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Preferential Liver Accumulation of Mercury Explains Low Concentrations in Muscle of *Caiman yacare* (Alligatoridae) in Upper Amazon

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Abstract

Caiman yacare is considered one of the top predators in the Amazon basin, and understanding pollutant distribution within its tissues may help its sustainable management. As a top predator, *C. yacare* should have the highest mercury concentrations, but has lower Hg concentrations than carnivorous fish (Rivera et al. 2016), which are part of their diet. We compared total Hg among liver, kidney, fat, and muscle of *C. yacare*, and whether trends in the distribution of Hg among tissues were like other crocodilians, aquatic birds, omnivorous, and carnivorous fish. Fat had the lowest concentrations (0.025 ± 0.03 mg kg^{-1}) followed by muscle (0.15 ± 0.06 mg kg^{-1}), kidney (0.57 ± 0.30 mg kg^{-1}) and liver (1.81 ± 0.80 mg kg^{-1}). Such preferential accumulation makes *C. yacare* meat a safer alternative for human consumption than carnivorous fish. The relation between Hg accumulation in liver and muscle is highest in crocodilians, which has evolutive and environmental implications.

Keywords Crocodilian · Mercury decontamination · Sustainable management · Mercury in kidney

Mercury contamintion has been a growing concern for a long time in tropical regions such as the Amazon basin (Nevado 2010; Vieira 2018). Riparian populations for whom fisheries are their most significant sources of protein are particularly exposed to such contaminant (Maurice-Bourgoin et al. 2000; Barbieri et al. 2009). Mercury in the Amazon is naturally abundant in the soil, and rivers (Kasper et al. 2018). The soil erosion, together with artisanal gold mining, installations of hydroelectric dams and deforestation, are important sources of inorganic mercury forms (Hg^0 and Hg^{2+}) (Veiga and Hinton 2002; Magarelli and Fostier 2005).

Mercury concentrations in the biota of the Amazon are generally high (Pouilly et al. 2004; Schneider et al. 2009; Souza-Araujo et al. 2015). Mercury in its organic form methylmercury (MeHg) is easily bioaccumulated and biomagnified through the food chain (Molina et al. 2010; Pouilly et al. 2013) and it is the most toxic form of mercury (Clarkson and Magos 2006). Thus, long-lived, larger organisms and those species higher in the food chain tend to have a higher concentration of mercury (Silva et al. 2005; Pouilly et al. 2013). Consequently, the top predator may have the highest mercury concentrations in its tissues (Rumbold et al. 2002). In addition to fish, human population living along the river also consume meat from crocodilians, which may be considered an added risk of exposure to mercury. Surprisingly, a risk analysis for alligators in the Brazilian Amazon suggest that its consumption does not represent a significant risk (Correia et al. 2014).

Caiman yacare (Yacare from now on) is considered one of the top predators in the Amazon basin (Sergio et al. 2014). Adults can reach ~ 2.5 m. Amazonian crocodilians'

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diet varies with ages, sex, season, habitat, year, and availability of prey (Santos et al. 1996; Grigg and Kirshner 2015). Yacare position in the trophic chain should result in high concentrations of mercury as seen for other species of South American crocodiles (Vieira et al. 2011; Schneider et al. 2015). However, Rivera et al. (2016) reported mercury concentration in the muscle tissue of the Yacare below the levels seen in carnivorous fish, which are part of the Yacare diet. Rivera et al. findings are against our general understanding of mercury biomagnification in the food web and mercury accumulation in crocodiles as top predators (Duvall and Barron 2000; Khan and Tansel 2000). It has been shown that mercury could be stored preferentially in different tissues of crocodilians (Rumbold et al. 2002; Buenfil-Rojas et al. 2018), which may explain Rivera's findings.

The population of Yacare is commercially exploited in Bolivia since 1999 under the National Program of Conservation and Sustainable trading of the *Caiman yacare* (MMAyA 2009; CIPTA and WCS 2010). For indigenous communities, Yacare and other caiman species constitute an essential source of meat for income and human consumption (Figueiredo et al. 2015). In this study, we investigated mercury concentrations in different tissues of Yacare, particularly those that could be a potential exposure pathway for Tacana people on the seasonal harvest, such as fat which they use for consumption as medicine, and the tail muscle since it is used for sale and for local consumption. We also analyzed the available published data to evaluate if the tendencies observed on mercury accumulation in Yacare are exclusive of such species or group of organisms.

Materials and Methods

Samples were collected at the Colorado-Majal lakes system within the Cachichira community (Fig. 1), which is part of the Tacana indigenous territory ("Tierra Comunitaria de Origen I", TCO Tacana I from now on). The TCO Tacana I is located in the north of La Paz Department, bounded on the east by the Beni River to the southwest with Madidi National Park and the northwest by the Undumo River.

The Beni river is a dynamic hydrological system with a large number of small and medium-sized oxbow lakes. Colorado-Majal lakes system is part of the floodplain of Beni river and is the same area which have been studied by Rivera et al. (2016). Yacare Caiman (*Caiman yacare*), Black Caiman (*Melanosuchus niger*), and river turtles such as Yellow-Spotted (*Podocnemis unifilis*) are common in the area.

Samples were collected in October 2017 during commercial harvest. The total length (TL) of harvested specimens ranged between 177 and 220 cm, and the weight was between 24 and 46 kg. Such measures correspond to those of sizeable male adult individuals, since the harvest program called Asociación Matusha Aidha (2016) and MMAyA (2009) only allows to kill males > 180 cm of TL which is equivalent to 90 cm of head-body length (HBL), corresponding to class IV, which are those individuals that correspond to measurements previously mentioned.

Immediately after Yacares were killed (n = 7), 28 samples were taken of four different tissues (liver, fat, muscle, and kidney). The samples were obtained using plastic knives one for each tissue. At least 1 g of each type of tissue was collected and placed into cryo-tubes and immediately frozen in liquid nitrogen for their conservation until analysis. Samples

Fig. 1 Sampling location inside Cachichira community (TCO Tacana I) area, upper Amazon

were analyzed using a variation of the method described by Rivera et al. (2016) (details in the supplementary materials).

Data were evaluated for normality with the Shapiro-Wilk test. Kruskal-Wallis one-way analysis of variance test was used to compare Total mercury (THg from now on) among tissues and a Turkey post-hoc analysis. We also evaluated the correlation of THg concentration between each kind of tissue (liver, muscle, fat and kidney) with a Spearman's non-parametrical correlation test (r_s). Mercury concentration in Yacare organs was compared with that of birds, carnivorous and omnivorous fish, and other species of crocodilians obtained from the published literature (Table S1). We used one-way ANOVA on Ranks to compare THg concentration in bird's muscle (BM), bird's liver (BL), carnivorous fish muscle (FCM), carnivorous fish liver (FCL), omnivores fish muscle (FOM), omnivorous fish liver (FOL), Crocodilian's muscle (CM) and Crocodilian's liver (CL). Finally, we evaluated the relationship between different organ concentrations using linear regression analysis after logarithmic transformation. We used an $\alpha = 0.05$ for all tests. All analyses were conducted in PASW SPSS® version 19.0 for Windows (SPSS Inc. Chicago. IL, USA) (IBM 2015), and SigmaPlot 12.0.

Results and Discussion

All Yacare samples tissues analyzed had a detectable concentration of THg. The median of THg concentration for liver was 1.81 ± 0.80 mg kg^{-1}, for kidney 0.57 ± 0.30 mg g^{-1}, for muscle 0.15 ± 0.06 mg kg^{-1} and for fat 0.025 ± 0.03 mg kg^{-1} wet weight (w.w). THg concentrations in different tissues were significantly different ($H = 24.340$, $p < 0.001$) (Fig. 2). Liver had higher concentrations than muscle and fat ($p < 0.05$), but not statistically difference with kidney ($p = 0.481$). Although, a review of the literature reported significant correlations between the hepatic and muscle tissues in species such as *Alligator mississippiensis* (e.g. Burger et al. 2000), no significant correlation (Table S2) was found among our samples. Changes in Hg concentrations with size or age (Lawson et al. 2020), or changes in diet (Schneider et al. 2012; Lázaro et al. 2015) may explain such inconsistency. Rivera et al. (2016) showed that there was significant correlation between Hg in muscle and animal size and weight, but the size range of Rivera and this study is too narrow for any further analysis. Since we collected all samples from the same type of habitats it is unlikely to have large variations in diet.

Metal accumulation varies among aquatic and terrestrial species depending on a variety of factors (Smith et al. 2007). Metals tend to have organ-specific affinities, and in turn, organs tend to serve as metal-specific locations for metal accumulation, which is known as organotropism (Norris 1997). In our study, the high Hg concentrations in the liver and the kidney are not surprising because they can accumulate divalent ions such as Hg^{2+} due to the presence of abundant cysteine, metallothionein and glutathione (Zalups 2000). The accumulation of Hg into the kidney of various reptiles has been well studied mainly in species of crocodilians. Consistently with our data, high Hg concentration has been shown to accumulate in the kidney of *Alligator mississippiensis, Alligator sinensis* and *Crocodylus moreletii* (Yanochko et al. 1997; Jagoe et al. 1998; Khan and Tansel 2000; Buenfil-Rojas et al. 2018) and also in marine turtles such as *Eretmochelys imbricata* and *Chelonia mydas* (Anan et al. 2001; Sakai et al. 2000). The presence of Hg in the kidney can lead to a Chronic kidney disease (CKD) (Orr and Bridges 2017) which characterized by a permanent loss of nephrons and an eventual decline in glomerular filtration rate (GFR) (Zalups and Diamond 1987). Future studies about detoxification pathway in kidney and liver of Yacare, will provided more information for a better understanding of pollution distribution and may help its sustainable management.

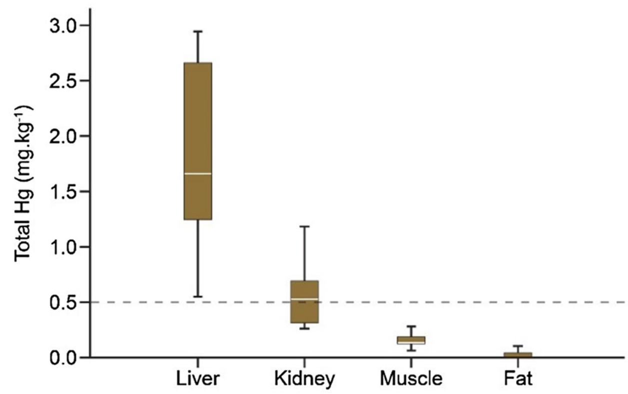

Fig. 2 Median with maximum and minimum values of total mercury concentration between different tissues of *Caiman yacare* harvested, in Colorado-Majal lakes system of TCO Tacana I. The horizontal line at 0.5 mg.kg^{-1} indicates the recommended limit by the WHO for human consumption

The highest concentrations of THg found in our Yacare liver samples relative to other tissues agrees with data from other species of crocodilians and also other vertebrate's (Rumbold et al. 2002; Lucia et al. 2010; Branco et al. 2011; Souza-Araujo et al. 2015; Schneider et al. 2015; Buenfil-Rojas et al. 2018) (Table S1). Hg may be initially retained in the liver before distribution to other tissues and also before excretion as a result of higher metallothionein production (Piotrowski et al. 1974; Zalups and Koropatnick 2000). Still, the ratio between liver and muscle Hg concentrations is much higher in crocodilians than in any other group (Fig. 3), suggesting that differential accumulation or retention of Hg in our liver samples is higher. The relatively high concentrations of THg in liver may be related to the role of liver

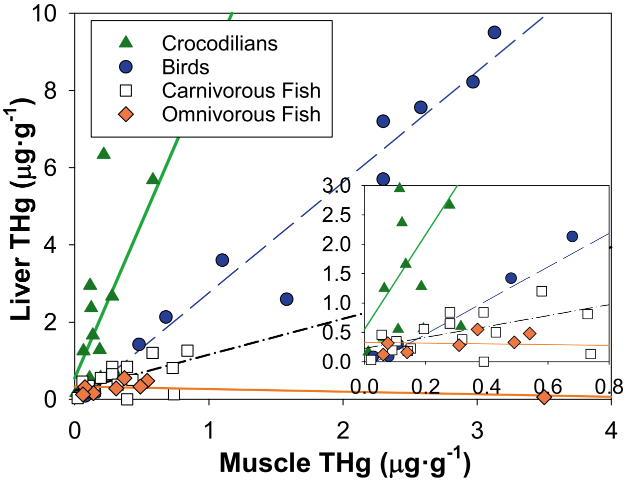

Fig. 3 THg concentration and regression lines between muscle and liver of crocodilians (green triangles and green solid line, including this study), birds (blue squares and dashed blue line), carnivorous fish (white squares and black dashed point line), and omnivorous fish (orange diamonds and orange solid line). Source references and data Table S1

in detoxification (Branco et al. 2011; Buenfil-Rojas et al. 2018).

Liver produces metallothioneins (MTs), a cysteine-rich metal-binding proteins, and Glutathione-s-transferase (GST) in response to Hg and other metal as a decontamination pathway (Cosson 1994; Thomas et al. 1994; Gunderson et al. 2016). The expression of such proteins is dependent on age, sex and metal exposure (Gunderson et al. 2016), which may explain the higher Hg concentration ratio between liver and muscle when compared to other crocodilians. Regardless, the low THg concentration in fat tissue of Yacare is good news for Tacana people because they can continue using it every seasonal harvest for oil preparation and its consumption as medicine (Azevedo et al. 2020).

The ratio of THg concentration between liver and muscle is highest in crocodilians and birds than in carnivorous and omnivorous fish (Fig. S1). The fish liver is a target organ for Hg accumulation (Hosseini and Bagher 2013). Still, in general, there is a relatively larger accumulation of Hg in muscles (Kaoud and El-Dahshan 2010), as also reported for birds (Lucia et al. 2010). The variation of Hg concentration between vertebrates may depend on the environmental conditions and intrinsic factors.

The MTs involvement in the sequestration, bioaccumulation, and detoxification of metals in teleosts, birds and mammals is not new (Hamer 1986; Nordberg and Nordberg 2009). However, published reports support our hypothesis that, there are differences between MTs production among vertebrates (Vasak et al. 2005; Trinchella et al. 2008; Buenfil-Rojas et al. 2015). While there is a clear relation between metal exposure and MT and GST concentrations in crocodilians (Gunderson et al. 2016; Buenfil-Rojas et al. 2018), there is no such relation at least in some fish species (Mieiro et al. 2011). Some studies claim that the measurement of MT concentrations may be a suitable tool for routine monitoring of metal exposure and toxicity and also understanding the relationship between MTs of vertebrates (Trinchella et al. 2008; Andreani et al. 2008). Since crocodilians order has been top predators for a very long time through their evolution (Grigg and Kirshner 2015), they may have been exposed to high MeHg concentrations and evolved more efficient or more extensive liver mediated decontamination processes.

Crocodiles were suggested as a very good biomonitor of mercury due to their high trophic status and ability to bioaccumulate Hg in tissues (Khan and Tansel 2000). However, Rivera et al. (2016) and our data suggest that a better understanding of Hg accumulation in crocodilians tissues is needed. Such information also has the potential to be useful to the sustainable management of species and overall, to species conservation. We suggest that further studies should consider physiological pathways of Hg on crocodilians.

Conclusions

Our results show that *Caiman yacare* accumulates most Hg in their liver and kidney, which means that Tacana population may continue the consumption of Yacare meat on the seasonal harvest and continue with its commercialization. Also, they can use Yacare fat for oil preparation and consumption. It is also noticeable that the information obtained from the available scientific literature shows that mercury accumulation in tissues is highly dependent on the vertebrate group, which has evolutive and environmental implications. Additionally, understanding pollutant distribution in different organisms may help its sustainable management.

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