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Mercury Concentrations in Tissues of Colombian Slider Turtles, *Trachemys callirostris*, from Northern Colombia

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Abstract This study determined the total mercury (THg) concentrations in pectoral muscle, blood and carapace tissue in turtles collected from Magangué and Lorica, Colombia. THg concentrations in µg/g (wet weight) were 0.39 ± 0.16 in muscle, 0.15 ± 0.08 in carapace and 0.07 ± 0.03 in blood for turtles from the Magdalena River and 0.25 ± 0.18 in muscle, 0.14 ± 0.09 in carapace and 0.06 ± 0.04 in blood for turtles from the Sinú River. Twenty-nine and ten percent of turtle muscle samples from Magangué and Lorica, respectively, exceeded the consumption advisory limit of 0.5 µg Hg/g for fish. There was a significant correlation between carapace length and THg levels for this specie, depending on the sample site. In addition, a significant correlation was observed in THg concentrations in carapace and muscle. However, significant differences were observed in the THg levels between the two study locations, with turtles caught in the Magdalena River having higher levels of THg.

Keywords Gold mining · Colombia · Mercury · *Trachemys callirostris*

Mercury (Hg) is a global problem due to its distribution, persistence, bioaccumulation, and toxicity in aquatic environments with multiple effects on biota and humans consumers of fishery products. Gold amalgamation, a common practice used by small scale artisanal miners, leads to the release of significant quantities of Hg in different environmental matrices. Fish from the northern region of Colombia have shown Hg levels higher than the WHO limit of 0.5 µg/g of Hg wet weight (Alvarez et al. 2012; Marrugo et al. 2008). However, little is known about Hg bioaccumulation in other organisms from aquatic ecosystems in northern Colombia, despite a widespread history of small scale and artisanal gold extraction operations in this area.

With their omnivorous diet, their mid to high trophic position in the food chain, their long life span, and relatively sedentary life styles, Colombian slider turtles, *Trachemys callirostris*, could be effective bioindicators of environmental contamination and potential human risk. The use of turtles as bioindicators has increased over the past two decades (Bergeron et al. 2007; Day et al. 2005; García-Fernández et al. 2009; Golet and Haines 2001; Sakai et al. 2000; Schneider et al. 2009; Storelli et al. 2005). In South America, although chelonians traditionally represent one of the most important sources of protein for humans, there are few studies that have analyzed heavy metal accumulation in tissues of river turtles (Piña et al. 2009; Schneider et al. 2009).

The Colombian slider, *T. callirostris* is listed in the national Colombia Red Book as Near Threatened and occurs in northern Colombia in the middle and lower Magdalena River drainage and its principal tributaries (lower Cauca and San Jorge rivers) and in other drainages such as the lower Sinú River (Bock et al. 2012). Environmental contamination and habitat alteration probably have influenced the distribution and local abundance of *T. callirostris*, but the single greatest threat to the species throughout its range of distribution is human exploitation.

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Little is known of the Hg levels in different tissues of Colombian turtles. It appears that the Hg level in blood is comprised of a transient component reflecting the dietary Hg intake over the previous days to weeks, whereas a more stable component is revealed by Hg analyses of other tissues, reflecting the total body burden from more long-term accumulation (Day et al. 2005). The relative importance of these two components would depend on the magnitude of the dietary pulses relative to the body burden of Hg at the time of sampling. Carapace samples may be noninvasively collected and should be stable enough to be used for live or dead animals that have been exposed to diverse conditions with a minimal risk of sample degradation (Day et al. 2005). In Colombia, the consumption of chelonians as food is a method of obtaining meat and protein, and forms part of the local culture. For this reason, information on Hg levels in muscle of *T. callirostris* is of particular importance because of the potential health risk to the local people as well as their possible usefulness as bioindicators. Furthermore, other studies have shown a high correlation for Hg concentrations between carapace and muscle (Golet and Haines 2001; Sakai et al. 2000), confirming the importance of this correlation analysis for non-invasive techniques.

Therefore, there were three primary objectives in the present study: the first was to determine total Hg (THg) concentrations in pectoral muscle, carapace and blood tissue in Colombian sliders, *T. callirostris*, collected from two different river systems affected by gold mining in Colombia. The second was to determine which non-lethal sampling method – blood or carapace – more closely related to muscle concentrations when attempting to ascertain human exposures to Hg via the consumption of turtle flesh. Given that the correlation between mercury content as a function of age and body size in turtles is not yet clear, a third objective of this research was to establish THg content/body size relationships to facilitate better risk assessments related to the consumption of this species. This study represents the first report of Hg concentrations in tissues of river turtles in Colombia.

Materials and Methods

Turtles were collected from February to May, 2012 from two sites (Fig. 1). Magangué is a city in the Bolivar Department of Colombia, located on the Magdalena River (9°13′N, 74°46′O), that drains a major portion of the country. It receives input from the San Jorge, Cesar, and Cauca rivers in the northern lowlands that produces a complex floodplain of wetlands. Santa Cruz de Lorica, also simple known as Lorica, is a town and municipality located in the northern portion of the Córdoba Department (09°14′N, 75°48′W) and in the lower portion of the Sinú River (Fig. 1).

Samples were collected from 60 individuals from Magangué and 30 from Lorica. In both sites, samples from female turtles were obtained directly from subsistence fishermen or at local marketplaces at the time the individuals were sacrificed. Hunters prefer adult females due to their larger size in this highly sexually dimorphic species. In *T. callirostris*, females are considered sexually mature adults at 15 cm straight-line carapace length (SCL) (Bock et al. 2012), and individuals that are harvested usually are mature females. For these reasons, this study only included females that exceeded this size.

A skinless boneless muscle sample of approximately 1.2 g was taken from the pectorals of the forelimbs. An equal quantity of carapace was taken from each turtle, and stored in polypropylene tubes. 1 mL of blood was drawn from the dorsal cervical sinus using a 22 gauge syringe and stored in vacutainers containing sodium heparin. All samples were immediately placed on ice while transported to the laboratory at the Universidad de Antioquia, Medellin, Colombia.

An aliquot of 0.6 g in duplicate from each individual was used for total mercury analyses for each tissue type. Prior to THg measurement, samples were digested with sulfuric acid (H_2SO_4), nitric acid (HNO_3) and potassium permanganate ($KMNO_4$; USEPA 1998). Total Hg content was determined by flameless atomic absorption spectrometry, using a Buck Scientific 410 Cold vapor Mercury Analyzer (Buck Scientific, Inc, Norwalk CT). THg concentrations are expressed as µg/g wet weight. Method accuracy was verified by using appropriate sample duplicates, calibration standards, blank samples and certified material (DORM-2 Dog fish Muscle Certified Reference Material for Trace Metals from National Research Council of Canada). The DORM-2 certified value for THg was 4.64 ± 0.26 µg/g. Our analyses yielded 4.30 ± 0.18 µg/g (n = 3). Recoveries were reported at 92 % for THg for the DORM-2 reference material. Variation coefficients for duplicate samples were estimated to be <5 %. The detection limit for THg in tissue was 0.05 µg/g.

Reported statistics are means and standard error in µg/g on a wet weight basis. Values that were below the limits of detection were substitute with 0.5 of the detection limit prior to statistical analyses (García-Fernández et al. 2009). Kolmogorov–Smirnov and Shapiro–Wilk's tests were performed to assess normality of the data distributions from Magangué and Lorica. Because the data were not normally distributed in any of the analyses ($p < 0.05$ in all cases), non-parametric statistics were used. Spearman correlations of THg and SCL values were calculated and

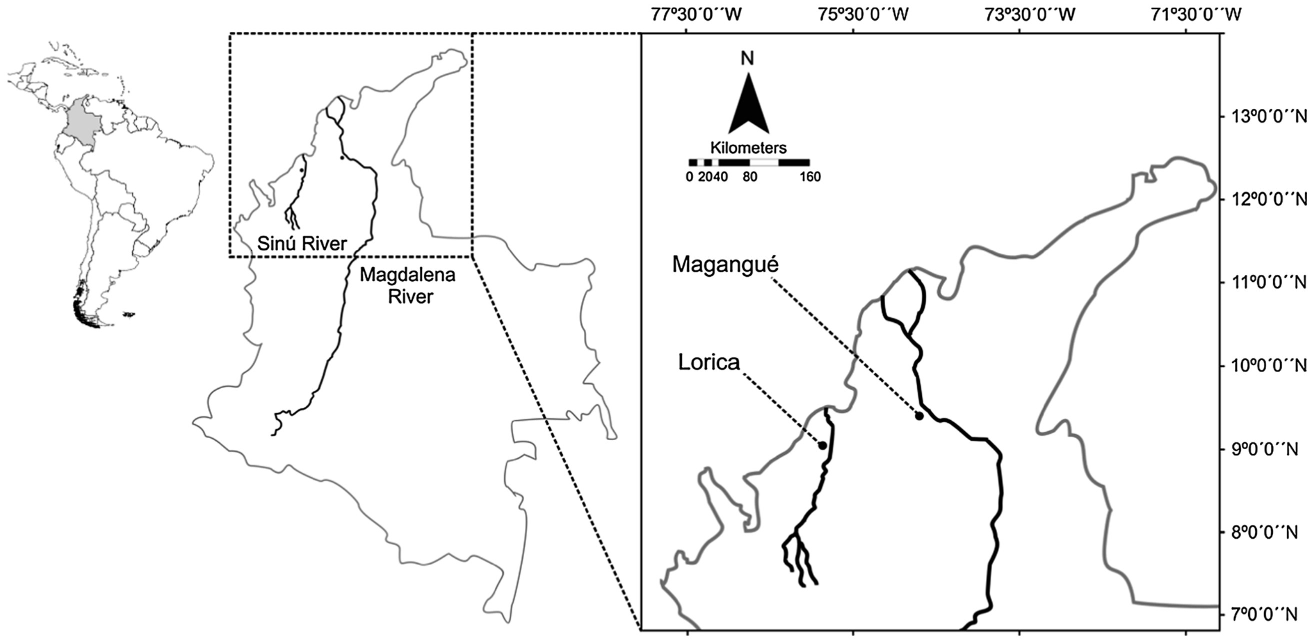

Fig. 1 Location of the sample collection sites (*black points*) of *T. callirostris* in Magangué (located along Magdalena River) and Lorica (located along the Sinú River) in 2012

Table 1 Turtle mean size measured as straight-line carapace length (SCL), and THg concentrations in tissues for individuals collected in Magangué and Lorica

Site	*N*	SCL (cm)		THg in muscle (μg/g)	THg in carapace (μg/g)	THg in blood (μg/g)
		Mean	Range			
Magangué	60	22	15.2–31.1	0.39 ± 0.16*	0.15 ± 0.08	0.07 ± 0.03
Lorica	30	22.77	15.0–31.2	0.25 ± 0.18*	0.14 ± 0.09	0.06 ± 0.04

Data reported as mean ± SE

* Significant differences ($p < 0.05$) in THg levels between two study sites

Kruskal–Wallis tests were used for multiple comparisons of THg levels in tissue types. In all cases, statistical significance was inferred if $p < 0.05$. Statistical analyzes were performed using the software Statgraphics Centurion.

Results and Discussion

The female turtles collected from Magangué and Lorica showed remarkably similar size distributions (Table 1). The number of turtles sampled, Hg concentrations in turtle tissues (muscle, carapace and blood) and corresponding mean body lengths for each study area are presented in Table 1. For all the tissue types, Hg concentrations were higher for samples collected in Magangué. The statistical analysis revealed only significant differences in THg levels in muscle tissue between the two study sites (Fig. 2). Of

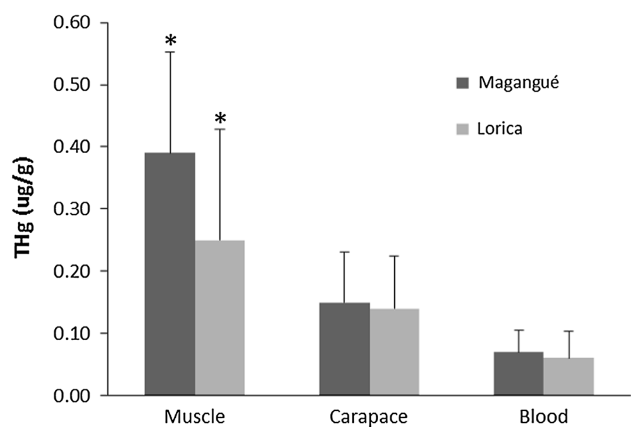

Fig. 2 THg levels in tissues of *T. callirostris* collected in Magangué and Lorica. *Significant difference in THg levels in each tissue between two study sites. The *error bars* represent the SE of the averaged medians

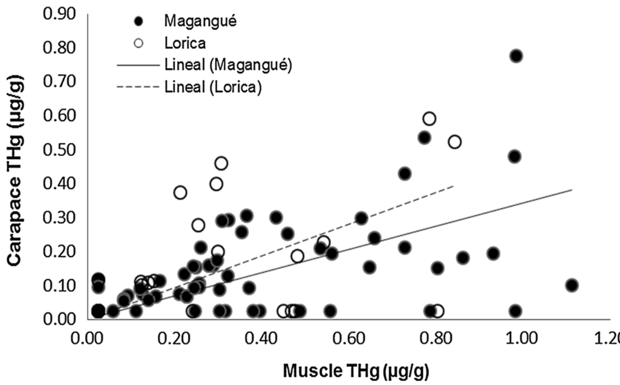

Fig. 3 Individual muscle and carapace THg concentrations for *T. callirostris* individuals collected in Magangué ($y = 0.2516x + 0.0538$; $r_s = 0.4437$; $p = 0.0007$) and Lorica ($y = 0.3685x + 0.0497$; $r_s = 0.5536$; $p = 0.003$)

the two study sites, the Magdalena River drainage has had a greater incidence of small scale and artisanal gold extraction operations, and thus probably suffers more effects from those activities.

In both sites, THg concentrations in muscle were higher than in carapace, which had higher concentrations than blood samples. This same tendency was reported for the sea turtles *Caretta caretta* and *Chelonia mydas* (Sakai et al. 2000). However, some authors have reported a higher concentration of THg in carapace than in blood or muscle (Day et al. 2005; Golet and Haines 2001; Schneider et al. 2009), attributing this to a possible strategy of sequestering Hg in this inert tissue as a mechanism to mitigate the impacts on the health of the individual.

Because obtaining carapace tissue requires a less invasive sampling procedure, and because its concentrations are more stable (Day et al. 2005), this tissue is ideal for monitoring exposure to environmental Hg in turtles on a long-term scale. It also permits sampling live individuals without having to sacrifice them, or using material from dead animals (local people often discard carapaces near their homes), in both cases with a minimal risk of sample degradation. For *T. callirostris*, the carapaces sample from Magangué and Lorica appeared to be a suitable material for Hg monitoring because there was a high correlation between carapace and muscle concentrations (Fig. 3).

No significant correlation was found between Hg levels in blood and the other tissues, probably due to many values for blood samples that were below the detection limit. Blood and muscle are likely to be more variable depending on daily intake and elimination of Hg, whereas carapace integrates mercury intake over longer periods of time (Day et al. 2005).

In addition, the importance of body size to mercury accumulation is widely recognized in aquatic organisms. Usually, older organisms show higher Hg levels as a consequence of a longer exposure time. A significant correlation was documented between SCL and THg concentrations in carapace ($r = 0.45$; $p < 0.001$) for the individuals obtained in Magangué, but not between SCL and THg concentrations in muscle ($r = 0.25$; $p = 0.06$) or blood ($r = 0.05$; $p = 0.70$) tissues there, or among SCL and any tissue type for the individuals obtained in Lorica (muscle: $r = 0.07$; $p = 0.69$; carapace: $r = 0.26$; $p = 0.15$; blood: $r = 0.05$; $p = 0.79$). The lack of correlations in Lorica may have been related to the smaller sample size there, as well as the relatively small range of body sizes included in this study. A lack of correlation between body size and mercury levels also was reported by Golet and Haines (2001) who studied *Chelydra serpentina*, and Schneider et al. (2009) who studied *Podocnemis erythrocephala*. They suggested that chelonians may eliminate mercury from body tissues at higher rates than fish, and thus may reach equilibrium with ingested mercury. This hypothesis is supported by the lack of correlation found in this study between body size and blood and muscle Hg levels in *T. callirostris*; there was, however, a strong correlation between carapace Hg concentrations and body size. The results of this study confirm that a correlation between Hg and turtle size can be expected when analyzing carapace tissue, but the mechanisms associated with tissue distributions of Hg in turtles and the role of the carapace as either a Hg storage compartment or as an elimination vector remains unknown.

Although all forms of mercury can accumulate to some degree, methylmercury (meHg) is absorbed and accumulates to a greater extent than other forms. This form accumulates in the tissues via biomagnification, where meHg concentrations increase as it moves from one trophic level to the next (USEPA 1997). According to the above, omnivorous or carnivorous species usually present higher levels of accumulation than herbivores (Bergeron et al. 2007). *T. callirostris* is a diurnal omnivore, consuming primarily algae and aquatic vegetation, but opportunistically also feeding on tadpoles, annelids, mollusks, insects and other arthropods, and even dead fish and nutrient-rich substrates (Bock et al. 2012). This diet makes the species susceptible to Hg bioaccumulation, especially from the Magdalena River fish species component of its diet, as high levels of Hg have been documented in tissues of a number of sympatric fish species in this drainage (Alvarez et al. 2012). Its feeding ecology also makes it susceptible to inorganic mercury exposures to a certain extent. Inorganic mercury can also be absorbed, but is generally taken up at a slower rate and with lower efficiency than is meHg (USEPA 1997). Therefore, study of Hg speciation in different body tissues and in carapace should be considered in future research.

The mean concentrations of THg documented for the tissues of *T. callirostris* in this study did not exceed the maximum level (0.5 µg/g Hg) recommended by the World Health Organization in fish species consumed by humans (WHO 2000), but 29.3 % and 10 % of the sampled turtle individuals from Magangué and Lorica, respectively, did exceed this level. Many turtle species are traditionally consumed in different regions of the country and may be important sources of protein for the local human populations.

Trachemys callirostris is relatively sedentary and does not exhibit extensive nesting migrations (Bock et al. 2012), making it a potentially excellent indicator species in terms of micro-habitat quality.

This is the first report of Hg accumulation in *T. callirostris* in Colombia. A long-term monitoring program should be established in the contaminated areas for the purpose of providing turtle consumption warnings to the public for those locations where such a warning is warranted.

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