Mercury Concentration in Tissues of a Captive Green Turtle (*Chelonia mydas* L.)

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Mercury (Hg) residence time in the ocean varies from approximately 30 years in the surface (0-200 m) layer to a century in the intermediate and deep water zones (UNEP 2013). During this period, Hg can be absorbed by marine biota, recycled in the water column and eventually transported to deep layers and deposited in sediments. Therefore, long-lived oceanic organisms, such as sea turtles, are exposed throughout their lives to the legacy of Hg pollution from anthropogenic sources of the Anthropocene as well as from long-term emissions from natural sources.

Green turtles (*Chelonia mydas* L.) are known as the only sea turtle species with a predominantly sea grass/algae-based diet as adults, although as juveniles they exhibit more omnivorous diet patterns (Bjorndal 1980). Considering the diet as the major Hg incorporation route for marine organisms and because Hg biomagnifies up the food web, adult *C. mydas* as herbivorous animals are exposed to a smaller risk of Hg contamination than other carnivorous/omnivorous sea turtle species, such as *Lepidochelys kempii* (Innis *et al.* 2008) and *Caretta caretta* (D'Ilio *et al.* 2011).

We report abnormally high Hg concentrations in external and internal tissues of a green sea turtle raised in captivity for four years. Based on these results, we offer recommendations for the prescribed diet of this species while in captivity in rehabilitation centers.

The studied animal was captured using free-diving methods near an artificial reef (known as *marambaia*) by local fishermen off Almofala beach (western coast of Ceará) in March 2008. The Ceará coast, northeastern Brazil, is an important feeding ground for green sea turtles in the Equatorial South Atlantic Ocean. This coastal region is characterized by low industrial development with no significant impact of Hg contamination reported in the literature (Lima *et al.* 2013; Marins *et al.* 2004; Monteiro-Neto *et al.* 2003). The animal was debilitated (i.e., cachexy, high epibiotic load, dehydration), weighing three kg with a curved carapace length (CCL) of 31 cm. After an unsuccessful rehabilitation period, this animal was maintained in an ambient temperature outdoor seawater pool at the Environmental Education Center of the Brazilian Sea Turtle Conservation Program (TAMAR/ICMBio) located in Ceará state for educational purposes (Lima 2001). The animal was fed twice daily with fresh sardines (*Opisthonema oglinum*) and marine algae (mostly Rhodophyceae), both collected from the surrounding region. The turtle's health status was established by veterinarians according to the protocols established by TAMAR/ICMBio. After four years in captivity, the turtle presented signs of illness (*i.e.*, positive buoyancy, anemia, appetite loss) and was sent to rehabilitation. On 12 July 2012 the animal died, measuring 52 cm CCL and weighing 14 kg. The necropsy showed tracheal secretions and black spots in the lungs and the cause of death was not determined. Muscle, kidney, liver and scute tissue samples were collected for further analyses, including total Hg content.

Total Hg was determined by cold vapor atomic absorption spectrophotometry (CVAAS). All samples were acid digested in duplicate according to Bezerra *et al.* (2012). Simultaneously, a reference standard (National Institute of Standard and Technology (NIST) Standard Reference Material® (SRM) 2976 Mussel Tissue) was similarly digested and analyzed as a quality control. The total Hg concentration measured in the SRM was 67.5 ± 9.6 ng.g^{-1} compared to a certified total Hg value of 61.0 ± 3.6 ng.g^{-1}. The limit of detection calculated according to USEPA (2000) guidelines was 3.0 ng.g^{-1}. All concentrations reported here are based on dry weight. From a previously published dataset (Bezerra *et al.* 2013), we selected four wild green turtles with a similar size and weight to the captive animal reported here for comparison of their total Hg concentrations in muscle, kidney, liver and scute (Table 1). Average water content measured in both wild and captive green turtles was 76.1% for muscle tissue, 79.6% for kidney tissue and 72.9% for liver tissue.

Total Hg concentrations found in the organs of wild and captive *C. mydas* are compared in Table 1. The total Hg concentrations in the organs and tissues of the captive animal were 8.4, 1.6, 8.0 and 3.9 times higher than those measured in the wild animals, for muscle, kidney, liver and scute, respectively. While total Hg concentrations in the organs of the wild green turtles were within the range reported by different authors (Kampalath *et al.* 2006), the concentrations found in liver of the captive animal (4,234 ng.g^{-1}) were in the same order of magnitude as those reported for carnivorous species such as *C. caretta* (Godley *et al.* 1999; Maffucci *et al.* 2005; Kampalath *et al.* 2006).

Mercury is known for its capacity to bioaccumulate and biomagnify in food webs, resulting in organisms at higher trophic levels accumulating a higher total Hg content (Gray 2002). Because the total Hg concentrations in muscle, kidney, liver and scute tissues found in the captive animal are comparable or even higher than those reported for carnivorous sea turtle species such as *Caretta caretta* (Godley *et al.* 1999; Maffucci *et al.* 2005; Kampalath *et al.* 2006) and *Lepidochelys kempii* (Innis *et al.* 2008), this suggests that the fish-based diet during captivity influenced the elevated total Hg content in the tissues of this animal. Unfortunately, it was not possible to quantify the levels of total Hg in the prey items (sardines and algae) of the captive green turtle. However, the total Hg concentration found in the muscle tissue of sardines from the same area (mean ± SD = 40.9 ± 3.1 ng.g^{-1}, Braga 2006) was 4 times higher than those measured in the local marine algae (9.5 ± 5.5 ng.g^{-1}, Bezerra *et al.* unpublished data), which is the regular food item consumed by wild green turtles. Therefore, due to bioaccumulation

Animal type	Sample size	CCL (cm)	Mass (kg)	Mean of total Hg concentrations on dry weight basis (ng.g-1) Muscle	Kidney	Liver	Scutes
Wild	4	46 ±6 (40-55)	10 ±6 (5-20)	97 ±95 (3-211)	753 ±339 (363-1,205)	529 ±261 (344-978)	296 ±204 (7-455)
Captive*	1	52	14	816 ±18 (798-834)	1,225 ± 5 (1,220-1,230)	4,234 ±385 (3.849-4,619)	1,155 ±26 (1,129-1,181)

Table 1. Biometric data and total Hg concentration of wild green turtles and a captive green turtle from the Ceará coast feeding grounds. Average values presented ± standard deviation and range in parentheses. *Total Hg variation from duplicate analysis of the same tissue sample.

and biomagnification of Hg, high contamination levels may be reached due to the high frequency of fish-based diets for animals raised in captivity.

In contrast, Suzuki *et al.* (2012) found no differences between Hg concentrations in the blood of wild and captive green turtles. These captive turtles were fed with fish, squid and vegetables, which is a different diet from that of wild green turtles. This is most likely a result of different Hg accumulation kinetics in blood versus other tissues, such as muscle, kidney and liver tissues (Schwenter 2007). Also, Kwon *et al.* (2013) showed a high increase of total Hg concentrations in muscle, kidney, liver and brain tissue of fish resulting from dietary changes, but did not observe the same increase in blood Hg concentrations. Unfortunately, we were unable to obtain blood samples, which are better able to reflect recent exposure than other tissues (Day *et al.* 2005) and would allow us to evaluate this apparent tendency of Hg accumulation between tissues of captive animals.

The results of this study are among the highest Hg concentrations reported in internal and external tissues of green turtles (Kampalath *et al.* 2006; van de Merwe *et al.* 2010). We hypothesize that these concentrations are likely related to the introduction of fish as a regular item in the animal's diet; however, we cannot suggest any causal relationship between Hg concentrations and the death of the animal based on the small sample size of this study. The adverse effects of Hg exposure in vertebrates (i.e., fish, birds, reptiles, mammals) are reported by numerous studies (Schneider *et al.* 2013). Day *et al.* (2007) reported a significant decrease in blood cell viability of loggerhead turtles with a methylmercury concentration ranging from 0.5 to 1.0 $\mu g.g^{-1}$. Perrault *et al.* (2011) also suggested that leatherback hatchlings (*Dermochelys coriacea*) may have reproductive limitations (decreased hatching and emergence success) resulting from low selenium and high total Hg ratios. In addition, Hopkins *et al.* (2013) provided evidence that total Hg exposure of female snapping turtles (*Chelydra serpentina*) is reflected in the reduction of reproductive success through increased infertility and embryonic mortality. Although we have not measured methyl Hg, which is the most toxic form of Hg, its concentration in animal tissues tends to vary according to the total Hg concentration. In general, methyl Hg concentrations correspond to over 90% of the total Hg content in muscle and adipose tissues, with a slightly lower percentage in liver and kidney tissues (Kampalath *et al.* 2006). Therefore, the high total Hg concentrations found in the captive green turtle in our study thus suggest high methyl Hg concentrations. Because the diet is the major pathway for Hg incorporation in marine organisms, it is important to avoid excessive changes in the diet of captive sea turtles, especially for the herbivorous *C. mydas*, to prevent the hazardous effects of increased Hg exposure.

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