Mercury and methyl mercury ratios in caimans (*Caiman crocodilus yacare*) from the Pantanal area, Brazil

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The Pantanal region is the largest floodplain area in the world and of great biological importance due to its unique flora and fauna. This area is continuously undergoing increasing anthropogenic threats, and has also experienced mercury contamination associated with gold mining and other anthropogenic activities. Pantanal caimans are top-level predators, and, as such, show great potential to accumulate mercury (Hg) by biomagnification. In this study 79 specimens from four locations in the Pantanal were analyzed for total Hg and methyl mercury (MeHg) by cold vapor atomic absorption spectrometry. Total Hg contents ranged from 0.02 to 0.36 μg g^{-1} (ww), and most specimens presented MeHg ratios above 70%. One of the sites, impacted by anthropogenic activities, presented significantly higher total Hg in comparison to three less impacted sites, supporting the hypothesis that caimans can, in fact, be considered effective bioindicators of ecosystem health.

1. Introduction

The Pantanal region is the largest floodplain area in the world. It extends over central-west Brazil, south-eastern Bolivia and northeastern Paraguay, covering approximately 200 000 km^2 during the rainy season. Due to its unique flora and fauna and great biodiversity, it is considered an area of world interest by UNESCO.[1,2] However, this area presently undergoes several anthropogenic threats, such as the use of agrochemicals, discharge of non-treated sewage water into its rivers and mercury (Hg) contamination due to former gold mining ("garimpo") activities.[2–6]

Hg is an important pollutant and considered one of the most toxic metals, presenting different toxicities depending on its forms.[7–9] In Brazil, gold mining has released more than 2500 tons of this element due to alluvial gold amalgamation during the last 25 years, mainly into the Amazon basin.[10] In artisan gold mining, Hg is used to amalgamate gold particles and separate them from the gangue. Large amounts of metallic Hg are directly released into the rivers and atmosphere as $Hg^0_{(g)}$, since the amalgam heating for gold recovery takes place in the open air. According to Artaxo and collaborators[11] the Pantanal area is an identified hotspot for Hg deposition, including atmospheric regional transport of a fraction of the Hg released in amalgam heating. In flood plains such as the Pantanal, Hg may also be present as dissolved and particulate mercury in river waters, and sorbed to soils particles, brought there by the rivers during the flood season.[5,6]

Despite being a protected area, the use of Hg in gold mining in some Pantanal areas has been well documented.[3,4,12–14] However, in comparison to the Amazon region, gold mining activities in the Pantanal region have been much more restricted and localized. Hg was used in gold mining in the Poconé region, for example, until 1994, when mining was forbidden. High Hg levels have been detected in carnivorous fish caught in some Pantanal sites,[15] however, their link to anthropogenic or natural processes has yet to be established.

Heavy metals have been recognized as a category of environmental contaminant affecting crocodilians,[16] and several researchers have reported the presence of Hg in crocodilian species.[17–20]

Pantanal caimans (*Caiman crocodilus yacare*) are top-level predators and, because of this, they may potentially accumulate substantial Hg burdens in their organs due to biomagnification. Thus, the study of Hg in this species is of the utmost importance. This species is the most widely distributed of the American crocodilians, ranging from southern Mexico to Peru and Brazil. It is also the most geographically variable species, with four or

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Environmental impact

The Pantanal region is an area of great biological importance, due to its unique flora and fauna. Impacts in the area receive much attention, due to possible severe environmental consequences. Biomonitoring studies provide important information on the presence and consequences of environmental contaminants, and it is extremely important to analyze mercury contamination in all environmental compartments. This includes biota, not only for environmental monitoring purposes but also potential consequences for caimans in captivity. The present study contributes to a more comprehensive data literature regarding crocodilians and, specifically, about Hg contamination in the Pantanal caiman, verifying risks for both the caiman populations and aquatic biota of the ecosystem and potential consequences for caimans in captivity.

five subspecies generally recognized, including the *Caiman crocodilus yacare*, the southern subspecies form. This subspecies is found from southern Brazil through the Pantanal region, up to Bolivia, Paraguay, and Argentina. In Brazil, this subspecies can also be found in the Amazon basin; however, the largest population is in the Pantanal region. These animals usually eat fish and other aquatic vertebrates as well as invertebrates (crabs, periwinkles and insects). Unlike other crocodilians, its feeding activity is governed by its habits instead of its size.[21]

Some studies related to Hg levels in crocodilians are found,[16–18,20,22–33] however, none of them specifically address the Pantanal caiman. Thus, the present study consists of an investigation of total Hg and methyl mercury (MeHg) concentrations in Pantanal caimans, and the investigation of their relationship with the capture sites, impacted by human activities in different ways.

2. Materials and methods

2.1. Study area and capture sites

Seventy-nine Pantanal caiman specimens from four different sites in the Pantanal region—Negro river, Nhecolândia, Cuiabá river and Bento Gomes river—were captured (Fig. 1). Caimans are territorial, and the animals do not move between the study sites. The Negro river is a tributary of the Paraguay river, situated in the southern part of the Pantanal, and samples from this site were obtained close to the Paraguay river mouth. This river is a large water flow, running from north to south, joining the Paraná river in Argentina to form the River Plate. Sampling site 2, Nhecolândia, is a permanently dry area, also located in the southern Pantanal. There, the specimens were captured in closed lagoons. No great anthropogenic impact is observed in both these areas, which are mainly occupied by natural landscape and extensive farms that do not conduct intensive agricultural activities, and do not impact the environment, since, in most cases, the land is left alone. Their population density is very low (4000 inhabitants), and no large urban concentration is observed. The other two sampling points are also located at tributaries of the Paraguay river, but in the northern part of the Pantanal region. The Bento Gomes river traverses the Poconé municipality, with approximately 31 000 inhabitants, where "garimpo" activities used to take place. This sampling site is located downstream to this former "garimpo" area. The Cuiabá river also flows in the direction of the Paraguay river and passes

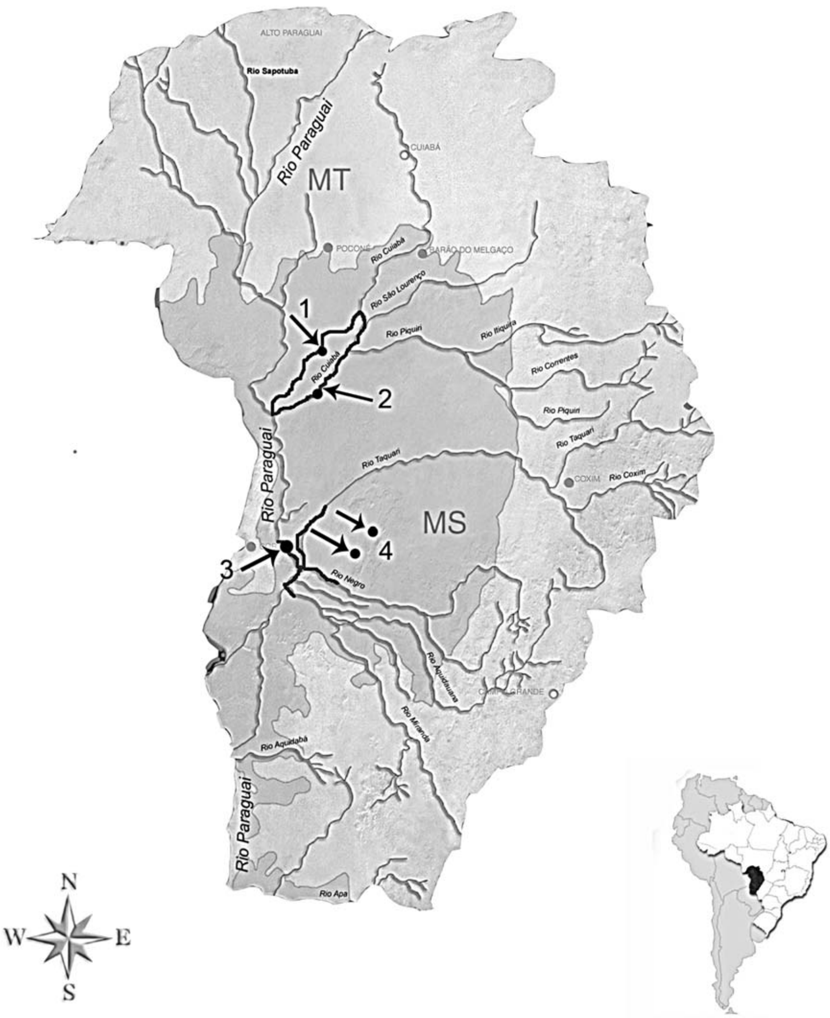

Fig. 1 The Pantanal region and the capture sites: (1) Bento Gomes river, (2) Cuiabá river, (3) Negro river and (4) Nhecolândia.

through the Cuiabá municipality, the capital of the State of Mato Grosso, with approximately 500 000 inhabitants. This sampling point is located downstream from Cuiabá city.

2.2. Chemicals

Ultrapure water (resistivity ≥ 18.0 MΩ) was used for all sample solution dilutions and preparation of the calibration solutions. Nitric (65%) and sulfuric (98%) acids, as well as sodium hydroxide, were from Vetec (Rio de Janeiro, Brazil). All other reagents were purchased from Merck (Darmstadt, Germany). All plastic and glassware were immersed in 20% v/v HNO_3 for at least 24 h, washed with Extran, rinsed 3 times with deionized water and finally rinsed with ultrapure water before analysis.

2.3. Quality assurance and laboratory sample representativeness

Quality assurance was assessed by the parallel analysis of DORM-2 dogfish muscle certified reference material (National Research Council of Canada) for total Hg and MeHg. In order to observe sample representativeness, twelve different aliquots taken from twelve different distinct locations from the tail of a same caiman specimen were analyzed.

2.4. Total and methyl mercury determination

For total mercury determination, muscle samples (0.5 g) were taken from the tail and digested with 10 mL of a HNO_3/H_2SO_4 (1 + 1) mixture in the presence of 0.1% (m/v) V_20_5, at 90 °C, for 1 hour. After cooling, the oxidation was completed by the addition of a sufficient volume of a 5% (m/v) $KMnO_4$ solution. Immediately prior to the instrumental determination by CVAAS, the oxidant excess was reduced by the addition of 50% (m/v) hydroxylamine. This solution was made up to 50 mL, homogenized and 5 mL aliquots taken to the instrument, where mercury was reduced by 1 mL of 20% (m/v) $SnCl_2$ in 50% (v/v) HCl solution.[34] The methyl mercury (MeHg) determination was carried out by a slightly modified[35] Magos procedure.[36] In short, muscle samples (0.2 g) were digested by 4 mL of 45% m/v NaOH, in the presence of 1 mL of 1% m/v cysteine at 90 °C, until a homogeneous medium was obtained. The sample was then made up to 10 mL with 1% m/v NaCl. Prior to the analysis, 5 mL of 1 + 1 H_2SO_4, 1 mL of 1% m/v cysteine, 1 mL of the reductant solution, 1 mL of the digestate, and 15 mL of 40% m/v NaOH were added to the reaction flask. The reaction flask was immediately closed and the mixture stirred for 1 minute. The reduced mercury was then carried to the absorption cell by a 1 mL min^{-1} air flow. Total mercury was determined using a Sn^{2+}/Cd^{2+} mixture as reductant. Inorganic mercury was determined using Sn^{2+} as reductant. Organic mercury was calculated by the difference between inorganic and total mercury. The instrumental measurements were performed using a Varian AA5 atomic absorption spectrophotometer, equipped with a modified Varian VGA 64 vapor generation system.[35] A mercury hollow cathode lamp operated at 5 mA was used as line source. External calibration was performed with calibration solutions prepared in the same media as sample blanks. The limits of detection (3 s) for the whole analytical procedure for total Hg and MeHg were 13 and 15 μg g^{-1} (ww), respectively.

2.5. Statistical analyses

Statistical analyses were performed using SPSS 8.0 for Windows. Descriptive statistics were conducted using box plots, and ANOVA was used to compare the data in relation to the capture sites. A degree of significance was accepted when $p < 0.05$. Linear regressions were used in order to test the relationships between caiman length and mercury concentrations at each capture site.

3. Results

3.1. Specimen characterization

All caiman specimens were characterized by the following morphological parameters: total length, snout–vent length (SVL), weight and sex. Animal weight in relation to the capture sites can be observed in Fig. 2, and animal SVL in relation to the capture sites can be observed in Fig. 3. Differences between weight, total length and SVL were non-significant. The number of captured males was higher than the number of females in all sampling sites. Four animals could not be sexed at the Bento Gomes sampling site, eight at Cuiabá river and one at Nhecolândia, and were not included in the statistical analysis. Males weighed more than females in all sampling sites, and also presented higher values for total and SVL lengths. In these comparisons, no significant differences could be assigned to sex.

Table 1 summarizes the physical characteristics of the caimans in relation to the sampling sites (shown as mean ± standard deviations).

3.2. Quality assurance and laboratory sample representativeness

For each batch, at least 3 blanks were analyzed in parallel. These were always well below the Hg level of the less concentrated caiman sample. For samples spiked with mercury and methyl mercury, the recoveries of both species were always between 90 and 110%, a range acceptable for the studied concentration levels. The accuracy of the analytical procedure was confirmed by the parallel analysis of a certified reference material (DORM-2 dogfish muscle). No statistically significant difference was

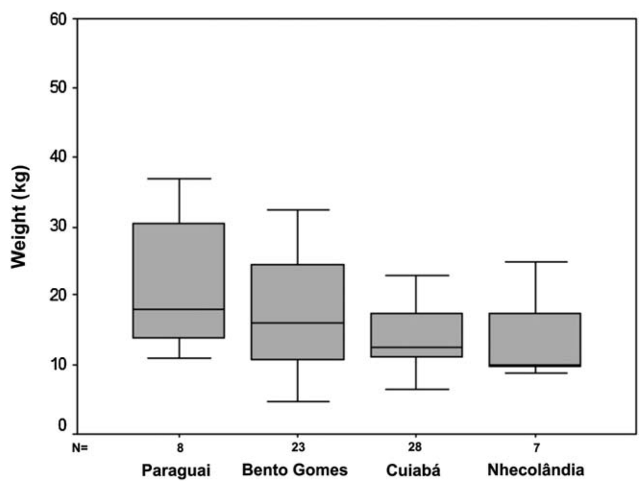

Fig. 2 Pantanal caiman weight in relation to the capture sites.

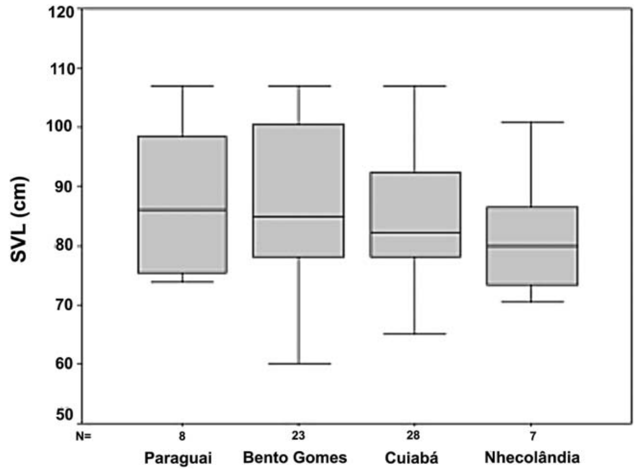

Fig. 3 Pantanal caiman SVL relation to the capture sites.

observed between found (4.46 ± 0.03 and 4.38 ± 0.03) and certified values (4.64 ± 0.26 and 4.47 ± 0.32) for total Hg and MeHg, respectively ($n = 6$).

3.3. Sample representativeness

Values for total Hg concentrations did not differ significantly between the twelve aliquots taken from different points of the tail of a same caiman specimen, and the final value of 0.100 ± 0.017 μg g^{-1} ww shows an uncertainty at the same level of the method uncertainty for this concentration level. Thus, this test was considered sufficiently repetitive to characterize the specimen. Therefore, in all analyses, only a small portion of caiman muscle tail was taken, enough for triplicate 0.5 g weightings.

3.4. Mercury and methyl mercury concentrations

Total mercury concentrations ranged from 0.02 to 0.36 μg g^{-1} ww. The highest concentrations were observed at the Bento Gomes sampling sites, with an average (±standard deviation) of 0.27 ± 0.08, followed by Cuiabá (0.14 ± 0.04 μg g^{-1} ww), Nhecolândia and the Paraguay river, of 0.12 ± 0.10 and 0.05 ± 0.01 μg g^{-1} ww, respectively. Concerning methyl mercury, animals from the Bento Gomes and Cuiabá sampling sites showed the highest MeHg percentages. The samples from the Bento Gomes river showed MeHg ranging from 52 to 90% (average of 75%), while the samples from the Cuiabá river ranged from 54 to 85% (average of 73%) of the total mercury concentrations. Samples from Nhecolândia showed MeHg percentages ranging from 23 to 78%, with an average of 53% of total Hg, while almost all animals from the Paraguay river showed MeHg < LD. The lowest MeHg ratios corresponded to the lowest total Hg concentrations, as shown in Fig. 4.

3.5. Relationships between sampling sites, morphological parameters and total mercury concentrations

No significant differences were observed ($p > 0.05$) when comparing specimen weights and SVL to the sampling sites (Fig. 2 and 3 respectively). Similarly, no significant inter-sex differences were observed ($p > 0.05$), although male average

Table 1 Specimen characterization

	n			Weight/kg			SVL/cm			Total length/cm		
Capture sites	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Paraguay	7	1	8	22.90 ± 9.57	11.40	21.46 ± 9.75	89.4 + 12.4	74.5	87.5 ± 12.6	175.8 ± 21.4	143.0	171.7 ± 23.0
Bento Gomes	20	7	27	20.44 ± 8.23	12.21 ± 7.20	17.93 ± 8.68	91.5 ± 14.1	78.5 ± 9.2	87.57 ± 14.0	168.7 ± 22.2	140.8 ± 12.3	160.2 ± 23.4
Cuiabá	30	6	36	17.20 ± 11.00	12.17 ± 1.51	16.12 ± 9.95	86.2 ± 12.1	80.1 ± 4.6	84.9 ± 11.2	163.9 ± 24.1	154.1 ± 6.9	161.8 ± 21.9
Nhecolândia	5	2	7	15.60 ± 7.08	9.90 ± 0.14	13.97 ± 6.42	84.2 ± 12.3	75.2 ± 6.7	81.6 ± 11.3	158.2 ± 25.8	142.5 ± 12.0	153.7 ± 22.9

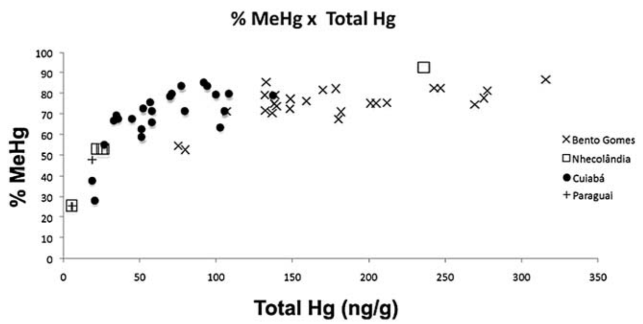

Fig. 4 Total mercury concentration in Pantanal caimans in relation to the capture sites.

values were systematically higher in comparison to those of females. When total Hg concentrations were analyzed in comparison to the sampling sites, significantly higher values ($p < 0.05$) were observed for the specimens captured at the Bento Gomes river (Fig. 5). No significant differences were found between the Hg concentrations found in the specimens caught at the Cuiabá, Nhecolândia and Paraguay rivers. To confirm this, data were normalized by weight or length, and the same pattern was observed, including no significant inter-sex differences. Significant correlations ($p < 0.05$) between length and mercury concentrations were observed between the specimens captured at the Bento Gomes and Cuiabá rivers, while the same was not observed ($p > 0.05$) for those captured in the other two sites (Fig. 6).

4. Discussion

4.1. Mercury and methyl mercury in caimans from the Pantanal area

Mercury is believed to be the heavy metal of greatest concern to crocodilians,[16,25] and studies show that these animals are capable of accumulating this element in muscle tissue. They are considered excellent indicators of Hg contamination in the environment, because they are very long-lived, occur in aquatic systems where mercury often accumulates, and are top-level predators.[16] However, no literature exists on Hg levels in the Brazilian caiman, *Caiman c. yacare*, even though this species occupies an

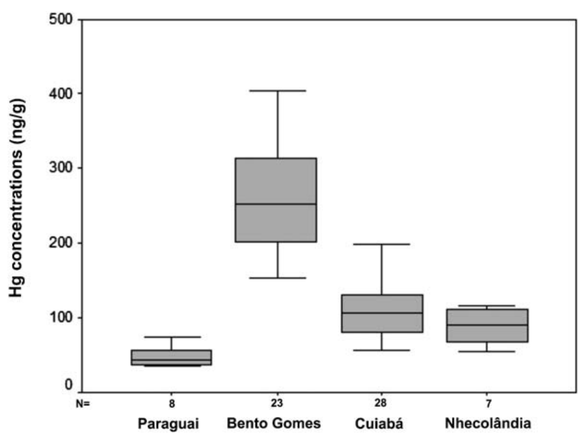

Fig. 5 Total mercury concentration in relation to the capture sites.

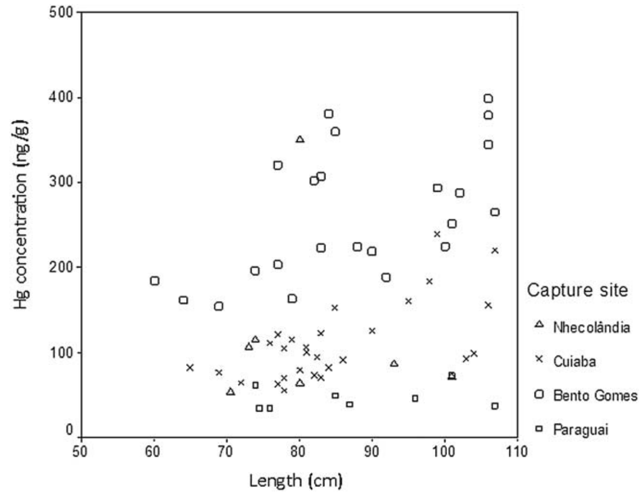

Fig. 6 Total mercury concentration in relation to the SVL and capture sites: (△) Nhecolândia, (×) Cuiabá river ($r = 0.66$, $p < 0.05$), (○) Bento Gomes river ($r = 0.56$, $p < 0.05$) and (□) Paraguay river.

important position in the trophic web of the Pantanal ecosystem and could be used as a bioindicator of ecosystem health, especially in the form of methyl mercury, since the Pantanal, as many other swamps and reservoirs, demonstrates enhanced Hg methylation, as a consequence of Hg biomethylation processes.[37,38]

The data found in the present study show that specimen weights, SVL and total lengths were not statistically different among the sampling sites, even after data normalization and, similarly, no significant differences regarding mercury concentrations could be assigned to sex, although male average values were systematically higher in comparison to those of females. However, as speculation exists that adult female alligators might exhibit lower levels of several types of contaminants than males since, in reptiles, some of these contaminants are removed when eggs are laid,[24] the results regarding sex differences in the present study were expected and are in agreement with the previous study.

Regarding specimens captured at the Bento Gomes river, where significantly higher mercury concentrations were found, it is important to note that this area is known for the presence of human activities, related to the presence of Poconé city (31 000 inhabitants), where former extensive "garimpo" activities occurred in the 1980 and 1990s. This implicated not only in the use of mercury for gold amalgamation and its direct release into the rivers and the atmosphere but also an intense river bench dismounting by high-pressure water jets. This operation facilitated lixiviation and metal transportation to the aquatic environment, both by its direct action and also by leaching behind large areas of naked land that can be washed anew in each new rainy season. In relation to specimens captured in the Cuiabá river, the release of sewage and day water, due to the proximity of Cuiabá city, that possesses many potential Hg contamination sources, such as gold retailers and dentists,[6] may, at least partially, respond for the mercury levels found in the area. However, other human activities, such as land use, must also be considered. Regarding correlations between length and total mercury concentrations, statistically significant correlations were found only in specimens captured at these sites (Fig. 6). This leads to the observation that Hg bioaccumulation processes were observed only in the sites where human activities are more intense, certainly due to higher mercury availability. Although this evidence of anthropogenic influences on the mercury concentrations of the Pantanal caimans exists, differentiated natural processes cannot be excluded and further studies are necessary for the estimation of actual human influence.

In contrast to the sampling sites where average mercury concentrations were the highest, no correlation between size and mercury concentrations were observed in the other two sampling sites. Although statistically non-significant ($p > 0.05$), the specimen from Nhecolândia showed mercury concentrations below those from the Cuiabá river. Nhecolândia is known for its low human density and no intensive agricultural activities, and at this site the animals were caught in closed lagoons, fed by relatively isolated drainage systems. MeHg ratios associated to specimens from this site were significantly lower in relation to those observed in the two more concentrated groups. Since flooding is known to enhance methylation processes,[39] and Nhecolândia is a permanently dry area, the flooding contribution to the methylation rate is not expected here and this may, therefore, contribute to the lower methyl mercury levels observed in this site. The Paraguay river specimens were those with the lowest Hg concentrations. This sampling site is an almost pristine area, with very low human impact. Also, any anthropogenic or natural contaminant coming from any upstream tributary (such as the Bento Gomes river) will be diluted in its impressive water flow, which ranges from 200 $m^3 s^{-1}$ during the dry season (June to September) to 2000 $m^3 s^{-1}$ during the humid season (January to April).[40]

As there is a lack of literature regarding mercury levels in the Brazilian caiman, comparisons to literature were made utilizing Hg levels in tail muscle reported for the American alligator (*Alligator mississippiensis*) and one study utilizing *Crocodylus porosus* (Table 2), in order to situate values obtained in the present study in a worldwide context. Although some results are expressed in dry or wet weight and no reference to this is found in other studies, some observations can be made; Hg values in *C. c. yacare* are higher than those values observed for *C. porosus* in Australia, and intermediary regarding *A. mississippiensis* from several different locations in the USA.

However, one must take into account differences among the species, such as different feeding habits and habitats: (a) *C. porosus* regular food items are crustaceans, mainly crabs and shrimps, and this species does not usually feed on fish, with the exception of one or two slow-moving species. However, animals larger than 120 cm in length usually ingest more vertebrate prey.[41] Also, the study concerning this species presented was conducted in Australia, in the pristine world heritage area of the Kakadu National Park and, therefore, low levels of contaminants are expected; (b) the diet of *A. mississippiensis* consists mainly of mollusks, invertebrates and crustaceans; arthropods and fish are more consumed in more saline environments;[42–44] and (c) *C. c. yacare* consumes mainly invertebrates and fish. Furthermore, caiman feeding habits are governed by their habitat, while alligator feeding habits, on the other hand, are governed by their size.[21]

4.2. Caiman Hg contamination—potential consequences in captivity

Caimans supply the vast majority of the reptile hide market in South America, which is considerable, and are also exploited for their meat. Sustainable programs for these reptiles have been developed in Latin America, and in Brazil *C. c. yacare* fresh meat has proven to be greatly accepted by humans.[45,46] Legal Pantanal caiman farming in Brazil has been stimulated and as a consequence, registers from Brazil from specialized and legal restaurants, show an increase in caiman consumption. In 1999 approximately 30 tons were consumed, at about 10 US dollars per kilo.[47] Meat harvested for human consumption almost exclusively comprises the tail and the dorsal fillets,[48] proving the importance of tail muscle Hg level monitoring in these animals. Concerning caiman management, several possibilities exist regarding wild caiman populations in the Pantanal area, from collecting eggs and/or young animals from the wild and rearing them in captivity to adult male management, without interfering with the reproductive potential of the population.[49] In Brazil, the current and legal model for caiman use is composed of egg

Table 2 Average total mercury concentrations (μg g^{-1}) observed in the literature for different crocodilian species

Reference	Study area	Number of samples	Total Hg content	Species
Rumbold *et al.*, 2002[20]	Everglades, USA	28	0.64 ± 0.54 (ww)	*Alligator mississippiensis*
	Everglades, USA	18	5.57 ± 0.47 (dw)	*Alligator mississippiensis*
Jagoe *et al.*, 1998[30]	Florida, USA	21	1.85 ± 0.35 (dw)	*Alligator mississippiensis*
	Okefenokee, USA	9	0.08 ± 0.12 (dw)	*Alligator mississippiensis*
	South Carolina, USA	49	4.83 ± 0.88 (dw)	*Alligator mississippiensis*
Yanochko *et al.*, 1997[23]	Everglades, Florida, USA	18	1.17 ± 0.36 (dw)	*Alligator mississippiensis*
Ruckel, 1993[22]	Georgia, USA	22	0.1 to 1.4 (ww)	*Alligator mississippiensis*
Delany *et al.*, 1988[24]	Florida, USA	32	0.04 to 0.61 (ww)	*Alligator mississippiensis*
Burger *et al.*, 2000[25]	Florida, USA	31	0.06 ± 0.02 (ww)	*Alligator mississippiensis*
Elsey *et al.*, 1999[32]	Louisiana, USA	42	0.047 ± 0.386 (ww)	*Alligator mississippiensis*
Facemire, *et al.*, 1995[29]	Everglades, Florida, USA	5	3.57	*Alligator mississippiensis*
Hord *et al.*, 1990[17]	Everglades, Florida, USA	18	2.36 ± 0.99	*Alligator mississippiensis*
Jeffree *et al.*, 2001[53]	Australia	75	<0.01 (dw)	*Crocodylus porosus*
Present study	Paraguay river, Brazil	79	0.05 ± 0.01 (ww)	*Caiman c. yacare*
	B. Gomes, Brazil		0.27 ± 0.08 (ww)	*Caiman c. yacare*
	Cuiabá, Brazil		0.14 ± 0.04 (ww)	*Caiman c. yacare*
	Nhecolândia, Brazil		0.12 ± 0.10 (ww)	*Caiman c. yacare*

extraction and rearing of young animals in captivity. As caiman farming comprises the entire Pantanal area, Hg levels in wild animals can be indicative of problems in captive ones, since wildlife species are highly sensitive to mercury toxicity during embryonic development and neonatal life.[50] The reason for concern is that studies have shown that crocodilian eggs also concentrate mercury, and that this mercury contamination in crocodilian eggs most likely results from maternal transfer.[19,51] Levels of mercury in different crocodilian species eggs from around the world have been shown to vary from 0.02 to 0.71 $\mu g\ g^{-1}$ (ww).[19] Therefore, developmental issues may occur in captivity, since these eggs may already be affected by above normal Hg levels. In the present study, mercury levels in Pantanal caiman tail muscle from all sampling sites were all above the limits considered harmful to aquatic biota, of 0.05 $\mu g\ g^{-1}$.[52]

5. Conclusions

The present study contributes to a more comprehensive data literature regarding crocodilians and, specifically, Hg contamination in the Pantanal caiman. The data obtained in the present study show important levels of Hg in this species, especially in animals from the Pantanal areas of former “garimpo” activities and in sites where other present anthropogenic activities take place. However, although this may support the role of human activities on mercury levels, one cannot exclude the contribution of natural processes, such as those associated to flooding, which is known to contribute to the increase of the methylation rate. In these areas, 80% of the captured specimens showed MeHg ratios over 70% of the total mercury content, which demonstrates valid concerns regarding the local aquatic biota. Total mercury levels found in Pantanal caimans are comparable to levels in the American alligator in several areas of the United States, but considerably higher than those from the estuarine crocodiles from northern Australia. However, this data comparison should be conducted with care, taking into account their different habitats, feeding habits and ecosystem dynamics that can be responsible for these Hg levels. Since this study has shown that, in areas of higher human activities, Hg levels in caimans were significantly higher, this fact, although not definite evidence of cause and effect, supports the hypothesis that caimans can in fact be considered effective bioindicators of ecosystem health concerning mercury. However, further studies must be performed before peremptory affirmations can be made.

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References

1 A. Sucksdorff, *Pantanal—um paraíso perdido*, Rio de Janeiro, 1984, p. 160.
2 L. D. Hylander, E. C. Silva, L. J. Oliveira, S. A. Silva, E. K. Kuntze and D. X. Silva, *Ambio*, 1994, **23**, 478.
3 L. D. Delacerda, W. C. Pfeiffer, R. V. Marins, S. Rodrigues, C. M. M. Souza and W. R. Bastos, *Water, Air, Soil Pollut.*, 1991, **55**, 283.
4 L. D. Delacerda, W. Salomons, W. C. Pfeiffer and W. R. Bastos, *Biogeochemistry*, 1991, **14**, 91.
5 L. D. Hylander, M. Meili, L. J. Oliveira, E. D. E. Silva, J. R. D. Guimaraes, D. M. Araujo, R. P. Neves, R. Stachiw, A. J. P. Barros and G. D. Silva, *Sci. Total Environ.*, 2000, **260**, 97.
6 L. D. Hylander, F. N. Pinto, J. R. D. Guimaraes, M. Meili, L. J. Oliveira and E. D. E. Silva, *Sci. Total Environ.*, 2000, **261**, 9.
7 O. Malm, M. B. Castro, W. R. Bastos, F. J. P. Branches, J. R. D. Guimaraes, C. E. Zuffo and W. C. Pfeiffer, *Sci. Total Environ.*, 1995, **175**, 127.
8 H. A. Kehrig, M. Costa, I. Moreira and O. Malm, *Mar. Pollut. Bull.*, 2002, **44**, 1018.
9 H. A. Kehrig, F. N. Pinto, I. Moreira and O. Malm, *Org. Geochem.*, 2003, **34**, 661.
10 S. Hacon, E. Yokoo, J. E. Valente, R. C. Campos, V. A. Silva, A. C. Menezes, L. P. Moraes and E. Ignotti, *Environ. Sci.*, 2003, **10**, 121.
11 P. Artaxo, R. C. de Campos, E. T. Fernandes, J. V. Martins, Z. F. Xiao, O. Lindqvist, M. T. Fernandez-Jimenez and W. Maenhaut, *Atmos. Environ.*, 2000, **34**, 4085.
12 CETEM, in ed. C. d. T. Mineral, Rio de Janeiro, 1989, p. 38.
13 CETEM, in ed. C. d. T. Mineral, Rio de Janeiro, 1991, p. 9.
14 S. Rodrigues and J. E. L. Maddock, *J. Geochem. Explor.*, 1997, **58**, 231.
15 L. M. Vieira, V. N. Silva and R. C. Campos, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)—Pantanal, in *IV Simposio sobre recursos naturais e socio-economicos do Pantanal*, Corumbá, Brazil, 2004, p. 1.
16 I. L. J. Brisbin, C. H. Jagoe, K. F. Gaines and J. C. Gariboldi, in *14th Working Meeting Crocodile Specialist Group*, 1998, p. 155.
17 L. J. Hord, M. L. Jennings and A. Brunell, in *Crocodiles—Proc 10th Working Meeting Croc Spec Grp, SSC-IUCN*, 1990, p. 229.
18 T. G. HeatonJones, B. L. Homer, D. L. HeatonJones and S. F. Sundlof, *J. Zoo Wildl. Med.*, 1997, **28**, 62.
19 T. R. Rainwater, B. M. Adair, S. G. Platt, T. A. Anderson, G. P. Cobb and S. T. McMurry, *Arch. Environ. Contam. Toxicol.*, 2002, **42**, 319.
20 D. G. Rumbold, L. E. Fink, K. A. Laine, S. L. Niemczyk, T. Chandrasekhar, S. D. Wankel and C. Kendall, *Sci. Total Environ.*, 2002, **297**, 239.
21 A. S. Santos, *Dieta e Nutrição de Crocodilianos*, Embrapa-CPAP, Corumbá, 1997, p. 59.
22 S. W. Ruckel, in *AnnualConference of Southeastern Fish and Wildlife Agencies*, 1993, p. 287.
23 G. M. Yanochko, C. H. Jagoe and I. L. Brisbin, *Arch. Environ. Contam. Toxicol.*, 1997, **32**, 323.
24 M. F. Delany, J. U. Bell and S. F. Sundlof, *J. Wildl. Dis.*, 1988, **24**, 62.
25 J. Burger, M. Gochfeld, A. A. Rooney, E. F. Orlando, A. R. Woodward and L. J. Guillette, *Arch. Environ. Contam. Toxicol.*, 2000, **38**, 501.
26 K. Vermeer, R. W. Risebrough, A. L. Spaans and L. M. Reynolds, *Environ. Pollut.*, 1974, **7**, 217.
27 F. J. Ware, H. Royals and T. Lange, in *Annual Conference of Southeastern Fish and Wildlife Agencies*, 1990, p. 5.
28 J. Yoshinaga, T. Suzuki, T. Hongo, M. Minagawa, R. Ohtsuka, T. Kawabe, T. Inaoka and T. Akimichi, *Ecotoxicol. Environ. Saf.*, 1992, **24**, 37.
29 C. Facemire, T. Augspurger, D. Bateman, M. Brim, P. Conzelmann, S. Delchamps, E. Douglas, L. Inmon, K. Looney, F. Lopez, G. Masson, D. Morrison, N. Morse and A. Robison, *Water, Air, Soil Pollut.*, 1995, **80**, 923.
30 C. H. Jagoe, B. Arnold-Hill, G. M. Yanochko, P. V. Winger and I. L. Brisbin, *Sci. Total Environ.*, 1998, **213**, 255.
31 W. E. Rhodes, in *Principles and Processes for Evaluating Endocrine Disruption in Wildlife*, ed. R. Kendall, R. Dickerson, J. Giesy and B. Suk, SETAC Press, Pensacola, FL, 1998, p. 301.
32 R. M. Elsey, V. A. Lance and L. Campbell, *Bull. Environ. Contam. Toxicol.*, 1999, **63**, 598.
33 B. Khan and B. Tansel, *Ecotoxicol. Environ. Saf.*, 2000, **47**, 54.
34 A. V. Yallouz, R. C. de Campos and S. Paciornik, *Fresenius J. Anal. Chem.*, 2000, **366**, 461.
35 A. M. Limaverde and R. C. Campos, *Quim. Nova*, 1999, **22**, 477.
36 L. Magos, *Analyst*, 1971, **96**, 847.
37 J. R. D. Guimaraes, M. Meili, O. Malm and E. M. D. Brito, *Sci. Total Environ.*, 1998, **213**, 165.

38 J. B. N. Mauro, J. R. D. Guimaraes and R. Melamed, *Appl. Organomet. Chem.*, 1999, **13**, 631.
39 C. A. Kelly, J. W. M. Rudd, R. A. Bodaly, N. P. Roulet, V. L. St. Louis, A. Heyes, T. R. Moore, S. Schiff, R. Aravena, K. J. Scott, B. Dyck, R. Harris, B. Warner and G. Edwards, *Environ. Sci. Technol.*, 1997, **31**, 1334.
40 M. L. Assine and A. Silva, *Geomorphology*, 2009, **113**, 189.
41 J. A. Taylor, *Aust. Wildl. Res.*, 1979, **6**, 347.
42 M. J. Fogarty and J. D. Albury, in *Annual Conference Southeastern Association Game Fish Commissioners*, 1967, p. 220.
43 R. H. Chabreck, in *Annual Conference Southeastern Association Game Fish Commissioners*, 1971, p. 117.
44 L. McNease and T. Joanen, in *Annual Conference Southeastern Association Fisheries and Wildlife Agencies*, Grand Chenier, 1977, p. 36.
45 J. Thorbjarnarson and A. Velasco, *Conserv. Biol.*, 1999, **13**, 397.
46 P. F. Romanelli, Propriedades Tecnológicas da Carne do Jacaré do Pantanal (*Caiman crocodilus yacare*), Universidade Estadual de Campinas (UNICAMP), 1995.
47 P. F. Romanelli, R. Caseri and J. F. Lopes-Filho, *Cienc. Tecnol. Aliment. (Campinas, Braz.)*, 2002, **22**, 70.
48 S. Magnino, P. Colin, E. Dei-Cas, M. Madsen, J. McLauchlin, K. Nockler, M. P. Maradona, E. Tsigarida, E. Vanopdenbosch and C. Van Peteghem, *Int. J. Food Microbiol.*, 2009, **134**, 163.
49 M. Coutinho and Z. Campos, *Preliminary report for a management system of yacare caiman in the Pantanal: A proposal for future research*, in Embrapa, 2006, p. 3.
50 R. Eisler, in *Bio Rep 85(1.10)*, USFWS, 1987.
51 M. F. Wolfe, S. Schwarzbach and R. A. Sulaiman, *Environ. Toxicol. Chem.*, 1998, **17**, 146.
52 P. Allen, *Comp. Biochem. Physiol.*, 1994, **108C**, 117.
53 R. A. Jeffree, S. J. Markich and J. R. Twining, *Arch. Environ. Contam. Toxicol.*, 2001, **40**, 236.

