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Short communication

Low total mercury in *Caiman yacare* (Alligatoridae) as compared to carnivorous, and non-carnivorous fish consumed by Amazonian indigenous communities[☆]S.J. Rivera^a, L.F. Pacheco^b, D. Achá^{c,*}, C.I. Molina^{c,d}, G. Miranda-Chumacero^{a,d}^a Wildlife Conservation Society, Greater Madidi-Tambopata Landscape Conservation Program, La Paz, Bolivia^b Colección Boliviana de Fauna, Instituto de Ecología, Universidad Mayor de San Andrés, P.O. Box 10077, La Paz, Bolivia^c Unidad de Calidad Ambiental, Instituto de Ecología, Universidad Mayor de San Andrés, P.O. Box 10077, La Paz, Bolivia^d Instituto de Ecología, Unidad de Limnología, Universidad Mayor de San Andrés, P.O. Box 10077, La Paz, Bolivia

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ABSTRACT

Mercury contamination in the River Beni basin is an important health risk factor, primarily for indigenous communities that live along the river. Among them are the Tacana, living in their original territory with sustainable use of their natural resources, consuming fish, *Caiman yacare*, and other riverine resources as their main source of protein. To assess mercury exposure to Tacana people, total mercury (THg) was evaluated in the muscle of seven commercial fish, and *Caiman yacare* (yacare caiman) during 2007 and 2008. THg was extracted by acid digestion and concentrations were determined by atomic absorption spectrometry. Mean mercury concentrations in *C. yacare* was $0.21 \pm 0.22 \mu\text{g g}^{-1}\text{Hg w.w.}$ (wet weight), which is lower than expected given its high trophic level, and its long life-span. It is possible that mercury in *C. yacare* is accumulated in other organs, not included in this study; but it is also possible that physiological mechanisms are involved that help caimans get rid of ingested mercury, or simply that *C. yacare*'s diverse diet reduces THg accumulation. Carnivorous fishes (*Pygocentrus nattereri*, *Pseudoplatystoma tigrinum*, *Zungaro zungaro*, *Plagioscion squamosissimus*, and *Leiarius marmoratus*) had the highest total mercury concentrations, ranging from 0.35 to $1.27 \mu\text{g g}^{-1}\text{Hg w.w.}$ moreover, most were above the limit recommended by WHO ($0.5 \mu\text{g g}^{-1}\text{Hg w.w.}$); except for *Leiarius marmoratus*, which presented a mean of $0.353 \pm 0.322 \mu\text{g g}^{-1}\text{Hg w.w.}$ The two non-carnivorous fish species (*Prochilodus nigricans*, and *Piaractus brachipomus*) present mean concentrations of 0.099 ± 0.027 , and $0.041 \pm 0.019 \mu\text{g g}^{-1}\text{Hg w.w.}$, respectively. Finally, recommendations on the consumption habits of Tacana communities are discussed.

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1. Introduction

Mercury pollution in the Amazon basin is a growing concern, mainly for indigenous riparian populations for whom fisheries are their major sources of protein (Barbieri et al., 2009; Benefice et al., 2008; Maurice-Bourgoin et al., 2000). Mercury in the Amazon is naturally abundant in soils, and erosion is thought to be an important source of inorganic mercury (Roulet et al., 1998), together with artisanal gold mining (Maurice-Bourgoin et al., 2000) and deforestation (Roulet et al., 1998).

Methylmercury (MeHg), an organic form of mercury, is one of

the most toxic chemical species (Lebel et al., 1998; Tamm et al., 2006), produced by the transformation of the inorganic mercury via biotic and abiotic processes. Sulfate-reducing bacteria (SRB) are frequently the main (though not the only) mercury methylators (Achá et al., 2011; King et al., 2000; Macalady et al., 2000). Mercury methylation in Amazonian Lakes takes place predominantly in the periphyton associated with different macrophytes (Acha et al., 2005; Guimarães et al., 2000a, 2000b). Periphyton is an important carbon source and could be the main entrance of MeHg to the food chain (Molina et al., 2010). In the aquatic food web, Hg is mainly found as MeHg.

Methylmercury is easily bioaccumulated and biomagnified through the food chain (Barbosa, 2003; Molina et al., 2010; Pouilly et al., 2013). Therefore, long-lived (Khan and Tansel, 2000), larger organisms (McArthur et al., 2003; Watras et al., 1998) and species higher in the food chain tend to have higher concentrations of

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mercury (Da Silva et al., 2005; Pouilly et al., 2013; Trudel and Rasmussen, 2001). Consequently, top predators may have the highest mercury concentrations (Cabana et al., 1994; Duvall and Barron, 2000; Rumbold et al., 2002) and potentially show the highest risk for human exposure to Hg. At the Amazon, mercury exposure is mostly related to fish consumption (Maurice-Bourgoin et al., 2000) and in particular to the consumption of carnivorous fish species (Passos et al., 2003), but little is known about the risk of *Caiman yacare* meat consumption. This information is particularly valuable for control methylmercury exposure in human populations since there are strong relationships between the quantity, frequency and type of fish consumed and mercury concentrations in human hair (Dolbec et al., 2001; Passos et al., 2003).

Caiman yacare (Yacare from now on) is considered as a top predator in the Amazon basin because it feeds mostly at the top of the food web (Table S1) and is almost free from predators (Sergio et al., 2014). Adults can reach ~2.5 m of total length and feed mainly on fish; although they are highly opportunistic predators that may also consume water birds and small mammals (Santos et al., 1996). Yacare populations are managed under a sustainable harvesting program in Bolivia (CIPTA and WCS, 2010). Yacare and other caiman species constitute an important source of meat for indigenous communities (Constantino et al., 2008; Figueiredo et al., 2015). Caiman consumption is growing so much that there is research on how to better process the meat (Romanelli et al., 2002; Telis et al., 2003). Yacare meat is rich in proteins, and it may be an important source of Omega-3 fatty acids (IBCE, 2010). Its position in the trophic chain generates a high concern of potential Hg exposure for the consumers, as high mercury concentrations have already been reported in other species of South American crocodilians (Schneider et al., 2015; Vieira, 2011). Here we investigate the concentrations of total mercury in *Caiman yacare* and compare it to the mercury concentration in seven fish species (five carnivorous and two non-carnivorous), which are all common in the diet of the Tacana people in the Beni river basin (Miranda-Chumacero et al., 2011). Under the hypotheses that Yacare occupies the highest trophic level, we expected to find the highest level of THg in *Caiman yacare*, followed by carnivorous fishes and non-carnivorous species.

2. Methods

2.1. Study area

All the sampling was conducted in the area of sustainable management of the indigenous Tacana land “Tierra Comunitaria de Origen Tacana I” (TCO Tacana I, Fig. 1). The area encompasses ~3719 km² within the Beni river basin. Legal harvesting of Yacare takes places within an area of about 1300 km² (CIPTA and WCS, 2002). A total of 17 locations were sampled, including a large river (Beni River), three smaller rivers and nine small lakes. The number of samples per location varied from one to nine.

The Beni is a “white waters” large river, characterized by neutral pH, low oxygen concentrations, and high concentrations of suspended particles (Gautier et al., 2007). The hydrological and geomorphological dynamics of the river generate a large number of small and medium-sized oxbow lakes, inhabited by Yacare, other common large reptiles like the black caiman (*Melanosuchus niger*), and yellow-spotted river turtles (*Podocnemis unifilis*) as well as a high diversity of fish species.

2.2. Yacare sampling

Since people consume mainly the tail of Yacare, all samples were collected from this part of the body. A total of 64 muscle samples were collected from individuals immediately after they were

captured during commercial hunting, between September and October of 2007 and 2008. Only animals larger than 1.8 m were sampled, since harvesting regulations only allow the capture of those individuals, under the assumption that most are males (Llobet et al., 2004). Capture site (GPS location, habitat type), and standard measurements (total length, sex, and weight) were recorded for each sampled individual. Samples were about 2 cm³, and their collection was as clean as possible, removing any fat, bone, and skin. Samples were placed into Teflon tubes and immediately cryo-frozen using liquid nitrogen for cryo-conservation.

2.3. Fish samples

Fish samples were obtained from specimens brought for sale by Tacana fishermen to Rurrenabaque, a small town close to the study site. A total of 35 muscle samples from seven different fish species were collected and grouped into carnivorous/piscivorous (C: *Pygocentrus nattereri*, *Pseudoplatystoma tigrinum*, *Zungaro zungaro*, *Plagioscion squamosissimus*, and *Leiarius marmoratus*) and not carnivorous (NC: *Prochilodus nigricans*, and *Piaractus brachipomus*). NC species includes herbivorous and omnivorous species that consume little or no other fish (Ibañez et al., 2007; Pouilly et al., 2004).

2.4. Total Hg determination

Samples were weighed both before, and after freeze-drying (lyophilized) to calculate the percentage of water content in each sample. Lyophilized tissues were ground and homogenized in an agate mortar. Mercury was extracted from 100 mg of the dry sample by acid digestion with 5 mL of HNO₃ (65%) and 0.5 mL of HCl (6 N), at 100 °C for 2 h. After cooling down, the extracts were further digested with 1 mL of H₂O₂ (50%) for 2 h. The final extract was completed to 30 mL with ultrapure water. Samples were digested alongside with reference samples (BCR: tuna fish and Tort-2: lobster hepatopancreas NRCC) (Pérez, 2000). Total Hg was detected after acidification with HCl (3%) and reduction with SnCl₂ (1.5%) with an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer 3110). Concentrations were determined using a standard curve and stability of the instrument was monitored with a standard every five sample readings.

2.5. Data analyses

Each set of data was evaluated for normality with Shapiro-Wilk test. Since most of our data sets fail to pass the normality test total mercury (THg) concentrations among carnivorous, non-carnivorous and Yacare were normalized applying the natural logarithm to compare them using one way ANOVA, followed by Tukey's post hoc pairwise comparison. The same procedure without normalization was applied to compare THg among the different species studied, assuming a normal distribution. Data transformation is always risky when using ANOVA. Therefore, we also performed Kruskal-Wallis one way ANOVA followed by Dunn's post hoc pairwise comparison. 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) or SigmaPlot 12.0.

3. Results and discussion

All 64 Yacare samples had detectable concentrations of THg (mean $0.21 \pm 0.22 \mu\text{g g}^{-1}\text{Hg w.w.}$, Table 1). Among them, about most had concentrations below the recommended limit of Hg content in freshwater fish meat for human consumption ($0.5 \mu\text{g g}^{-1}\text{Hg w.w.}$; WHO, 1991). Only 6 had concentrations higher

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