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Non-lethal sampling for mercury evaluation in crocodilians

Wilkinson L. Lázaro [a], Robson F. de Oliveira [a], Manoel dos Santos-Filho [a], Carolina J. da Silva [a], Olaf Malm [b], Áurea R.A. Ignácio [a], Sergi Díez [c,*]

[a] Mato Grosso State University, UNEMAT, Biology Department, Cáceres, Brazil
[b] Federal University of Rio de Janeiro, Carlos Chagas Filho Biophysics Institute, Eduardo Penna Franca Radioisotopes Laboratory, Rio de Janeiro, Brazil
[c] Environmental Chemistry Department, Institute of Environmental Assessment and Water Research, IDAEA-CSIC, Barcelona, Spain

HIGHLIGHTS

- Hg in crocodilians was successfully studied by means of non-lethal tissues.
- Claws and caudal scutes are useful for estimating Hg bioaccumulation over time.
- Highest Hg in claws was related to the flood season and mining gold sites.
- Claw tissue is a better indicator to assess both levels and spatial patterns of Hg.

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ABSTRACT

Mercury (Hg) is a ubiquitous environmental contaminant that poses potential threats to ecosystems due to its toxicity to humans and wildlife. The development of non-lethal sampling techniques is a critical step for evaluation of Hg in threatened species in tropical floodplain environments, where most of Hg found is the result of land use and gold mining activities, and more methylation sites are available. We evaluated the spatial and seasonal effectiveness of caudal scutes and claws to estimate Hg bioaccumulation in crocodilians (*Caiman yacare*), in the scarcely documented Pantanal. Hence, we investigated the potential for Hg bioaccumulation in top predators according to its proximity to mining sites, and in water bodies with different hydrological characteristics and connectivity with the main river during two phases of the flood pulse (dry and flood). The highest Hg concentrations were detected in caimans captured close to mining activities, in claws (2176 ng g^{-1} ww) and caudal scutes (388 ng g^{-1} ww). THg concentration in claws was related to the flood season and its mean concentration was thirteen fold higher than Hg concentration in scutes during whole year. Both tissues were found to be effective as non-lethal sampling techniques for measuring Hg bioaccumulation in reptiles over time. Nevertheless, claw tissue seems to have a more consistent result, since its constitutional chemical characteristics makes it a better indicator of spatial patterns that influence on Hg exposure.

1. Introduction

The problem of environment contamination of mercury (Hg) has been an issue in Brazil, especially in the Amazon and Pantanal, which are two of the world's largest floodable areas (Akagi et al., 1995; Malm et al., 1995; Hylander et al., 2000; Guimarães et al., 2000; Del Lama et al., 2011; Lázaro et al., 2013). Informal gold prospecting in the Amazon region has released nearly 2500 tons of Hg directly into water systems and the atmosphere, in different Hg forms from the burning of the gold amalgam (Malm, 1998). Part of that Hg released into the environment reaches the Pantanal area through atmospheric deposition (Artaxo et al., 2000).

The Mato Grosso Pantanal was declared a National Heritage Site in 1988 and a World Natural Heritage Site by UNESCO in 2000, making it the world's third largest floodplain reserve of its kind and the largest continuous floodable area in the planet (Da Silva and Girard, 2004). In the Upper Paraguay River Basin (the Pantanal), gold mining has been documented by Lacerda et al. (1991a,b) and Rodrigues and Maddock (1997). However, in addition to this activity, the conversion of natural areas into grazing and agriculture systems has been pointed out as potential sources of Hg for the aquatic systems of the Pantanal, due to erosion and subsequent remobilization of the soil environment towards the

* Corresponding author.
E-mail address: sergi.diez@idaea.csic.es (S. Díez).

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floodplain from run-off and biomass burning (Artaxo et al., 2000; Cordeiro et al., 2002; Godoy et al., 2002).

Despite this scenario, only a handful of studies have been conducted on monitoring the effects of Hg environmental contamination on Pantanal wildlife. The main works deal with fishes and birds, excluding one of the main representatives of Pantanal fauna: *Caiman yacare* (Daudin, 1802), a large scavenger and predator of the Pantanal. This species are listed on the IUCN Red List of Threatened Species (IUCN, 2013). Estimates indicate over 4 million *C. yacare* populate the 140,000 km^2 of flooded area in the region (Mourão et al., 2000). Moreover, *C. yacare* is one of the preferred prey of jaguars (*Panthera onca*) the largest feline in the Americas (Azevedo and Verdade, 2012).

Given their long lifespan and high position in the aquatic and aquatic–terrestrial trophic chains, Alligatoridae are susceptible to exposure and accumulation of environment contaminants released in the atmosphere or discharged directly into their water system habitats. The specific characteristics of the group allow an accurate diagnosis of the conditions imposed by these pollutants in the study areas. Several different xenobiotics – primarily metals and organochlorine pesticides – have been detected in Alligatoridae at countless locations worldwide (Ding et al., 2001; Rumbold et al., 2002; Campbell, 2003; Roe et al., 2004; Almli et al., 2005; Jeffree et al., 2005; Presley et al., 2006; Wu et al., 2006; Xu et al., 2006; Yoshikane et al., 2006).

This study has two main objectives: (1) to evaluate concentrations of THg in *C. yacare* at flood-prone areas of the northern Pantanal, and relate the variation in Hg of the specimens to areas with and without a history of gold mining and to hydrological cycles (flood and drought) and (2) to assess the relationships between Hg concentrations in caudal scute and claw to test which of these non-lethal sampled tissues is the best predictor of Hg concentration in crocodilians.

2. Methodology

2.1. Study area

The Pantanal is a vast floodplain within the Upper Paraguay River Basin, located in the Center West region of Brazil, east of Bolivia, and northeast from Paraguay. This humid area is still in pristine condition and its ecological integrity is highly related to hydrology and its monomodal flood pulse. The floodplain regulation of the water balance affects biogeochemical cycles and the biology and ecology of the Paraguay-Paraná watershed (Da Silva and Girard, 2004; Junk et al., 2011).

This work was carried out at two points of the Upper Paraguay River Basin, northern Pantanal: one location in the Paraguay River around Descalvados Farm, municipality of Cáceres-MT, located at 57°30′00″W and 16°40′00″S, with no artisanal and small-scale gold mining (ASGM) influence; another in the Bento Gomes River, near the city of Poconé-MT, located at 56°30′00″W and 16°20′00″S, in an area with a history of influence from gold prospecting (Fig. 1).

2.2. Sample capture and collections procedures

Removal of claws and scutes in crocodilians is a marking technique commonly used in the field and in captivity (Richardson et al., 2002; Campos et al., 2014), but which can also be used as non-lethal techniques to acquire tissue samples for environmental contaminant analyses. Then, biological marker samples such as claws and caudal vertical scutes were employed as an alternative to the use of (lethal) muscle tissue or (invasive) blood. They are relatively simple to collect and should contain high concentrations of Hg due to their high keratin content (Alibardi, 2003). These tissues are also devoid of bones or muscles, which could act as sources of varying concentrations of Hg (Richardson et al., 2002; Schneider et al., 2015).

Wild caiman exemplars were captured at night, with the aid of a specially fashioned loop. A total of 39 animals were captured – 7 individuals during the flood period and 10 during the drought period in the Paraguay River, and 9 in the flood and 13 during the drought in the Bento Gomes River. The animals were roped by the neck and immobilized, and afterwards we measure the snout-vent-length (SVL), placing the caiman on its back and measure the distance from the tip of the snout to the end of the cloacal scales with a measuring tape. We identified the sex of the caiman by cloacal examination of the genitalia. Finally, biological tissue samples were collected by clipping one or two of the raised single caudal scutes and claws from the rear right foot with the aid of scalpels and pliers, respectively.

The samples were packed individually in sealed plastic bags, labeled and stored in a freezer, where they were kept frozen until Hg quantification. Crocodiles were then released at the site of capture.

2.3. Analytical methods

Analyses used samples with ~0.5 g fresh weight of tail scutes and ~0.05 g dry weight of claws. The samples were subjected to acid digestion using 4 mL of sulfonitric solution (H_2SO_4:HNO_3, 1:1 *v*/*v*) (Merck p.a.) and 1 mL hydrogen peroxide (H_2O_2) (Merck p.a.) in a 5 mL centrifuge tube. They were kept in water bath at 60 °C for approximately 45 min. Next, 5 mL of $KMnO_4$ solution at 5% (Merck p.a.) were added, then returned to water bath at 60 °C for 15 min, left to rest overnight, then neutralized by adding 1 mL of 12% hydroxylamine chloride (NH_3ClOH + NaCl).

Total Hg concentrations in biological samples were determined in the Laboratório de Radioisótopos Eduardo Penna Franca, Federal University of Rio de Janeiro (UFRJ) by cold vapor atomic absorption spectrometry with a Flow Injection Mercury System (FIMS) and FIAS 400 (Perkin Elmer, USA) equipped with auto sampler AS90 (Perkin Elmer, USA) and using sodium borohydride as a reducing agent (Kehrig et al., 2008).

The detection threshold was 0.01 ppb, and the method was validated in accordance to the standardized material (DORM-2, *Dogfish muscle sample*) provided by the National Research Council – Canada. Total Hg quantified in the reference material was within 95% and 98% of the mean certified values. The overall reproducibility for the analysis period was determined from the results obtained using certified samples. The coefficient of variation (SD/mean) for the duplicate samples was less than 10%.

2.4. Statistical analyses

In order to assess the variation in THg concentrations of claws and caudal scutes in relation to collection sites and periods of the water cycle, we used One-Way Analysis of Variance (One-Way ANOVA). Post-hoc Tukey-HSD tests were performed to identify the areas and/or periods where Hg concentrations differed significantly. THg variations in the target tissues with regard to sex of the individuals were evaluated using *F* test. Analysis of covariance (ANCOVA) was used to compare Hg concentrations in various tissues and sites, with SVL as a covariate to account for potential effects of size. All analyses were carried out using *R* (R Development Core Team, 2011) statistical interface, version 3.0, with an attributed significance of 0.5%. Normality and homoscedasticity were verified and when a variable did not meet these assumptions, then it was log transformed (Log_{10}). All THg concentration values referenced in the text are given as means and standard deviation in wet weight (ww).