($p < 0.05$) in scutes Hg concentration with an increase of Hg

concentration in the whole body, estimated from the total Hg burdens in whole body and body weight. Day et al. (2005), evaluating six loggerhead sea turtles captured in the USA east coast also found significant correlation ($p < 0.015$) between scutes Hg and liver, muscle, blood and dorsal spine Hg concentrations. These results suggest that Hg concentrations in keratinized fragments could reflect Hg content in internal tissues, like muscle and liver of *C. caretta*. Van de Merwe et al. (2010) investigating Hg concentrations in blood and internal organs of green sea turtles found significant correlations between them, which indicate that blood also seems to reflect internal Hg concentration in this species. However, Innis et al. (2008) did not found such correlations in *L. kempii*, although muscle tissue was not assessed by these authors. The authors ascribed this result to an insufficient amount of samples. Generally, the major hindrance for these studies is the insufficient sample size to allow effective statistical tests and conclusive evaluations to be performed.

In conclusion, the results showed that keratinized fragments are indeed good matrices for monitoring Hg concentrations in the green sea turtle *C. mydas*. Monitoring Hg using this approach allows a better health assessment of still alive sea turtles making conservation guidelines more effective. This management tool may provide valuable information on Hg contamination in the oceans and wildlife, besides reinforcing sea turtles as flags species for nature conservation and truly sentinels of the oceans.

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