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Variation of Total Mercury Concentrations in Different Tissues of Three Neotropical Caimans: Implications for Minimally Invasive Biomonitoring

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Abstract

Mercury (Hg) is a global environmental contaminant that affects ecosystems. It is known to biomagnify through food webs and to bioaccumulate especially in the tissues of top predators. Large-scale comparisons between taxa and geographic areas are needed to reveal critical trends related to Hg contamination and its deleterious effects on wildlife. Yet, the large variety of tissues (keratinized tissues, internal organs, blood) as well as the variability in the units used to express Hg concentrations (either in wet- or dry-tissue weight) limits straightforward comparisons between studies. In the present study, we assessed the moisture content that could influence the total Hg (THg) concentrations measured in several tissues (claws, scutes, total blood, and red blood cells) of three caiman species. We evaluated the moisture content from the different tissues to provide information on THg concentrations in various matrices. Our results show a difference of THg concentrations between the tissues and intra- and interspecific variations of moisture content, with the highest THg values found in keratinized tissues (scute keratinized layers and claws). For the three species, we found positive relationships between body size and THg concentration in keratinized tissues. In the blood, the relationship between body size and THg concentration was species-dependent. Our results emphasize the need for a standardized evaluation of THg concentration and trace elements quantification based on dry weight analytical procedures. In addition, the use of both blood and keratinized tissues offers the possibility to quantify different time scales of THg exposure by non-lethal sampling.

Mercury (Hg) is one of the major contaminants of concern in ecosystems (Ericksen et al. 2003; Fitzgerald et al. 2007; Selin 2009). In addition to naturally present geological Hg, human activities, such as deforestation, fossil fuel combustion, and gold mining activities, have been shown to increase the level of Hg in the environment, particularly in aquatic ecosystems (Ericksen et al. 2003; Scheuhammer and Sandheinrich 2007; Hsu-Kim et al. 2018). In anoxic conditions, aquatic microorganisms can transform inorganic Hg into methylmercury (MeHg), the most bioavailable and toxic form of Hg (Jensen and Jernelöv 1969; Benoit et al. 2003). Importantly, MeHg biomagnifies through food webs and bioaccumulates in the tissues of top predators, which makes them particularly vulnerable to this contaminant (Eagles-Smith et al. 2018).

Crocodilians belong to the world's largest predators, and as such, they have important functions in ecosystems and can constitute indicators of ecosystem health (Somaweera et al. 2020). As apex predators, crocodilians bioaccumulate environmental contaminants that biomagnify across food webs, and thus can be particularly vulnerable to their toxicity (Cook et al. 1991; Camus et al. 1998; Rainwater et al. 2007). Therefore, they are relevant bioindicators regarding environmental contamination (Guillette et al. 1994; Manolis et al. 2002; Campbell et al. 2003; Chumchal et al. 2011; Schneider et al. 2015). In addition, such an evaluation is useful to determine the relatively poorly known detrimental

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effects of contaminants on this taxon, such as physiological and reproductive impairment and DNA damages (Guillette et al. 2000; Siroski et al. 2016; Burella et al. 2018; Lemaire et al. 2021a).

Crocodilians bioaccumulate Hg at various concentrations, depending on the species and on the location (Elsey et al. 1999; Rumbold et al. 2002; Campbell et al. 2010; Vieira et al. 2011; Nilsen et al. 2017a; Lemaire et al. 2021a). However, interspecific variability across the whole group remains still poorly understood to date as most of the available studies on Hg concentrations have disproportionately focused on two species, the American Alligator, *Alligator mississippiensis* and the Morelet's Crocodile, *Crocodylus moreletii* (Yanochko et al. 1997; Jagoe et al. 1998; Elsey et al. 1999; Burger et al. 2000; Rainwater et al. 2007; Horai et al. 2014; Trillanes et al. 2014; Nilsen et al. 2017a, 2019; Buenfil-Rojas et al. 2018, 2020). Some recent studies, however, have focused on several other species (Almli et al. 2005; Vieira et al. 2011; Lázaro et al. 2015; Schneider et al. 2015; Marrugo-Negrete et al. 2019; Lemaire et al. 2021a,b). Furthermore, Hg concentrations in crocodilians have been determined in a variety of tissues (blood, muscles, internal organs, or keratinized tissues), depending on the study, which limits robust comparisons between them. It is worth noting that the associated methodologies (e.g., sample preparation and tissues studied) are variable, which also limits straightforward comparisons between studies (Schneider et al. 2015). Furthermore, total Hg (THg) concentrations in these papers are either presented as the concentration relative to the wet- or dry-weight of sampled tissues. Clearly, such discrepancy prevents direct comparison between studies, especially as the amount of tissue moisture content, and its variation within and across tissues, on Hg concentration has not yet been thoroughly investigated. In addition, one of the most studied tissues in crocodilians is the muscle, most likely because it is used for human consumption (Delany et al. 1988; Elsey et al. 1999; Eggins et al. 2015; Rivera et al. 2016). Although assessing Hg contamination in such tissue can be useful, many species are now classified by the IUCN as in danger of extinction, which supports both the reduction of crocodile hunting per se, and the development of specific, non-lethal sampling methods to assess their Hg contamination levels and its effects (as methods have shown for other reptile species, Day et al. 2005; Lemaire et al. 2018; Beau et al. 2019).

In this context, the goals of the present study were two-fold: first, we assessed the moisture content of different tissues (claws, scutes and total blood) and investigated the influence of sample preparation on THg quantification to provide recommendations to harmonize Hg assessment across multiple matrices. Second, we compared THg concentrations between several tissues (full scutes, scute keratin layers, claws, total blood, and red blood cells) to evaluate the use of non-lethal sampling methods to facilitate future comparisons of contamination in crocodilians.

Materials and Methods

Sample Collection

Our study was conducted in French Guiana between April 2016 and February 2020 (Fig. 1). We sampled 51 individuals of smooth-fronted caiman, *Paleosuchus trigonatus* (Schneider, 1801) from 6 different sites, 48 individuals of spectacled caiman, *Caiman crocodilus* (Linnaeus, 1758) from 2 different sites, and 13 individuals of dwarf caiman, *Paleosuchus palpebrosus* (Cuvier, 1807) from 4 different sites. *P. trigonatus* were captured in small forest streams. *C. crocodilus* and *P. palpebrosus* were captured in marsh or stream habitats.

The snout-vent length (SVL) and total length (TL) of each individual were measured. We took claw (randomly at the posterior legs) and scute samples (in all samplings < 1 cm, and never exceeding half of the scute) of all caimans using pliers for clipping the sample. Clipping tail scutes is a common marking method in crocodilians. The scutes which were clipped in order to mark the captured individuals were therefore not the same for all animals as they followed the consecutive identification code. Claw and scute samples were placed in dry plastic containers. Blood was collected on a subsample of individuals depending on the field possibilities ($N = 24$ for *P. trigonatus*, $N = 40$ for *C. crocodilus*, and $N = 7$ for *P. palpebrosus*). Blood samples (0.2–3 ml) were drawn from the lateral tail vein using 27 gauges (25 mm) or 21 gauges (50 mm) (depending on the size of the individual animal) heparinized needles (heparin sodium). Each blood sample was separated into two tubes and kept at cold temperatures (4 °C) until being processed at the laboratory (always < 3 h after collection). The first tube, containing total blood, was frozen at − 28 °C in the lab. The second was centrifuged at 6,500 rpm for 5 min to separate red blood cells (RBCs) and plasma, after which both fractions were then frozen at − 28 °C in the lab until analysis.

After sampling, each individual was released at its capture location. *C. crocodilus, P. trigonatus*, and *P. palpebrosus* are protected by the French law (Ministerial decree NOR: TREL1933710A of 08/10/2018) and a permission to capture individuals, draw blood, and sample claws and scutes was granted by the French authorities (Direction Régionale des Territoires et de la Mer) after evaluation by the CSRPN, the regional scientific committee (Permit: R03-2016-06-21-010; R03-2019-01-09-001; R03-2019-10-24-007, www.guyane.developpement-durable.gouv.fr).

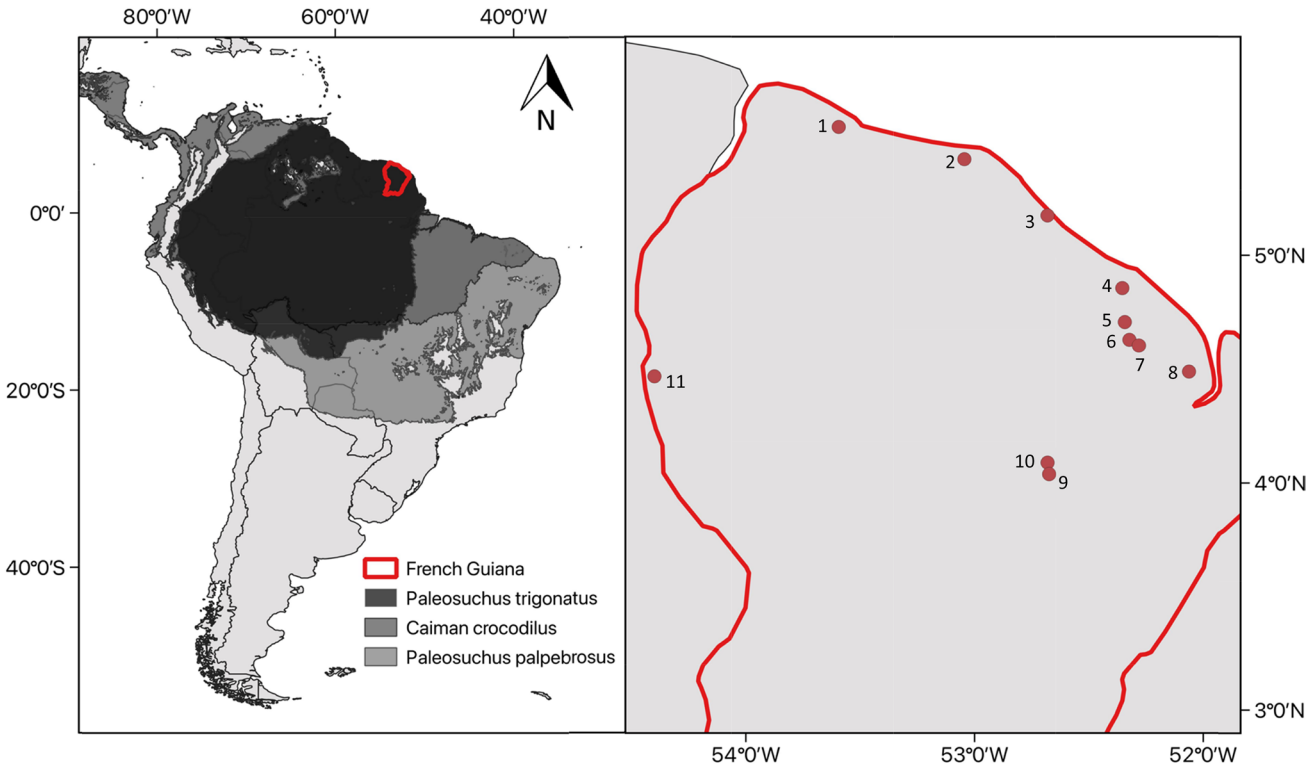

Fig. 1 Location of French Guiana, distribution ranges and capture locations of the spectacled caiman, *Caiman crocodilus* (sites 2, 8), the smooth-fronted caiman, *Paleosuchus trigonatus* (sites 3, 4, 7, 9, 10, 11), and the dwarf caiman, *Paleosuchus palpebrosus* (sites 1, 2, 5, 6). (Data for distribution ranges provided by The IUCN Red List of Threatened Species)

Sample Preparation

Claws and scutes were cleaned for 5 min in an ultrasonic bath of ultrapure water to remove all external dirts and rinsed 3 times, as described in Lemaire et al. (2021b). To assess the wet weight of claws and scutes, we weighed cleaned samples after elimination of water surplus with absorbent paper. Because cleaning procedures were relatively short and because caimans are semiaquatic species that spend most of their lifetime immerged in water, our cleaning procedures were not likely to influence wet weight assessment. In order to assess dry weight, each sample was dried in an oven for 48 h at 45 °C, which was found to be sufficient to reach stable weight (no difference was found when dried at 45 °C for 48 h, 72 h, and 96 h, data not shown). Moisture content was calculated as wet weight minus dry weight and expressed as a proportion of wet weight for further analyses. A subsample of dried scutes ($N=26$ for *C. crocodilus*, $N=4$ for *P. palpebrosus*, and $N=4$ for *P. trigonatus*) was used to perform comparison of Hg concentrations between these matrices. We separated the external layer, which is composed of keratin (corneoscute), from the underlying connective tissue, which is the link between bone/cartilage (osteoscute) and the keratin layer. For the remaining scute samples, only the keratin layer was analysed. To assess the moisture of total blood and RBC samples, we weighted the samples before and after the freeze-drying process (48 h). Freeze-dried samples were then ground into a homogeneous powder. The moisture content of total blood and RBC samples was calculated as wet weight minus dry weight and then expressed as a proportion of wet weight.

Instrumental Method and Quality Control

For all samples, total Hg (THg) was determined using an atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254; Altec®). At least two replicates of 0.3–1.0 mg dry weight (dw) were analysed for each sample. The reproducibility for duplicate samples was approved when relative standard deviation (RSD) was below 10%. The analyses of certified reference material (CRM) TORT-2 (Lobster hepatopancreas from the National Research Council of Canada; certified Hg concentration: 0.27 ± 0.06 µg g^{-1} dw) and TORT-3 (Lobster hepatopancreas from the National Research Council of Canada; certified Hg concentration: 0.292 ± 0.022 µg g^{-1} dw) was performed at the beginning and at the end of the analytical cycle and every 10 samples, for the validation of the method. The TORT-2 measured value was 0.243 ± 0.015 µg g^{-1} dw ($n=20$), giving a recovery of 90.2 ± 5.7%. The TORT-3 measured value

was 0.289 ± 0.007 µg g^{-1} dw ($n = 30$), giving a recovery of 99.0 ± 2.3%. Blanks were included at the beginning of analytical runs and the limit of quantification of the AMA was 0.05 ng. THg concentrations are expressed in µg g^{-1} dw.

Statistical Analysis

All statistical analyses were performed using the software R, v.3.6.1 (R development Core Team 2013). All data were checked for normality and homogeneity of variances. Depending on the results, parametric or nonparametric tests were used. The differences of moisture content in each tissue between species, as well as the differences of moisture content between different tissues for each species, and the differences of moisture content for each species and each tissue between capture sites were all investigated by Kruskal–Wallis test.

Paired *t*-tests were used to compare THg concentrations between full scutes (a combination of keratin, bone and connective tissue) and scute keratin layers for each species. To access the relationship between THg concentrations in full scutes and scute keratin layers, we built a predictive equation for *C. crocodilus*. The predictive equation was built for a significant relationship between both tissues, using slope and intercept derived from the parametric linear regression line. To validate the models, bootstrapping with 1,000 iterations were applied (Harrell 2015). We did not build predictive equations for *P. palpebrosus* and *P. trigonatus* due to the restricted number of samples.

The difference of THg concentrations between tissues was assessed by Friedman ANOVA and Pairwise Bergmann–Hommel comparisons for each species. We performed Spearman rank tests to assess the relationships between body size (SVL and TL) and THg concentrations in the tissues, independently for each species. The significance level for statistical analyses was always set at $p < 0.05$.

Results

Moisture Content

The results of the moisture content of blood, claws, and scutes for each species are summarized in Table 1. We found significant differences in moisture content between the species for claws (Kruskal–Wallis: $\chi^2 = 22.79$, $p < 0.001$, $n = 57$) and full scutes (Kruskal–Wallis: $\chi^2 = 10.66$, $p < 0.005$, $n = 58$), but not for total blood (Kruskal–Wallis: $\chi^2 = 1.11$, $p = 0.57$, $n = 31$) (Table 1; Fig. 2). We did not find any geographic variation in moisture content of the different tissues for the three species (all $p > 0.18$).

THg Concentrations in Scutes

Our results show significantly higher concentrations of THg in scute keratin layers than full scutes in two species (Paired *t*-test: *C. crocodilus*, $t = -5.44$, $p < 0.001$; *P. trigonatus*, $t = -4.71$, $p = 0.02$; *P. palpebrosus*, $t = -2.18$, $p = 0.12$; Fig. 3).

Our results do not show significant differences of THg concentration between the two capture sites (site 2, $n = 11$; site 8, $n = 15$; Fig. 1) for full scutes and scute keratin layers of *C. crocodilus* (*t*-test: $t = 0.24$, $p = 0.815$ and $t = 0.15$, $p = 0.882$, respectively). Because no significant differences were found between sites, all *C. crocodilus* were pooled. A positive significant relationship was found between THg concentrations in full scutes and the scute keratin layers for *C. crocodilus* (linear regression: $F_{1-24} = 93.67$, $R^2 = 0.78$, $p < 0.001$; Fig. 4). The significant level of the predictors and the upper and lower bounds of the 95% confidence interval (CI) are given by the equation ($R^2 = 0.788$; $p < 0.001$; 95% CI 0.518–0.938): THg scute keratin layers = 0.525 × THg full scute + 0.321. THg in keratin layers and full scutes are expressed in µg g^{-1} dw.

Table 1 Proportion of moisture (%, mean ± SD (coefficient of variation), [min–max] in claws, scutes and total blood of smooth-fronted caiman, *Paleosuchus trigonatus*, spectacled caiman, *Caiman crocodilus* and dwarf caiman, *Paleosuchus palpebrosus* from French Guiana

Species	Moisture content in claws (*n*)	Moisture content in scutes (*n*)	Moisture content in total blood (*n*)
Caiman crocodilus	25.7 ± 4.5 (17.5) [15.3–47.1] (29)	39.9 ± 19.3 (48.3) [13.5–81.3] (28)	72.1 ± 9.5 (13.2) [57.3–88.7] (20)
Paleosuchus palpebrosus	20.7 ± 4.1 (19.8) [15.5–26.0] (5)	26.4 ± 4.4 (16.7) [20.1–31.5] (5)	78.9 ± 0.2 (0.3) [78.8–79.0] (2)
Paleosuchus trigonatus	46.1 ± 15.2 (33.0) [14.8–65.0] (23)	55.3 ± 21.6 (47.2) [14.4–90.1] (25)	72.9 ± 19.2 (26.3) [39.2–90.3] (9)

n number of samples

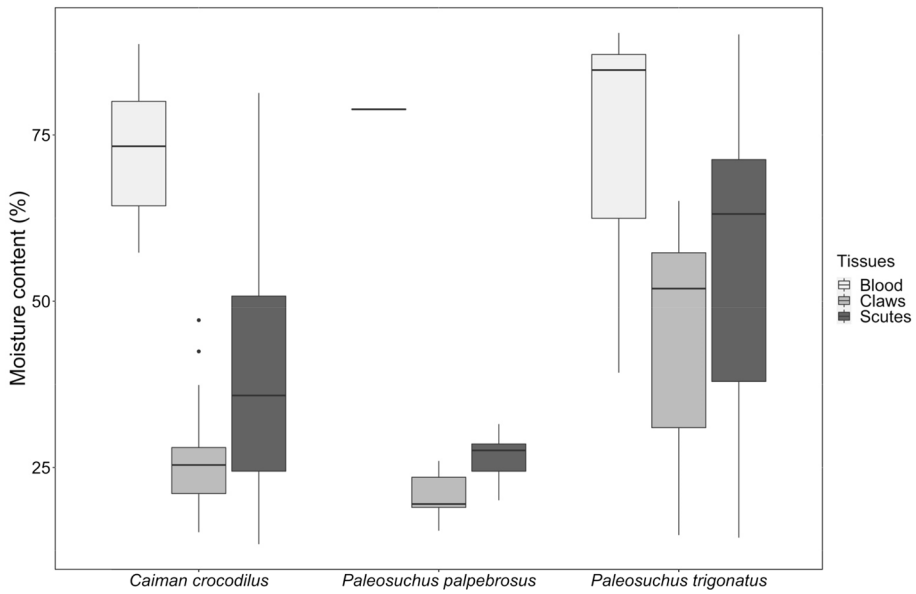

Fig. 2 Moisture content measured in total blood, claws and scutes (%) of spectacled caiman, *Caiman crocodilus*, dwarf caiman, *Paleosuchus palpebrosus*, and smooth-fronted caiman, *Paleosuchus trigonatus*, in French Guiana. Top and bottom of the boxes represent the first and last quartiles. Line across the box represents the median. Whiskers represent the 5th and 95th percentiles. Circles represent outliers

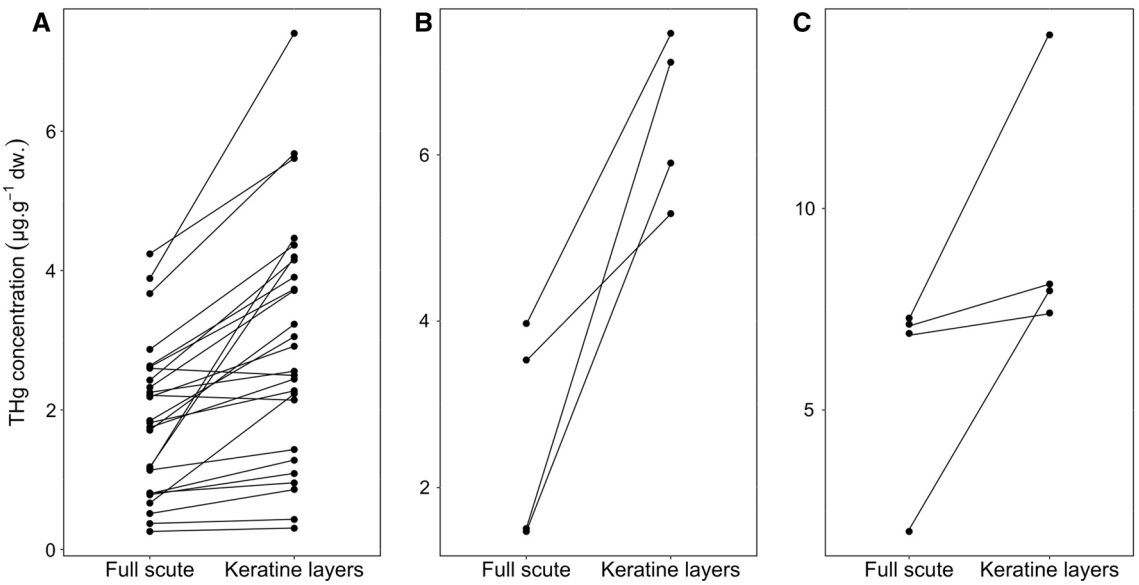

Fig. 3 THg concentrations measured in full scutes and scute keratin layers (µg g^{-1} dw) of spectacled caiman, *Caiman crocodilus* (**a**: $n=26$, paired *t*-test: $t=-5.44$, $p<0.001$), smooth-fronted caiman, *Paleosuchus trigonatus* (**b**: $n=4$, paired *t*-test: $t=-4.71$, $p=0.02$) and dwarf caiman, *Paleosuchus palpebrosus* (**c**: $n=4$, paired *t*-test: $t=-2.18$, $p=0.12$), in French Guiana. Each pair of connected dots correspond to one individual

Relationship Between Tissues

Our results show a significant difference of THg concentrations between tissues within each of the three species (Friedman ANOVA: *P. trigonatus,* $\chi^2=20$, $p<0.001$; *C. crocodilus,* $\chi^2=91.33$, $p<0.001$; *P. palpebrosus,* $\chi^2=19.04$, $p<0.001$, respectively; Table 2). The THg concentrations were always higher in claws and scutes than in total blood and RBCs (Fig. 5). The relationships between body size and THg concentrations in the different tissues, and the relationships between tissues were assessed by Spearman rank test for the three species and summarized in Table 3.

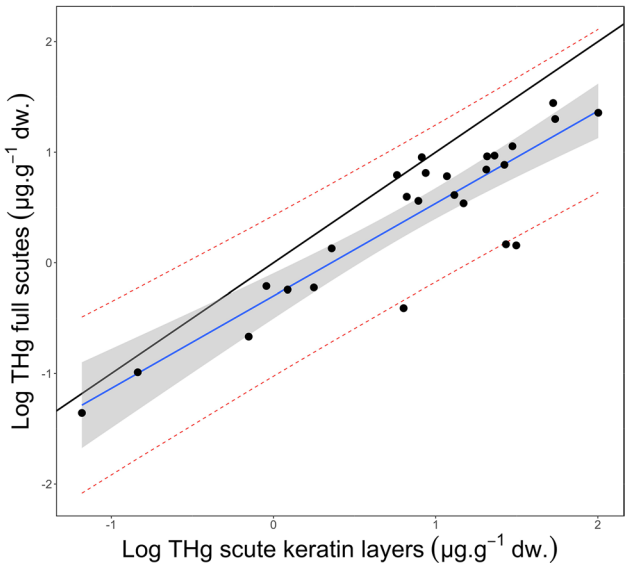

Fig. 4 Linear regression between the mercury (THg) concentration in full scutes and scute keratin layers (in µg g^{-1} dw) of the spectacled caiman (*Caiman crocodilus*; $n=26$; $F_{1-24}=93.67$, $R^2=0.78$, $p<0.001$, in blue). Regression lines (in red) with 95% confidence intervals indicate highly significant relationships between two tissues. Black line represents isometric scaling (ratio 1:1 between the two tissues)

Discussion

Moisture Content

Our results show a strong inter- and intraspecific variability of moisture content between tissues (Table 1; Fig. 2). Indeed, claws contain both alpha- and beta-keratin and present the lowest moisture content, whereas scutes are mainly composed of beta-keratin in association with osteoscutes and connective tissues, which increases water content (Richardson et al. 2002). In contrast to the study of Yanochko et al. (1997), in which study sites were separated by a very large distance, we did not find any geographic differences regarding moisture content in the examined tissues, which may be linked to the more restricted area of our study. Our results suggest that the relatively large variation of moisture content in fresh samples can induce variations in terms of trace element quantification, including THg concentration. This seems particularly the case for keratinized tissues, whereas moisture content in blood samples was less variable. Overall, our assessment of the moisture content in various tissues clearly suggests that the use of dry samples for trace elements quantification should be favoured to facilitate future large-scale comparisons of contamination in crocodilians. Alternatively, the moisture content of the tissues should be specified when trace element concentrations are expressed in wet weight to allow straightforward conversion between units.

THg Concentration: Scute Keratin Layers Versus Full Scutes

Clipping tail scutes is a common sampling method in crocodilians which further allows identification of the animal and can be used for DNA, stable isotopes and contaminant analyses (Jagoe et al. 1998; De Thoisy et al. 2006; Rainwater et al. 2007; Machkour-M'Rabet et al. 2009; Trillanes et al. 2014; Pacheco-Sierra et al. 2016; Santos et al. 2018; Lemaire et al. 2021a). The skin of crocodilians is composed of bony scutes covered by connective tissue and keratin layers (Richardson et al. 2002; Alibardi 2003). Although several studies reported concentrations of Hg in crocodilian scutes (Jagoe et al. 1998; Lázaro et al. 2015; Schneider et al. 2015; Buenfil-Rojas et al. 2018), detailed information on the actual part of the scute which had been used for analyses is generally missing, precluding further comparisons (Schneider et al. 2015). With the exception of two animals, our results show that THg concentrations significantly differ between full scutes (combination of keratin, bone, and connective tissues) and scute keratin layer, which can be explained by the poor affinity of Hg for bone tissue (Schneider et al. 2015),

Table 2 Biometric data (cm) (mean ± SD, min—max (n)) and THg concentrations (µg g^{-1} dw; mean ± SD, min—max (n)) in the tissues of smooth-fronted caiman, *Paleosuchus trigonatus*, spectacled caiman, *Caiman crocodilus* and dwarf caiman, *Paleosuchus palpebrosus,* in French Guiana. *SVL* Snout Vent Length; *TL* Total Length; *RBCs* red blood cells; *n* number of samples

Species	SVL	TL	THg claws	THg scutes	THg RBCs	THg Total blood
Paleosuchus trigonatus	33.32 ± 19.81 10.90–81 (51)	62.11 ± 36.42, 22.8–143 (51)	2.420 ± 1.905[a], 0.147–7.509 (50)	3.332 ± 3.066[a], 0.087–9.859 (48)	0.447 ± 0.270 [b], 0.049–0.774 (11)	0.300 ± 0.178 [b], 0.032–0.738 (24)
Caiman crocodilus	32.70 ± 13.57 14.5–103 (48)	66.60 ± 24.11 31.0–176.0 (48)	2.692 ± 1.608[a] 0.321–8.807 (48)	2.638 ± 1.497[a] 0.307–7.407 (47)	0.963 ± 0.612 [b] 0.145–2.244 (26)	0.605 ± 0.380 [b] 0.089–1.532 (40)
Paleosuchus palpebrosus	38.12 ± 15.29 16.5–62 (12)	79.42 ± 33.22 34.2–150 (13)	8.351 ± 4.965[a] 2.028–20.042 (13)	7.647 ± 4.742[a] 0.789–15.628 (13)	2.364 ± 1.884 [b] 0.447–5.775 (6)	1.376 ± 0.986 [b] 0.540–3.415 (7)

THg values marked with the same letter (a or b) are not statistically different (Pairwise Bergmann–Hommel test, $p<0.05$), test performed independently for the three species

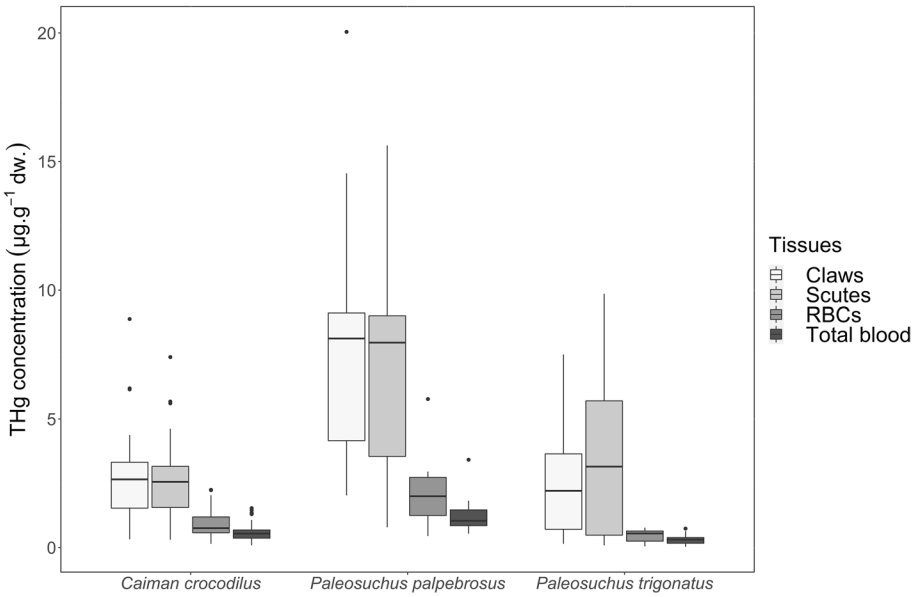

Fig. 5 THg concentrations measured in claws, scutes, red blood cells (RBCs), and total blood (µg g^{-1} dw) of spectacled caiman, *Caiman crocodilus*, dwarf caiman, *Paleosuchus palpebrosus*, and smooth-fronted caiman, *Paleosuchus trigonatus*, in French Guiana. Top and bottom of boxes represent the first and last quartiles. Line across box represents the median. Whiskers represent the 5th and 95th percentiles. Circles represent outlier

Table 3 Relationship between body size (cm) and THg concentration (µg g^{-1} dw) in tissues, and THg concentration between tissues of smooth-fronted caiman, *Paleosuchus trigonatus*, spectacled caiman, *Caiman crocodilus* and dwarf caiman, *Paleosuchus palpebrosus*

	TL	SVL	THg scutes	THg claws	THg RBCs
Paleosuchus trigonatus					
THg scutes	**0.877** (48)	**0.885** (48)	–	–	–
THg claws	**0.842** (50)	**0.850** (50)	**0.945** (48)		–
THg RBCs	0.451 (11)	**0.623** (11)	0.614 (10)	0.614 (10)	–
THg total blood	**0.730** (24)	**0.769** (24)	**0.897** (21)	**0.914** (23)	0.771 (6)
Caiman crocodilus					
THg scutes	**0.459** (47)	**0.472** (47)	–	–	–
THg claws	**0.468** (48)	**0.475** (48)	**0.909** (47)	–	–
THg RBCs	0.353 (26)	0.388 (26)	**0.894** (25)	**0.811** (26)	–
THg total blood	**0.386** (40)	**0.399** (40)	**0.882** (39)	**0.870** (40)	**0.992** (25)
Paleosuchus palpebrosus					
THg scutes	**0.831** (13)	**0.846** (12)	–	–	–
THg claws	**0.945** (13)	**0.937** (12)	**0.857** (13)	–	–
THg RBCs	0.771 (6)	0.600 (5)	0.829 (6)	0.657 (6)	–
THg total blood	0.750 (7)	0.600 (6)	0.643 (7)	0.607 (7)	0.900 (5)

Values refer to ρ (Spearman rank test) and significant relationships are in bold. Sample sizes are given in parenthesis. *TL* total length; *SVL* snout vent length; *RBCs* red blood cells

which results in the highest concentrations in the keratin layer (Fig. 3). In contrast, keratinized tissues of vertebrates, such as hairs/fur, feathers, and claws, generally display high Hg concentrations due to the affinity of Hg for the sulfhydryl groups contained in keratins (Appelquist et al. 1984; Schneider et al. 2012; Benjamin et al., 2018; Treu et al., 2018; Albert et al., 2019). Standardization of the analysed layers when scutes are used for Hg biomonitoring is therefore required to enable comparison between studies. In this respect, our results suggest that selecting the keratin layer should be favored, because this avoids including an unknown quantity of other tissue types (e.g., bone, connective tissues), thereby improving the evaluation of the actual environmental contamination status.

By providing a predictive equation of the relationship between THg concentrations found in full scutes and scute keratin layers of *C. crocodilus*, our study gives an objective tool to compare studies using both tissues (both dry) for this species (Fig. 4).

Relationships Between Tissues

THg concentrations were higher in scutes and claws than in the RBCs and total blood (Table 2; Fig. 5). Blood is involved

in the transport of Hg to different organs and represents the recent Hg exposure of the animal via its diet—one source of variation in Hg contamination in crocodilians (Lemaire et al. 2021a). In crocodilians, Hg concentrations in the blood are known to be related to Hg concentrations of internal tissues due to dynamic transfer in tissues involved in elimination (keratinized tissues), excretion (kidneys), detoxification (liver), and storage (muscles) (Eggins et al. 2015; Nilsen et al. 2017b). In contrast to blood, keratinized tissues are known to reflect the long-term exposure due to the non-reversible binding of Hg to the sulfhydryl residues of the keratins (Schneider et al. 2015; Lázaro et al. 2015; Marrugo-Negrete et al. 2019). A combination of non-lethal sampling of several tissues in caimans provides information on the recent (blood) and the long-term (keratinized tissues) Hg contamination of the individual, as well as the dynamics of Hg contamination over time, which had already been shown in other reptiles (Lemaire et al. 2018).

Our results show a positive correlation between the body size (SVL and TL) of the three species included in the study and the THg concentrations in claws and scutes (Table 3). In contrast, the THg concentration of RBCs was positively related to SVL in *P. trigonatus* solely. The THg concentration in total blood showed a positive relationship with the SVL and TL of *P. trigonatus* and *C. crocodilus*, but not with *P. palpebrosus* (Table 3). The variable relationship between blood Hg and body size is likely related to short-term variations in the diet of the individuals. Accordingly, such relationship was found in some studies on crocodilians (Eggins et al. 2015; Buenfil-Rojas et al. 2018; Lemaire et al. 2021a) but not in others (Yanochko et al. 1997; Eggins et al. 2015; Lawson et al. 2020). This emphasizes the complementarity of assessing Hg concentrations related to both long-term integration (i.e., keratinized tissues such as claws and scutes) and short-term exposure (i.e., blood) in biomonitoring studies.

Conclusions

Our results highlight the need to standardize the evaluation of Hg concentrations in crocodilians between studies. The variation in moisture content between tissues and individual animals increases the variation in the reported Hg concentrations and hence precludes robust comparison between studies. To avoid such shortcomings, researchers should perform trace elements quantification to report their results based on dry weight analytical procedures or should provide the information on the tissue moisture contents when studies report concentrations based on wet weight. In addition, our results emphasize the need to analyze scute keratin layer rather than full scutes to provide less variable and more reliable values of long-term Hg contamination of caimans. Finally, the simultaneous use of both blood and keratinized tissues in crocodilians offer the possibility to quantify different time scales of Hg exposure by non-lethal sampling.

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Authors' Contributions JL, FB, OM, RM, PB conceived and designed experiments. JL, FB performed statistical analysis. JL performed chemical analysis. JL, FB, OM, RM, PB wrote the manuscript.

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Declarations

Conflict of interest The authors have no competing interests to declare.

Ethics Approval Permission to capture individuals, draw blood and sample claws and scutes was granted by the French authorities (Direction Régionale des Territoires et de la Mer) after evaluation by the CSRPN, the regional scientific committee (Permit: R03-2016–06-21–010; R03-2019–01-09–001; R03-2019–10-24–007, www.guyane.developpement-durable.gouv.fr)

Availability of Data and Materials Data are available from the corresponding author (jeremy.lemaire@univ-lr.fr).

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