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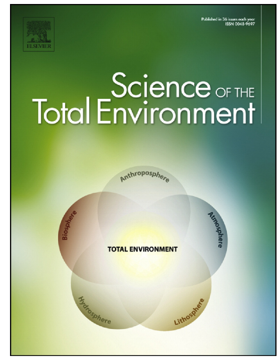

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Relationships between stable isotopes and trace element concentrations in the crocodilian community of French Guiana

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Abstract

Trace elements in the blood of crocodilians and the factors that influence their concentrations are overall poorly documented. However, determination of influencing factors is crucial to assess the relevance of caimans as bioindicators of environmental contamination, and potential toxicological impact of trace elements on these reptiles. In the present study, we determined the concentrations of 14 trace elements (Ag, As, Cd, Cr, Co, Cu, Fe, Hg, Pb, Mn, Ni, Se, V, and Zn) in the blood of four French Guiana caiman species (Spectacled Caiman *Caiman crocodilus* [n = 34], the Black Caiman *Melanosuchus niger* [n = 25], the Dwarf Caiman *Paleosuchus palpebrosus* [n = 5] and the Smooth-fronted Caiman *Paleosuchus trigonatus* [n = 20]) from 8 different sites, and further investigated the influence of individual body size and stable isotopes as proxies of foraging habitat and trophic position on trace element concentrations. Trophic position was identified to be an important factor influencing trace element concentrations in the four caiman species and explained interspecific variations. These findings highlight the need to consider trophic ecology when crocodilians are used as bioindicators of trace element contamination in environmental studies.

Keywords: Caiman, Trophic ecology, Tropical ecosystem, Blood, Contaminant

INTRODUCTION

All over the globe, anthropogenic activities release a variety of contaminants into ecosystems (Bard, 1999; Pacyna and Pacyna, 2001; Lewis *et al.*, 2011; Tkaczyk *et al.*, 2020). Industrial processes, mining activities and fossil fuel combustion mainly contribute to environmental contamination by releasing trace elements (Pacyna *et al.*, 2007; Pirrone *et al.*, 2010; Vereda *et al.*, 2019). While trace elements are naturally present in the environment, their levels drastically increase due to human activities. In South America, trace elements are naturally present in high concentrations in the soils; mining activities and industrial processes have strongly increased, which results in massive discharges of trace elements such as cadmium (Cd), mercury (Hg), and lead (Pb) into the environment (Smolders *et al.*, 2003; Guédron *et al.*, 2009; Burger *et al.*, 2018). The major concern linked to elevated concentrations of trace elements is related to their persistence and toxicity in different ecosystem compartments: trace elements, and particularly non-essential elements such as Hg and Pb, bioaccumulate in organisms, and Hg further biomagnifies through the trophic webs, exposing top predators to relatively high concentrations of this contaminant.

High trophic-level predators such as fish, birds, reptiles or mammals have successfully been used as bioindicator organisms to monitor trace element contamination in different habitats by using a variety of tissues that integrate contaminants over different periods of time, depending on their physiological role (Silva *et al.*, 2018; Kalisińska, 2019; Albuquerque *et al.*, 2021; Lemaire *et al.*, 2021a). More recently, the use of non-lethal sampling methods (e.g., sampling of blood, hair, scales, claws and feathers) has been emphasised as a welcome effort to decrease potential impacts of sampling on wildlife (Carravieri *et al*., 2014; Guillot *et* *al.*, 2018; Treu *et al.*, 2018; Lettoof *et al.*, 2021). Additionally, the use of wildlife species to monitor environmental contamination requires information on the influence factors. In most vertebrates, foraging habitats and trophic position are important factors influencing trace element bioaccumulation (Le Croizier *et al.*, 2016; Sebastiano *et al.*, 2017). Stable isotope ratio of nitrogen (δ^{15}N) has become a standardised tool to determine the trophic position of an organism in the food web (Post, 2002; Boecklen *et al.*, 2011). Tissues of consumers are ^{15}N-enriched as a function of their diet, meaning that consumers at the top of the food web show higher δ^{15}N values (Minagawa and Wada, 1984; Peterson and Fry, 1987). In a complementary manner, the stable isotope ratio of carbon (δ^{13}C) is intensively used to determine foraging habits of organisms, allowing to discriminate the preferential feeding habitats (Post, 2002). Thus, the combined use of δ^{15}N and δ^{13}C offers a powerful tool to access an organisms' trophic ecology.

Crocodilians can be used to monitor the environmental contamination through trace elements (Nilsen *et al.*, 2019). They have a lifespan of several decades leading to long-term accumulation of contaminants. Furthermore, they have a comparatively low metabolic and tissue turn-over rates which increases the bioaccumulation of contaminants (Campbell, 2003). Consequently, trace elements such as Hg and Pb among others, accumulate in crocodilian tissues (Jeffree *et al.*, 2001; Almli *et al.*, 2005; Warner *et al.*, 2016; Quintela *et al.*, 2020). Among the factors which influence Hg contamination in crocodilians, body size appears important in some species (Jagoe *et al.*, 1998; Schneider *et al.*, 2012; Buenfil-Rojas *et al.*, 2015). The trophic position in the food web appears also as a key factor (Lemaire *et al.*, 2021b). However, studies combining trace elements and trophic information are lacking though it is crucial to understand species-specific contamination.

The present study considers the four caiman species living in French Guiana, the Spectacled Caiman *Caiman crocodilus*, the Black Caiman *Melanosuchus niger*, the Dwarf Caiman *Paleosuchus palpebrosus* and the Smooth-fronted Caiman *Paleosuchus trigonatus* to investigate trace element contamination at a large spatial scale in the region. We determined the concentrations of 14 trace elements in caiman blood as blood concentrations of many trace elements are good predictors of other tissues such as muscles or liver, and as blood is a dynamic matrix which reflect circulating trace elements between other tissues (Eggins *et al.*, 2015; Nilsen *et al.*, 2017). We further investigated the influence of the body size and of the trophic ecology on trace element concentrations using stable isotopes of carbon and nitrogen as proxies.

MATERIAL AND METHODS

Sample collection

84 individuals of four caiman species were captured at 8 different sites in French Guiana (Fig. 1). Spectacled Caimans (*Caiman crocodilus*, n = 34) were captured at the "Pripris de Yiyi" (n = 18) and "Kaw river" (n = 16) sites, Black Caimans (*Melanosuchus niger*, n = 25) were captured at the "Mare Agami" site, Dwarf Caimans (*Paleosuchus palpebrosus*, n = 5) were captured at "Pripis de yiyi" (n = 2), "Matoury" (n = 1) and "Mana" (n = 2) sites, and Smooth-fronted Caimans (*Paleosuchus trigonatus*, n = 20) were captured at the sites "Nouragues station" (n = 15), "Mont Grand Matoury" (n = 4) and "French Guiana space centre" (n = 1) (Fig. 1).

Total length (TL) of all individuals was measured ventrally, and body mass was recorded. We drew blood samples (0.2 – 3 mL) either through occipital venous sinus puncture, using a syringe with a 30 gauge – 50 mm heparinized needle (heparin sodium), or through the lateral tail vein with a 27 gauge - 25 mm or 21 gauge - 50 mm heparinized needle (heparin sodium), depending on the size of the animal. Blood samples were immediately stored at 4°C and further kept at -21°C. Whole blood was freeze-dried for 48 hours to eliminate water and then ground into a homogeneous powder before further analysis.

All individuals were released at the place of capture immediately after sampling. Capture and sample collection were performed under permits from French authorities (Direction Régionale des Territoires et de la Mer) after evaluation by the CSRPN, the regional scientific committee (Permit: N°155/DEAL/2013, N°2014114-006, N°2014114-007, N°2015034-008, R03-2019-01-09-001, R03-2019-10-24-007).

Trace element analyses

Total mercury (THg) was quantified using an atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254; Altec®). Two replicates of 0.5 - 3.0 mg dry weight (dw) were analysed for each sample. Reproducibility for duplicate samples was approved when the relative standard deviation (RSD) was below 10%. The method was validated by the analysis of certified reference material (CRM) TORT-3 (Lobster hepatopancreas from the National Research Council of Canada (NRCC); certified Hg concentration: 0.292 ± 0.022 $\mu g.g^{-1}$ dw) at the beginning and the end of the analytical cycle and after every 5 samples. Measured values for TORT-3 were 0.292 ± 0.006 $\mu g.g^{-1}$ dw (n = 20), with a recovery of 99.85 ± 2.13 %. Blanks were included at the beginning of each analytical run and the limit of quantification of the AMA was 0.05 ng Hg.

Silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), vanadium (V) and zinc (Zn) were determined using Inductively Coupled Plasma (ICP) Optical Emission Spectrometry (Varian

Vista-Pro ICP-OES) and Mass Spectrometry (Series II Thermo Fisher Scientific ICP-MS) on mineralized aliquots (mass: 5 - 150 mg dw) as described in Bustamante *et al.* (2008). Aliquots were microwave-digested in a mixture of 6 mL 65% HNO_3 (VWR Quality SUPRAPUR) and 2 mL 30% HCl (VWR Quality SUPRAPUR), except for samples with a weight below 100 mg where volumes of HNO_3 and HCl were divided by half. Samples were then diluted to 50 mL (25 mL for samples with a weight below 100 mg) with ultrapure water. To avoid trace element contamination, all utensils used were soaked in a bath of diluted nitric acid for 48h, rinsed with ultrapure water, and dried. Two CRM (DOLT-3, Dogfish liver, NRCC, and TORT-2, Lobster hepatopancreas, NRCC) were treated and analysed in the same way as the samples. Results were in agreement with the certified values and displayed recoveries ranging from 88% to 116% (n = 10), proving repeatability of the method. All trace element concentrations are presented in $\mu g.g^{-1}$ dw.

Isotope analysis

Except for *M. niger* stable isotopes, which were analysed as described in Caut *et al.* (2019), all nitrogen and carbon stable isotopes were determined in freeze-dried whole blood (aliquots mass: ~0.3mg) with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA1112). Results are presented in the usual δ notation relative to the deviation from standards (Pee Dee Belemnite for δ^{13}C and atmospheric nitrogen for δ^{15}N), in parts per thousand (‰) following the formula δ^{15}N or δ^{13}C = $[(R_{sample}/R_{standard})-1]x1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$ for δ^{15}N or δ^{13}C. Replicate assays of internal laboratory standards (n = 56) indicated maximum measurement errors of ± 0.14 ‰ for nitrogen, and ± 0.18 ‰ for carbon isotope measurements.

Statistical analysis

All statistical analyses were performed using the Software R v.3.6.1 (R core Team, 2019).

Statistical analyses were only performed on trace elements with concentrations above the limit of quantification (LOQ) in a minimum of 70% of individuals. All data were checked for normality and homogeneity of variances and log-transformed if necessary. Differences in body size between species were assessed by ANOVA. Differences in isotope composition and trace element concentrations between species were performed by ANOVAs or ANCOVAs with body size as a cofactor. Then post-hoc Tukey's honestly significant difference (HSD) was applied to evaluate the variation of contaminant and isotope values between species. Relationships between trace elements, isotopes and body size were performed by general regression models (simple or polynomial). Principal component analysis (PCA) was performed on log-transformed trace elements to detect covariance and the contaminants that reflect most of the total variance. Generalised linear models (GLM) were used to test relation of feeding ecology (using stable isotopes), sites and species on trace element concentrations. Forward selection using Akaike's Information Criterion (AICc) was applied, and the effect of variables affecting contaminants was inferred through Akaike's weights and R^2 adjusted.

RESULTS

Trace element concentrations

Among the 14 targeted trace elements, only the essential elements Cu, Fe, Mn, Se and Zn, and non-essential elements Hg and Pb were detected in the blood for more than 70% of individuals (Table S1). Ag and V always remained below the LOQ and As, Cd, Co, Cr, and Ni were only detected in few individuals (Table S1). Arsenic was exclusively detected in *C. crocodilus* (0.34 ± 0.15 µg.g^{-1} dw) which were captured in the "Kaw river" estuary, and in a single subadult *M. niger* (0.24 µg.g^{-1} dw) from "Mare Agami" (Table S1). Additionally, Cr was found in *P. trigonatus* (0.34 ± 0.01 µg.g^{-1} dw) and *C. crocodilus* (0.36 ± 0.14 µg.g^{-1} dw). Apart from Fe concentrations, all trace element concentrations varied significantly between species (ANOVAs and ANCOVAs, all $p < 0.05$, Table 1). *P. trigonatus* showed the highest concentrations for Cu (8.32 ± 5.41 µg.g^{-1} dw), Mn (0.34 ± 0.26 µg.g^{-1} dw), Se (4.05 ± 1.43 µg.g^{-1} dw) and Zn (68.9 ± 53.6 µg.g^{-1} dw). The highest Hg concentrations were found in *M. niger* (1.56 ± 0.65 µg.g^{-1} dw) while *P. palpebrosus* showed the highest concentrations of Pb (1.35 ± 1.43 µg.g^{-1} dw, Table 1).

The Se:Hg molar ratio differed between species with the highest values for *P.trigonatus* (31.42 ± 14.28, Table 1).

Relationships with isotope values and sites

The PCA analysis which included Cu, Fe, Hg, Mn, Pb, Se, and Zn, explaining 60,8% of the total variance, revealed strong species segregation in the ordination space (Fig. 4). Fe and Hg were not strongly associated with other trace elements. Most parsimonious GLM models selected by AICc showed that the species and the δ^{15}N values explained most of the total variation in Cu, Fe, Hg, and Zn concentrations in the four caiman species while the sites and the species explained Pb concentrations and finally the sites and the δ^{13}C values explained Se concentrations (Table 2).

δ^{15}N and δ^{13}C values varied significantly between species (ANCOVAs, all $p < 0.05$, Table 1); *P. trigonatus* showed the highest values for δ^{15}N (8.28 ± 0.94 ‰) and δ^{13}C (-26.38 ± 1.21 ‰).

The relationship between the δ^{15}N value and body size was positive for *P. palpebrosus* and *C. crocodilus* (R^2 = 0.781, p = 0.047 and R^2 = 0.364, p < 0.001, respectively). For δ^{13}C, a positive relationship with body size was only found for *C. crocodilus* (R^2 = 0.128, p = 0.038, Table S2). In this species, positive relationships were found between δ^{13}C values and the concentrations of Mn (R^2 = 0.217, p = 0.005) and Pb (R^2 = 0.535, p < 0.001), and a negative relationship for Zn (R^2 = 0.142, p = 0.028). *C. crocodilus* also had negative relationships between δ^{15}N values and the concentrations of Cu (R^2 = 0.129, p = 0.037), Se (R^2 = 0.304, p = 0.011), and Zn (R^2 = 0.225, p = 0.005), but δ^{15}N values correlated positively with Mn (R^2 = 0.263, p = 0.002).

For *P. trigonatus*, positive relationships were found between δ^{13}C and δ^{15}N with Hg and Se concentrations (respectively R^2 = 0.229, p = 0.033 and R^2 = 0.611, p < 0.001 (Fig. 3), and R^2 = 0.290, p = 0.038 and R^2 = 0.289, p = 0.039).

For *P. palpebrosus*, a negative relationship was found between the δ^{13}C value and Cu (R^2 = 0.880, p = 0.018), a positive relationship for Hg concentration (R^2 = 0.978, p = 0.002), and a positive relationship between the δ^{15}N and Hg (R^2 = 0.877, p = 0.019; Fig. 3), and negative for Cu (R^2 = 0.920, p = 0.010) and Mn (R^2 = 0.935, p = 0.007).

For *M. niger*, a negative relationship was found between the δ^{15}N values and Cu concentrations (R^2 = 0.171, p = 0.040), and positive relationship for Hg (R^2 = 0.391, p < 0.001; Fig. 3).

Relationship with body size

The relationship between Hg concentration and body size was positive for *P. trigonatus* (R^2 = 0.353, p = 0.006) and *M. niger* (R^2 = 0.628, p < 0.001) (Fig. 2), and marginally positive for *P. palpebrosus* (R^2 = 0.768, p = 0.051) and *C. crocodilus* (R^2 = 0.112, p = 0.053) (Table S2).

Selenium concentrations showed a negative relationship with the body size of *P. trigonatus* (R^2 = 0.639, $p < 0.001$). In *M. niger*, Fe and Mn concentrations showed negative relationships with body size (respectively, R^2 = 0.229, p = 0.016 and R^2 = 0.180, p = 0.034). The relationship between the Se:Hg molar ratio and the body size was negative for *P. trigonatus* (R^2 = 0.306, p = 0.042), *C. crocodilus* (R^2 = 0.162, p = 0.033) and *M. niger* (R^2 = 0.648, $p < 0.001$).

DISCUSSION

The present study is the first to investigate trace element concentrations and their relationship to body size and stable isotopes as proxies of foraging habitat and trophic position, in the crocodilian community of French Guiana. Our results showed that trophic ecology influence trace element concentrations, an aspect which needs to be evaluated when caimans are used as bioindicators.

Trace element concentrations

In the present study, Hg was detected in all samples, which indicates that all caiman species in French Guiana are under Hg contamination. This finding is not surprising considering that Hg is a widespread environmental contaminant that affects ecosystems worldwide (Chen *et al.*, 2018). Additionally, forest soils of the area are known to present high natural Hg concentrations, with an average of 0.3 $\mu g.g^{-1}$ dw (Richard *et al.*, 2000). In the present study, *C. crocodilus* presents a higher average blood Hg concentration than individuals of similar body size from Brazil and Colombia (Eggins *et al.*, 2015; Marrugo-Negrete *et al.*, 2019; Table 3). Blood Hg concentrations in *M. niger* from French Guiana are also higher than reported values from Brazil (Eggins *et al.*, 2015; Table 3). We cannot perform comparison regarding blood Hg concentration in *P. trigonatus* and *P. palpebrosus* as there is no published data for the *Paleosuchus* genus besides French Guiana. Mercury is well known for its ability to biomagnify through the food web and to bioaccumulate in top predators with age due to a low excretion rate (Lavoie *et al.*, 2013). Elevated concentrations found in caimans from French Guiana, in comparison to other studies, might be caused by high environmental Hg concentrations in the associated food chain, and/or are the result of a long trophic chain that can increase the biomagnification process (De Almeida Rodrigues *et al.*, 2019).

Lead was found in more than 90% of samples with the highest values in *P. palpebrosus* (1.35 ± 1.43 $\mu g.g^{-1}$ dw) followed by *P. trigonatus* (0.58 ± 0.77 $\mu g.g^{-1}$ dw). The lack of data on Pb concentrations in the blood of crocodilians does not allow for a thorough comparison. However, the relatively high Pb concentrations we have found in *P. trigonatus* are concerning because the study sites seems undisturbed by any known anthropogenic activities. Nevertheless, Pb concentrations were found in other caiman species, and our results have identified that both the sites and the species were related to Pb variation (Table 2). In French Guiana, the background levels of Pb in soil have recently been identified as elevated with very high values in some areas without any apparent relation to anthropogenic activities (S. Guédron, personal communication). The geological background appears to be the main explanation for Pb found in the blood of *P. trigonatus*, which may thus reflect a natural contamination. Additionally, for *P. palpebrosus*, such natural contamination may have been affected by anthropogenic activities at some of our study sites (i.e., French Guiana international airport and rice cultivation at “Mana”, Sites #5 and #1 in Fig. 1) because of the use of specific products containing Pb (e.g., pesticides, herbicides, Defarge *et al.*, 2018). Natural Pb contamination was already reported in the Nile Crocodile *Crocodilus niloticus* from a pristine environment without any known anthropogenic Pb sources, with concentrations of Pb and size of individuals being both higher than in the present study (Warner *et al.*, 2016, Table 3). Pb concentrations, which appear to originate from natural sources, may be of concern as the metal is an extremely toxic element for human and wildlife that affects reproductive systems, renal and hepatic functions, and endocrine processes (Wani *et al.*, 2015; Pain *et al.*, 2019, Lemaire et *al.*, 2021c). As crocodiles efficiently assimilate Pb (Hammerton *et al.*, 2003), they may suffer Pb poisoning. Future studies are required to determine the source of this contamination in French Guiana by using Pb stable isotopes and to further investigate the consequences of this toxic element in crocodilians.

In the present study, Se concentrations were quite different between species, with *P. trigonatus* and *P. palpebrosus* having higher Se concentrations than *C. crocodilus* and *M. niger* (Table 1). While Se is an essential trace element involved in the metabolism of living organisms (Kieliszek and Blazejak, 2016), it negatively affects body condition in the American alligator, *Alligator mississippiensis* (Finger *et al.*, 2017). However, Se by its antagonist interaction with Hg can play a major role in the reduction of Hg toxicity in vertebrates(Rahman *et al.*, 2019, Manceau *et al.*, 2021). In this order, the Se:Hg molar ratio is important to inform on potential capacities to protect an organism from Hg toxicity. *P. trigonatus* showed the highest Se:Hg molar ratio compared to the other caiman species in French Guiana (Table 1). Sources of Se are closely related to the diet (Rayman *et al.*, 2008), suggesting that trophic ecology can influence variation of Se concentration. The diet of *P. trigonatus,* which is composed of terrestrial prey, can explain differences with the other species, which mainly forage in aquatic habitats (see below; Magnusson *et al.*, 1987; Villamarín *et al.*, 2017).

Among the essential trace elements, only Fe concentrations were in the same range for all species while Cu, Mn and Zn concentrations were different between the species (Table 1). The lack of variation for Fe relates to its key role in haemoglobin, suggesting that Fe regulation is comparable between species. As the according data is limited in crocodilians, we cannot make a robust comparison.

Influence of body size

Hg was the only non-essential trace element that showed a positive relationship with body size for all species. Crocodilians as most ectothermic vertebrates have an indeterminate growth, and consequently, age and size are generally correlated (i.e., Campos *et al.*, 2013; Eaton and Link, 2011). This highlights that larger – presumably older - caimans present higher Hg blood concentrations than smaller – hence younger - ones. Indeed, the positive relationship between Hg concentration and body size of *M. niger* is in accordance with the study from Eggins *et al.* (2015) in Brazil, though contrasts with previous studies for *C. crocodilus* in Brazil and Colombia, where Hg concentrations and body size were not correlated (Eggins *et al.*, 2015; Marrugo-Negrete *et al.*, 2019). In most vertebrates, Hg blood concentration is considered to represent a relatively recent contamination (Monteiro and Furness, 2001; Fournier *et al.*, 2002; Schneider *et al.*, 2015) that reflects variations in the environmental Hg contamination or modifications in the origin of prey. Such variations may explain the non-consistence in the relationship between body size and Hg contamination in some cases. However, the consistence of the relationships in our results can be related to a constant environmental Hg contamination and/or a stability in the origin of prey in the examined caiman populations.

Additionally, our results show a negative relationship between the Se:Hg molar ratio and body size of *C. crocodilus*, *M. niger* and *P. trigonatus*. As previously discussed, body size is generally related to age in crocodilians which leads to an accumulation of Hg over time in the tissues. A decrease in the Se:Hg molar ratio can indicate that dietary change between juveniles and adults leads to lower Se concentrations in the prey of adults. Additionally, this decrease can be explained by the increase of Hg contamination, which needs to be detoxified to cope with its toxic effect. The main detoxification process in vertebrates involves Se for demethylation of MeHg, and its co-precipitation with Se to form tiemannite nanoparticles in the liver, but also in other tissues and organs such as muscles, kidneys and brain (Korbas *et al.*, 2010; Manceau *et al.*, 2021; Renedo *et al.*, 2021). Demethylation requires a substantial amount of Se and leads to a depletion of the available Se for the organism (Manceau *et al.*, 2021). The levels of the different physiochemical forms of Hg and their relation to the Se proteins deserve further studies to understand the consequences of Se:Hg molar ratio variation in blood, and potential implications on physiological processes and health status of caimans.

Concerning other trace elements, Fe and Mn concentrations negatively related to the body size of *M. niger* only. Mn bioconcentrates significantly in the aquatic ecosystem at low trophic levels (Briand *et al.*, 2018), and the change in diet between juveniles and adults may lead to such a decrease of Mn concentrations in adults compared to juveniles.

Influence of trophic ecology

Our results show that variations of Hg concentrations were explained by the species and the δ^{15}N (Table 2), which is a proxy of consumers' trophic position (Post, 2002). Additionally, the increase of blood Hg concentrations with δ^{15}N for *P. trigonatus*, *P. palpebrosus* and *M. niger* reflects the biomagnification process which is already described in several aquatic and terrestrial ecosystems (Cristol *et al.*, 2008; Rimmer *et al.*, 2010; Lavoie *et al.*, 2013). Differences in δ^{15}N values between species can result from the interspecific competition which leads to a different trophic ecology of the four caiman species (Magnusson *et al.*, 1987; Moldowan *et al.*, 2016; Villamarín *et al.*, 2017) (Fig. 5). Our results highlight that trophic levels assessed via the δ^{15}N values are key factors driving Hg contamination in caimans. Elevated Hg concentrations are particularly concerning regarding the deleterious effects of this metal as already reported in several taxa, such as reproduction impairment and alteration of physiological functions (Day *et al.*, 2007; Evers, 2018; Morcillo *et al.*, 2017). Thus, the trophic level is an important factor that should be considered when mercury contamination and its toxicity are assessed in caimans.

In contrast to Hg, Se concentrations were mostly explained by the sites and the δ^{13}C. As δ^{13}C does not vary significantly across the trophic chain and depends on the primary sources, it informs on the foraging habitat. The diet of *P. trigonatus* consists of more than 50% of terrestrial animals such as snakes, rodents, monkeys and other herbivorous animals; their consumption of plants, fruits and prey that are rich in Se can explain high Se concentrations in the blood of this species (Magnusson *et al.*, 1987; Ortiz *et al.*, 2013; Moldowan *et al.*, 2016; Villamarín *et al.*, 2017; Mangione *et al.*, 2020). In this respect, the caimans which consume terrestrial prey may have an advantage in the Hg detoxification as discussed before, due to an elevated intake of Se.

Most of the observed trace element variations were explained by the species, and the δ^{13}C for Mn, and the δ^{15}N for Cu and Zn. Caimans with a diet composed of δ^{13}C-enriched prey were consequently more likely to have high Mn concentrations. The trophic level of individuals influences Cu and Zn concentrations. These results highlight that the feeding preferences of caimans drive most trace elements, as it was already demonstrated in other wild vertebrates such as fish, seabirds or mammals (Lahaye *et al.*, 2007; Bodin *et al.*, 2017; Carravieri *et al.*, 2020).

CONCLUSION

In the present study, trophic position was identified to influence trace elements in the four caiman species and explains the interspecific concentrations, which highlights the necessity to evaluate trophic ecology when crocodilians are used as bioindicators of trace element contaminations in environmental studies. Additionally, Hg and Pb concentrations are relatively high in comparison to concentrations already reported in other studies and are concerning regarding their toxic effects on wildlife. In the genius *Paleosuchus* sp., Pb needs particular attention in French Guiana because of the high concentrations found in the blood of caimans at some sites. Lastly, the interspecific variability of the Se:Hg molar ratio deserves future study to understand the potential impact regarding protective effect against Hg and the role play by the diet.

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Table 1. Biometric data, trace element (µg.g^{-1} dw), δ^{15}N and δ^{13}C (‰) values in the whole blood of the Spectacled Caiman (*Caiman crocodilus*), the Black Caiman (*Melanosuchus niger*), the Dwarf Caiman (*Paleosuchus palpebrosus*) and the Smooth-fronted Caiman (*Paleosuchus trigonatus*) in French Guiana. N = number of individuals

	Caiman crocodilus			*Melanosuchus niger*			*Paleosuchus palpebrosus*			*Paleosuchus trigonatus*		
	N	**Mean ± SD**	**Min / Max**	**N**	**Mean ± SD**	**Min / Max**	**N**	**Mean ± SD**	**Min / Max**	**N**	**Mean ± SD**	**Min / Max**
Total length (cm)	34	72.3 ± 24.7	40.6 / 176.0	25	176.4 ± 72.2	71.0 / 326.0	5	75.3 ± 44.6	35.5 / 150.0	20	82.8 ± 32.7	27.0 / 143.0
Weight (g)	34	2154 ± 5556	173 / 33200	17	10612 ± 19559	970 /85000	4	1095 ± 10[illegible]6	130 / 2500	18	3875 ± 3779	305 / 13300
δ^{15}N	34	6.02 ± 0.96 [a]	4.12 / 8.23	25	6.98 ± 0.62 [b]	4.93 / 7.88	5	7.[illegible]0 ± 0.86 [b,c]	6.36 / 8.44	20	8.28 ± 0.94 [c]	6.10 / 9.20
δ^{13}C	34	–27.14 ± 2.46 [a,b]	–30.72 /–21.84	25	–27.58 ± 0.74 [a]	–29.45 /–26.36	5	–[illegible]7.75 ± 2.10 [a]	–30.28 /–25.11	20	–26.38 ± 1.21 [b]	–26.20 / –23.9
Hg	34	0.61 ± 0.39 [a]	0.09 / 1.53	25	1.56 ± 0.65 [b]	0.54 / 2.89	5	1.50 ± 1.18 [b]	0.54 / 3.42	20	0.35 ± 0.15 [c]	0.10 / 0.70
Se	28	1.36 ± 0.28 [a]	0.76 / 1.92	24	1.14 ± 0.15 [b]	0.85 / 1.[illegible]7	3	3.53 ± 1.60 [c]	2.45 / 5.37	15	4.05 ± 1.43 [c]	2.30 / 7.90
Se:Hg (molar)	28	7.59 ± 5.27 [a]	1.45 / 21.54	24	2.38 ± 1.41 [a,b]	[illegible].04 / 6.00	3	6.26 ± 6.01 [b]	1.82 / 13.09	15	31.42 ± 14.28 [c]	9.80 / 62.30
Cu	34	5.95 ± 4.86 [a]	2.99 / 31.07	25	3.61 ± 0.68 [b]	2.60 / 5.21	5	6.24 ± 2.44 [b,c]	3.24 / 7.97	20	8.32 ± 5.41 [c]	4.20 / 23.10
Fe	34	1520 ± 167 [a]	1201/ 1972	25	1519 ± 1[illegible]6 [a]	1281 / 1896	5	1353 ± 131 [a]	1172 / 1495	20	1476 ± 237 [a]	867 / 1856
Mn	34	0.23 ± 0.09 [a,b]	0.13 / 0.52	25	0.[illegible]0 ± 0.06 [c]	0.05 / 0.34	5	0.18 ± 0.08 [b]	0.09 / 0.30	19	0.34 ± 0.26 [c]	0.10 / 1.30
Pb	32	0.12 ± 0.08 [a]	0.03 / 0.38	23	0.0[illegible] ± 0.09 [b]	0.02 / 0.44	5	1.35 ± 1.43 [c]	0.07 / 3.67	19	0.58 ± 0.77 [c]	0.10 / 2.70
Zn	34	30.6 ± 8.5 [a]	18.5 / 51.1	25	24.7 ± 3.9 [a]	19.2 / 31.7	5	56.2 ± 20.9 [b]	38.9 / 90.8	20	68.9 ± 53.6 [b]	26.2 / 184.0

Letters indicate significant differences (isotopes and trace elements: Tukey's HSD with total length in cofactor, all $p < 0.05$)

Table 2. AICc model ranking of selected trace element concentrations in blood of the four French Guiana caiman species. k = number of parameters, AICc = Akaike's Information Criteria, w_i = AICc weights, *gdf* = goodness of fit.

Models	k	AICc	ΔAICc	w_i	R^2 adj	*gdf*
Hg, GLM, Log-transformed						
Species + $\delta^{15}N$ + Species:$\delta^{15}N$	9	152.14	0.00	0.71	0.56	1
Species + $\delta^{15}N$	6	154.40	2.27	0.23	0.53	1
Species + $\delta^{13}C$ + Species:$\delta^{13}C$	9	158.12	5.99	0.04	0.52	1
Se, GLM, Log-transformed						
Sites + $\delta^{13}C$ + Sites:$\delta^{13}C$	13	-23.25	0.00	0.39	0.88	1
Sites + $\delta^{13}C$	9	-22.78	0.46	0.31	0.87	1
Sites + Species	9	-20.41	2.84	0.09	0.87	1
Pb, GLM, Log-transformed						
Sites + Species	10	197.04	0.00	0.48	0.58	1
Sites + Species + Sites:Species	10	197.04	0.00	0.48	0.58	1
Species + $\delta^{13}C$ + Species:$\delta^{13}C$	9	202.51	5.47	0.03	0.54	0.98
Zn, GLM, Log-transformed						
Species + $\delta^{15}N$	6	106.82	0.00	0.31	0.43	1
Species	5	107.15	0.34	0.26	0.42	1
Species + $\delta^{13}C$	6	107.40	0.58	0.23	0.42	1
Cu, GLM, Log-transformed						
Species + $\delta^{15}N$	6	97.62	0.00	0.27	0.28	1
Species + $\delta^{15}N$ + Species:$\delta^{15}N$	9	98.14	0.52	0.21	0.30	1
Sites + $\delta^{15}N$	9	99.25	1.63	0.12	0.29	1
Mn, GLM, Log-transformed						
Species + $\delta^{13}C$ + Species:$\delta^{13}C$	9	98.15	0.00	0.57	0.57	1
Species + $\delta^{15}N$ + Species:$\delta^{15}N$	9	99.90	1.75	0.24	0.56	1
Species + $\delta^{13}C$	6	102.99	4.84	0.05	0.53	1
Fe, GLM, Log-transformed						
$\delta^{15}N$	3	-108.15	0.00	0.35	0.03	1
$\delta^{13}C$	3	-107.19	0.95	0.22	0.02	1
Species + $\delta^{13}C$	6	-105.82	2.32	0.11	0.04	1

Table 3. Review of Hg and Pb concentrations (µg.g^{-1} dw) reported in crocodilians. TL stands for Total Length and SVL for Snout-Vent-Length (cm). [a] Original data reported in wet weight, transformed in dry weight using a factor of 3.6 according to Lemaire *et al.*, (2021a).

Species	Location	n	Length (cm)	Hg	Pb	Reference
Spectacled Caiman ***Caiman crocodilus***	La Mojana, Colombia	22	57.2 ± 3.5 (TL)	0.23 ± 0.08[a]	-	Marrugo-Negrete et al. (2019)
Spectacled Caiman ***Caiman crocodilus***	La Mojana, Colombia	23	57.5 ± 6.8 (TL)	0.05 ± 0.02[a]	-	Marrugo-Negrete et al. (2019)
Spectacled Caiman ***Caiman crocodilus***	Rio Purus, Brazil	11	80 ± 14 (SVL)	0.22 ± 0.23[a]	-	Eggins et al. (2015)
Spectacled Caiman ***Caiman crocodilus***	French Guiana	34	72.3 ± 27.4 (TL)	0.61 ± 0.39	0.12 ± 0.08	Present study
Black Caiman ***Melanosuchus niger***	Rio Purus, Brazil	16	102 ± 27 (SVL)	0.17 ± 0.12[a]	-	Eggins et al. (2015)
Black Caiman ***Melanosuchus niger***	Kaw swamp, French Guiana	25	176.4 ± 72.2 (TL)	1.56 ± 0.65	0.06 ± 0.09	Present study
Nile crocodile	KwaZulu-Natal, South	34	134.5 (SVL)	-	2.29 ± 6.30[a]	Warner et al. (2016)

Crocodylus niloticus	Africa					
Smooth-fronted Caiman *Paleosuchus trigonatus*	French Guiana	20	82.8 ± 32.7 (TL)	0.35 ± 0.15	0.58 ± 0.77	Present study
Dwarf Caiman ***Paleosuchus palpebrosus***	French Guiana	5	75.3 ± 44.6 (TL)	1.50 ± 1.18	1.35 ± 1.43	Present study

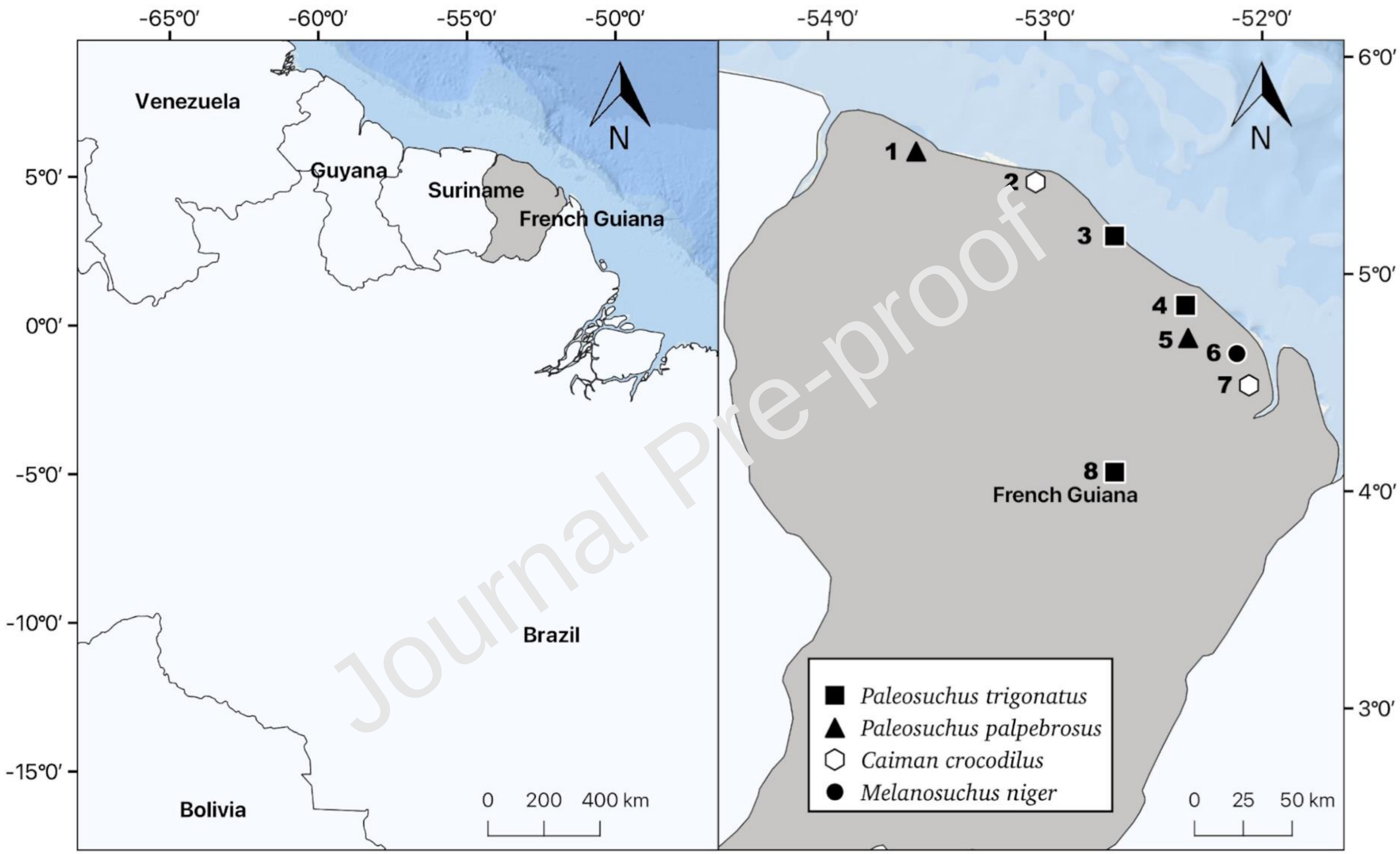

Figure 1. Geographic location of the 8 study sites in French Guiana and distribution of captured caiman species. Sites are 1: "Mana"; 2: "Pripris de Yiyi"; 3: "French Guiana space centre"; 4: "Mont-Grand Matoury"; 5: "Matoury"; 6: "Mare Agami"; 7: "Kaw river"; 8: "Nouragues station".

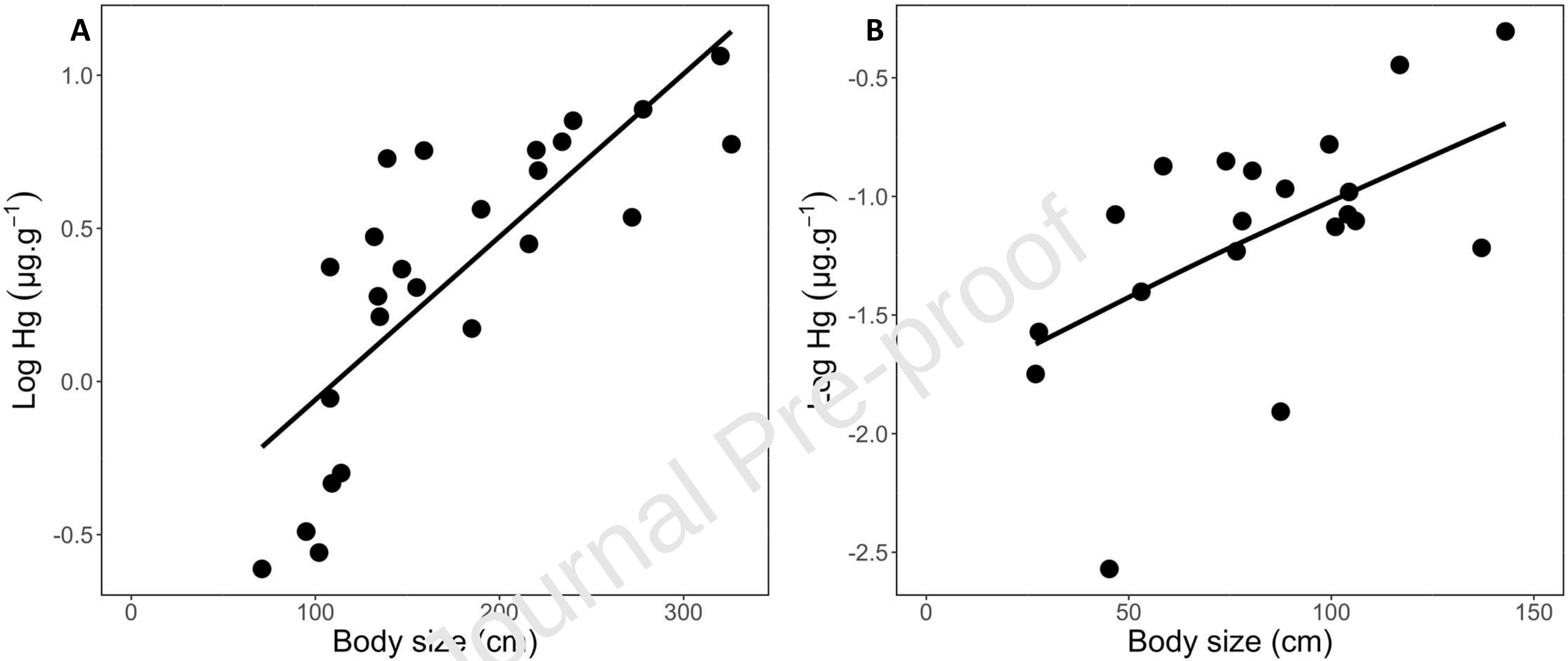

Figure 2. Relationships between body size (total length in cm) and Hg concentration (µg.g^{-1} dw) in the whole blood of the Black Caiman, *Melanosuchus niger* (A: R^2 = 0.625 , p < 0.001, n = 25) and the Smooth-fronted Caiman, *Paleosuchus trigonatus* (B: R^2 = 0.353, p = 0.006 , n = 20), from French Guiana.

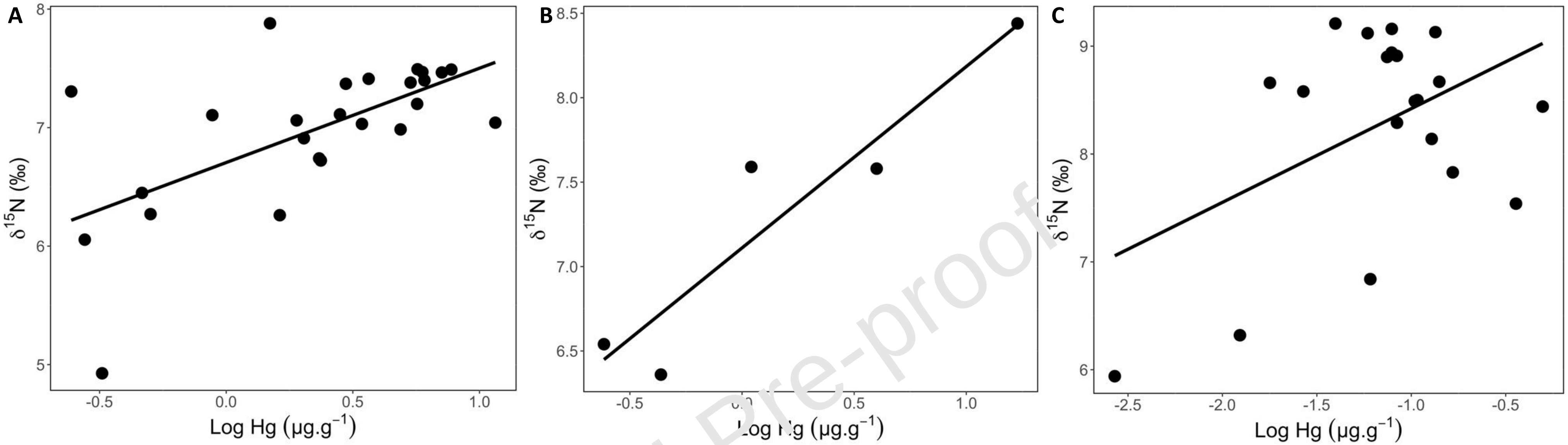

Figure 3. Relationships between Hg concentration (µg.g^{-1} dw) and the δ^{15}N (‰) in the whole blood of the Black Caiman, *Melanosuchus niger* (A: R^2 = 0.391 , $p < 0.001$, n = 25), the Dwarf Caiman *Paleosuchus palpebrosus* (B: R^2 = 0.877, p = 0.019, n = 5) and the Smooth-fronted Caiman, *Paleosuchus trigonatus* (C: R^2 = 0.611, $p < 0.001$, n = 20) in French Guiana.

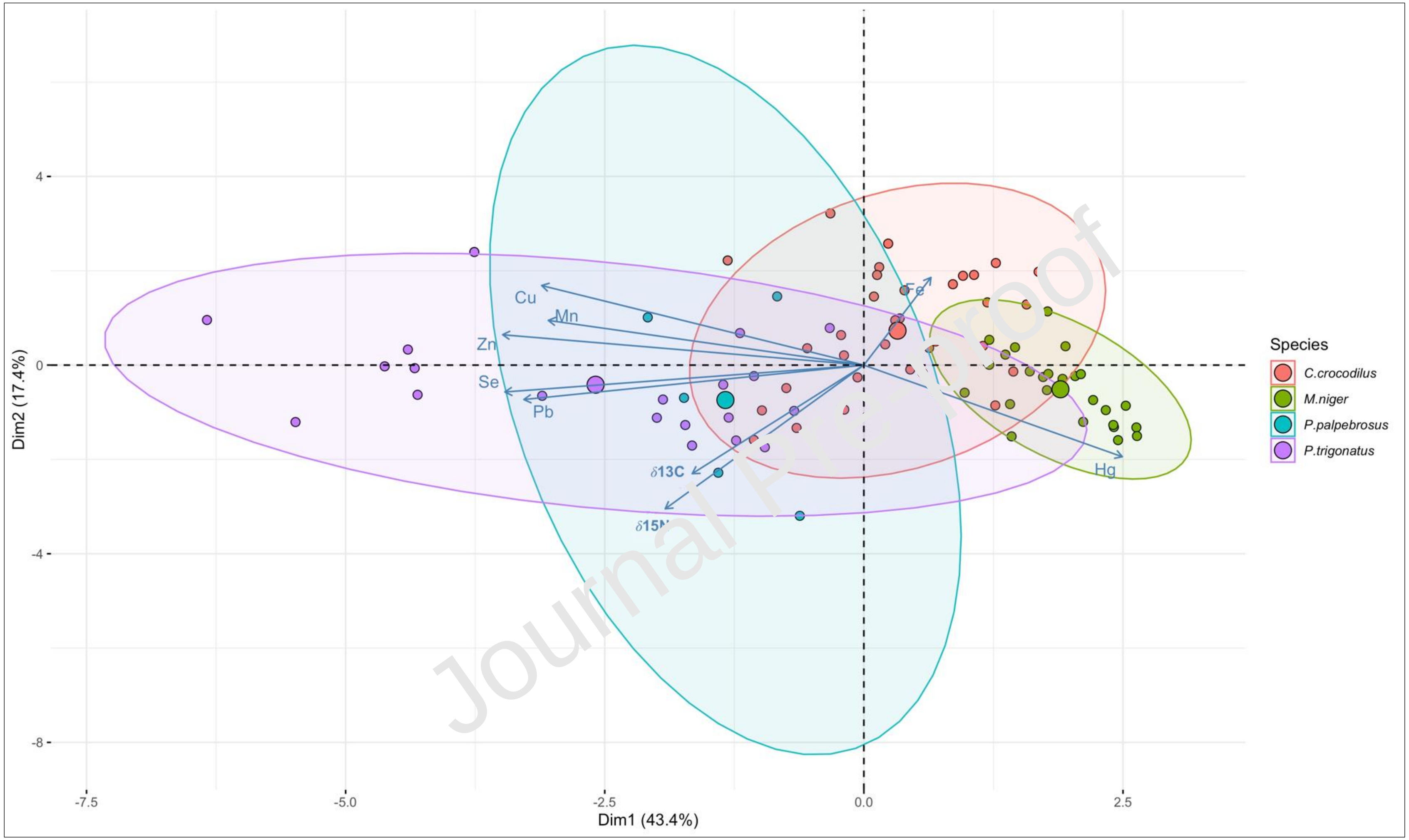

Figure 4. Biplot of individual scores extracted by principal component analyses (PCA) and elements loading on the two principal axes with trace elements of the four French Guiana caiman species (Spectacled Caiman, *Caiman crocodilus*; Black Caiman, *Melanosuchus niger*; Dwarf Caiman,

Paleosuchus palpebrosus and Smooth-fronted Caiman, *Paleosuchus trigonatus*). Ellipses represent confidence interval (95%) of the estimated group position.

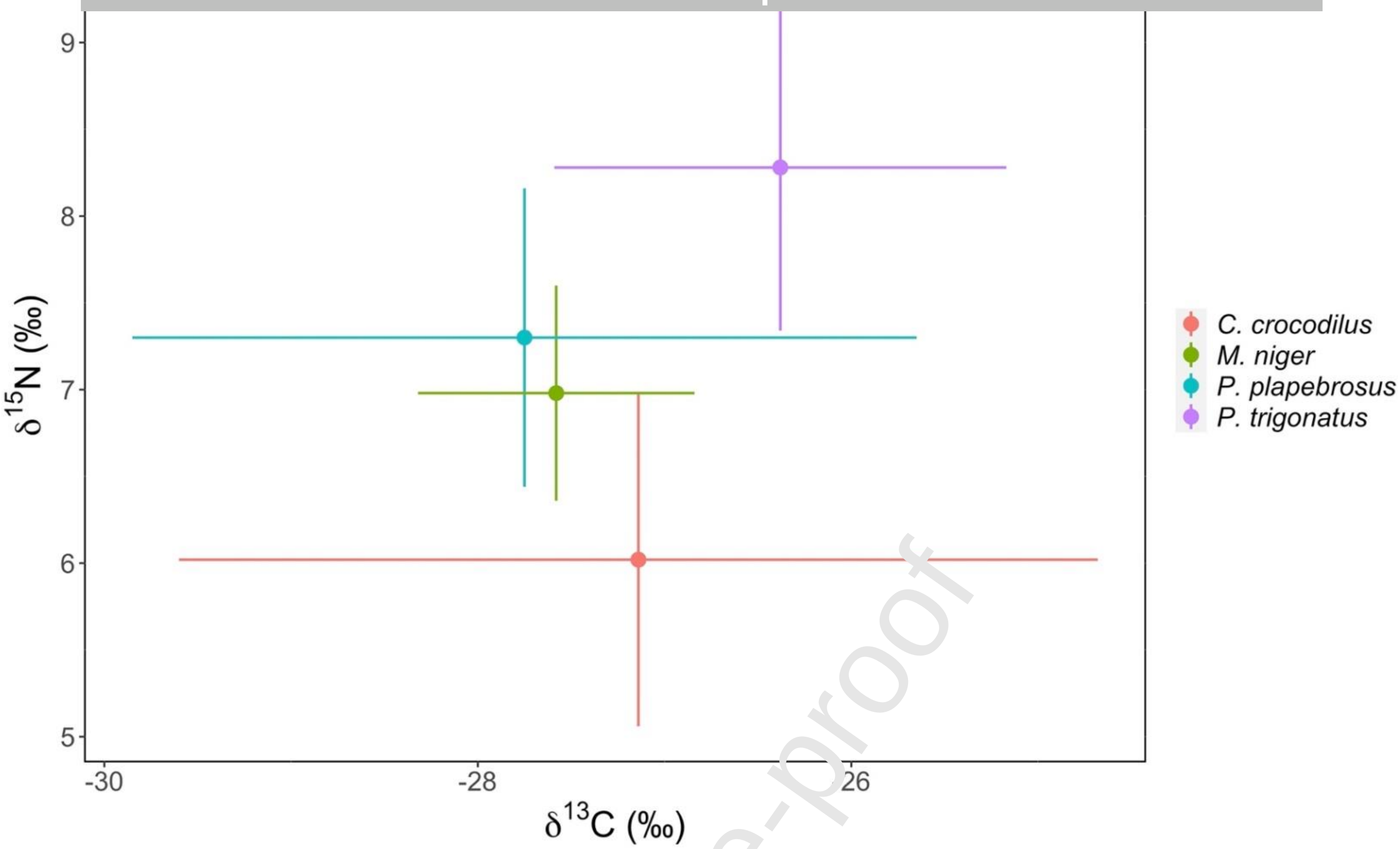

Figure 5. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) values (‰; Mean ± SD) of blood of the four caiman species (Spectacled Caiman, *Caiman crocodilus*; Black Caiman, *Melanosuchus niger*; Dwarf Caiman, *Paleosuchus palpebrosus* and Smooth-fronted Caiman, *Paleosuchus trigonatus*), from French Guiana.

Credit Authors statement:

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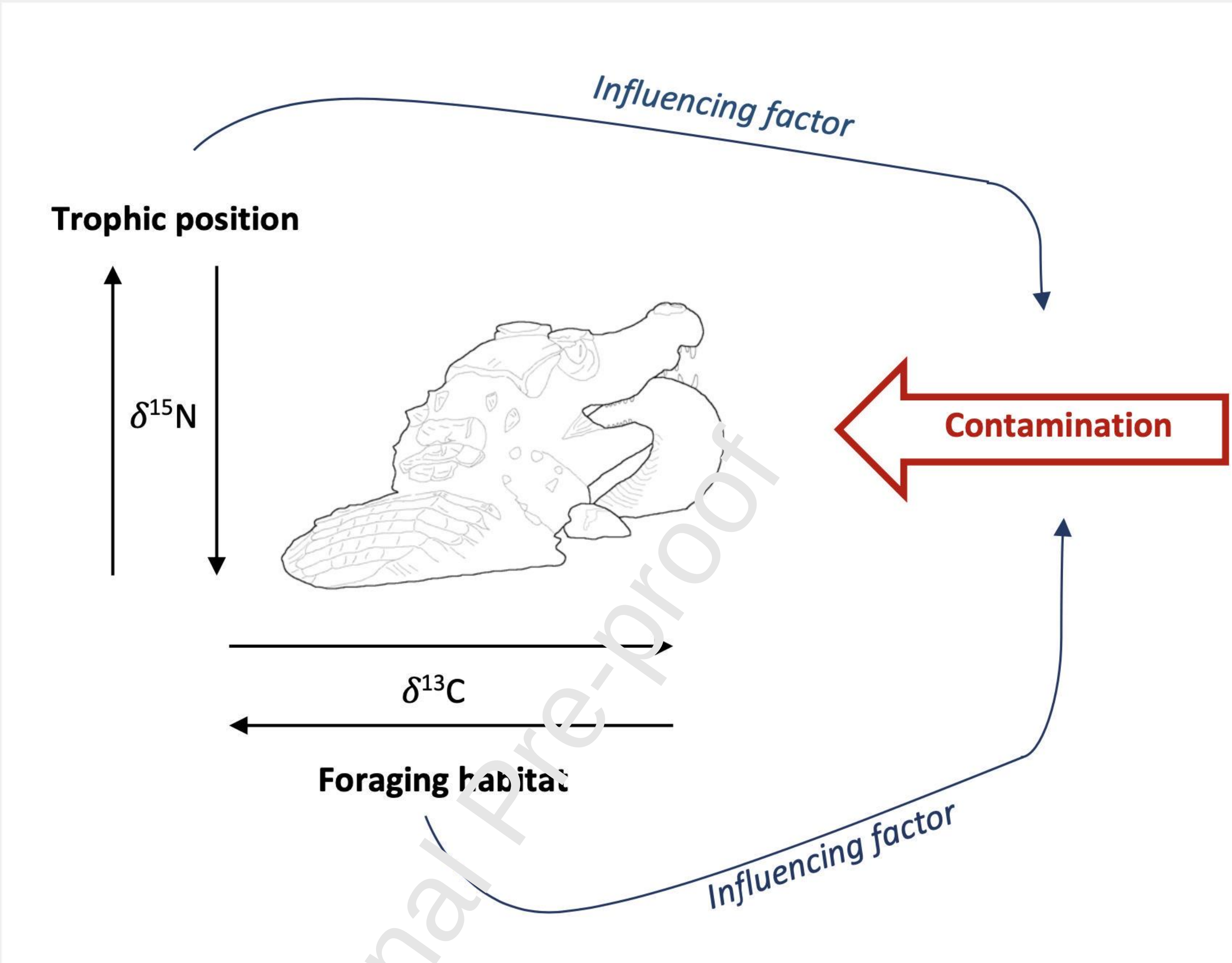
Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



Graphical abstract

Highlights

- Trace element concentrations vary between caiman species

- Trophic ecology influences trace element concentrations in all four caiman species

- High lead concentrations in the *Paleosuchus* genius