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Lead, mercury, and selenium alter physiological functions in wild caimans (*Caiman crocodilus*)☆

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ABSTRACT

Environmental contaminants affect ecosystems worldwide and have deleterious effects on biota. Non-essential mercury (Hg) and lead (Pb) concentrations are well documented in some taxa and are described to cause multiple detrimental effects on human and wildlife. Additionally, essential selenium (Se) is known to be toxic at high concentrations but, at lower concentrations, Se can protect organisms against Hg toxicity. Crocodilians are known to bioaccumulate contaminants. However, the effects of these contaminants on physiological processes remain poorly studied. In the present study, we quantified Hg, Pb and Se concentrations in spectacled caimans (*Caiman crocodilus*) and investigated the effects of these contaminants on several physiological processes linked to osmoregulatory, hepatic, endocrine and renal functions measured through blood parameters in 23 individuals. Mercury was related to disruption of osmoregulation (sodium levels), hepatic function (alkaline phosphatase levels) and endocrine processes (corticosterone levels). Lead was related to disruption of hepatic functions (glucose and alanine aminotransferase levels). Selenium was not related to any parameters, but the Se:Hg molar ratio was positively related to the Na^+ and corticosterone concentrations, suggesting a potential protective effect against Hg toxicity. Overall, our results suggest that Hg and Pb alter physiological mechanisms in wild caimans and highlight the need to thoroughly investigate the consequences of trace element contamination in crocodilians.

1. Introduction

Environmental contamination is recognized to be a widespread phenomenon, leading to increasing concerns as to its potential impact on biodiversity (Fleeger et al., 2003; Eagles-Smith et al., 2018; Brühl and Zaller, 2019; Ferronato and Torretta, 2019; Pain et al., 2019; Kasonga et al., 2021). Environmental contaminants have multiple sources, and their recent increase mainly relates to anthropogenic activities such as fossil fuel combustion, chemical production and use, and mining activities (Richardson and Kimura, 2017). In addition, global changes have recently been identified as a cause of modification in the distribution and behaviour of environmental contaminants at a global scale (Noyes et al., 2009; Obrist et al., 2018). Providing information on environmental contamination levels and distribution across geographical areas, biomes or species is critical. One of the current challenges is to quantify how environmental contaminants affect physiological functions in various organisms and to understand the potential consequences for wildlife and human health.

Mercury (Hg) and lead (Pb) are two non-essential trace elements which are present in ecosystems worldwide and induce deleterious effects on humans and wildlife (Clarkson and Magos, 2006; Wani et al., 2015; Evers, 2018; Pain et al., 2019). They naturally occur in the environment, though human activities increase their levels (e.g., fuel combustion, mining activities and ammunition from hunting, Fisher et al., 2006; Pirrone et al., 2010; Beckers and Rinklebe, 2017; Fry et al., 2020). In addition, Hg and Pb bioaccumulate in various tissues of

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organisms (Vizuete et al., 2018; De Almeida Rodrigues et al., 2019; Pain et al., 2019). They have multiple adverse effects on reproductive, renal and hepatic functions, and are recognized as immune and endocrine disruptors (Eisler, 1988; Fingerman et al., 1996; Pattee and Pain, 2003; Zahir et al., 2005; Scheuhammer et al., 2008; Tan et al., 2009; Grillitsch and Schiesari, 2010; Bergeron et al., 2011; Hopkins et al., 2013; Tartu et al., 2013; Whitney and Cristol, 2017; Monclús et al., 2020).

Selenium (Se) is an essential trace element which is involved in functions of various physiological processes, such as immune system and thyroid hormone homeostasis (Roy et al., 1995; Ramauge et al., 1996; Köhrle et al., 2005; Avery and Hoffmann, 2018; Qian et al., 2020). Its sources are both natural and anthropogenic with major releases into the environment from coal combustion (Rowe et al., 2002; Lemly, 2004; He et al., 2018; Ullah et al., 2019). A disruption of the normal Se balance can negatively affect metabolic processes (Rayman, 2000; Taylor et al., 2009; Finger et al., 2016). Selenium toxicity is known to affect growth, reproduction, and the immune system (Heinz et al., 1987; Hoffman et al., 1991; Hoffman, 2002; Hopkins et al., 2004; Naderi et al., 2021). Nevertheless, due to its high affinity to Hg, Se qualifies as a natural antagonist: low Se concentrations can protect organisms against toxic effects of Hg by reduction or even elimination of its toxicity (Sugiura et al., 1978; Gajdosechova et al., 2018; Rahman et al., 2019).

Crocodilians, being apex predators, accumulate high levels of metal contaminants due to their life history characteristics (e.g. aquatic habitat, long lifespan, high trophic level, high tissue conversion rate) and can serve as indicators of ecosystem health (Yanochko et al., 1997; Vieira et al., 2011; Schneider et al., 2015; Somaweera et al., 2020; Lemaire et al., 2021a). However, many crocodilians have a concerning conservation status, and populations of several species are currently decreasing (Targarona et al., 2008; Ferreira and Pienaar, 2011; Bezuijen et al., 2012; Van Weerd et al., 2016; Balaguera-Reina et al., 2018; Ortiz et al., 2020). Few studies which focused on a limited number of species have evaluated Hg and Pb concentrations in crocodilians (Burger et al., 2000; Jeffree et al., 2001; Correia et al., 2014; Trillanes et al., 2014; Nilsen et al., 2017b). Further knowledge on their negative effects is restricted to four crocodilian species (*Alligator mississippiensis, Caiman crocodilus, Paleosuchus trigonatus* and *Crocodylus niloticus*) and few markers (morphology, DNA and reproduction; Lance et al., 2006; Warner et al., 2016; Nilsen et al., 2017a; Marrugo-Negrete et al., 2019; Lemaire et al., 2021b). To our knowledge, no studies have investigated the combined effects of Hg, Pb and Se, and the possible protective effect of Se on physiological parameters of caimans, particularly in French Guiana, where said contaminants are highly abundant in the environment.

To investigate potential effects of such contaminants on physiological functions of humans and wildlife, blood chemistry analysis is a relevant tool. Blood integrates indices of most organisms' physiological processes. Therefore, blood chemistry offers a generalist approach to investigate several physiological functions (e.g., energetic metabolism, reproduction, detoxification, osmoregulation; Aguiree and Balazs, 2000; Brischoux and Kornilev, 2014; Barão-Nóbrega et al., 2018; Hudson et al., 2020) and can additionally be used for contamination level assessment (Clark et al., 2000). Because blood sampling is non-lethal and performed relatively easily, the effect of trace elements on biochemical parameters can be monitored over time.

In the present study, we assessed the concentrations of selected trace elements (Hg, Pb, and Se) and 15 physiological parameters indicative of osmoregulatory (e.g., sodium, chlorine), metabolic (e.g., glucose, calcium), hepatic (e.g., aspartate aminotransferase, alanine aminotransferase), endocrine (e.g., corticosterone) and renal (e.g., total protein) functions in the blood of *Caiman crocodilus* from French Guiana. Additionally, we evaluated relationships between Hg, Pb, and Se with blood chemistry and corticosterone to investigate the effects of these trace elements on crocodilian physiology.

2. Material and methods

2.1. Sample collection

Between November 2019 and February 2020, we captured and sampled 23 Spectacled Caimans *Caiman crocodilus* at “Pripis de Yiyi” (N 05°25′25″, W 53°02′50″) and the nature reserve “Kaw-Roura” (N 04°29′20″, W 52°03′38″) in French Guiana. Each individual was measured (Snout-Vent Length, SVL) and weighed. Blood was drawn from the lateral tail vein using a 2.5 mL syringe and a 23 or 21 gauge - 50 mm heparinized needle (heparin sodium). Due to logistical constraints inherent to field procedures and the study species, time between the successful capture of an individual and the end of blood sampling was 14 ± 3 min (range 10–19 min). Blood samples were immediately placed in cold temperatures (4 °C) until being processed at the laboratory. After biochemistry analysis (see below), half of the whole blood was frozen at −28 °C, the second part was centrifuged at 6500 rpm for 5 min, then plasma and red blood cells were separately frozen at −28 °C.

After sampling, all caimans were released at their capture locations. Captures and sample collection were performed under a permit from the French authorities (Direction Régionale des Territoires et de la Mer) after evaluation by the CSRPN, the regional scientific committee (Permit: R03-2019-01-09-001 & R03-2019-10-24-007).

2.2. Blood biochemistry analysis

On average of 4 h after sampling, we analyzed blood parameters using VetScan VS2 Chemistry Analyzer (Abaxis, Inc., Union City, California, USA) by applying 100 μL of whole blood on “Avian-Reptilian” and/or “Preventive Care Profile Plus” designated rotors to measure total calcium (Ca^{2+}), potassium (K^+), sodium (Na^+), chloride (Cl^-), phosphorus (P), uric acid (UA), glucose (GLU), total bilirubin (TBIL), total protein (TP), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), total carbon dioxide (tCO_2), blood urea nitrogen (BUN), bile acids (BA), creatinine (CRE) and globulin (GLOB). Five blood biochemistry parameters (ALB, BUN, CRE, BA and GLOB) could not be quantified as their levels were under the detection limit of the VetScan.

2.3. Corticosterone analysis

We measured plasma corticosterone levels using radioimmunoassay, as described in Lormée et al., (2003). Corticosterone was measured after ethyl ether extraction using a commercial antiserum. After adding dextran-coated charcoal and centrifugation, the free corticosterone was separated. Bound fraction containing corticosterone was measured using a liquid scintillation counter. The minimum detectable corticosterone concentration was 0.28 ng ml^{-1}, and the inter-assay coefficients of variation were 6.24% and 9.63%, respectively (samples were assayed in duplicate, in two assays).

2.4. Trace element analysis

Whole blood was freeze-dried for 48 h, ground and homogenized. Total Hg concentrations were determined by direct measurement in the whole blood, using an atomic absorption spectrometer AMA-254 (Advanced Mercury Analyzer-254; Altec®). Certified Reference Material (CRM) TORT-3 (lobster hepatopancreas; certified Hg concentration: 0.292 ± 0.022 μg g^{-1} dry weight (dw), NRCC) was analyzed at the beginning and at the end of the analytical cycle to validate the method. For each individual, two replicates of blood (~0.6 mg dw) were analyzed, and the reproducibility for replicate samples was approved when the relative standard deviation (RSD) was <10%. Measured values for TORT-3 were 0.292 ± 0.006 μg g^{-1} dw (n = 20), with a recovery of 100.1 ± 1.9%. The limit of quantification was 0.05 ng.

Se and Pb were analyzed in freeze-dried whole blood using

Inductively Coupled Plasma Mass Spectrometry (ICP-MS II Series Thermo Fisher Scientific), (aliquots mass: 8–139 mg dw). Whole blood samples were microwave-digested in a mixture of 6 mL of 70% HNO_3 (VWR SUPRAPUR Quality) and 2 mL of 37% HCl (VWR SUPRAPUR Quality); for samples weighing less than 100 mg, the volumes of HNO_3 and HCl were divided by half. Samples were further diluted with ultrapure water to 50 mL (25 mL for samples $<$ 100 mg). To avoid contamination, all utensils used were soaked in a bath of diluted nitric acid for at least 48 h, rinsed in ultrapure water and dried. Certified Reference Materials (CRM; dogfish liver DOLT-3, NRCC, and lobster hepatopancreas TORT-2, NRCC) were treated and analyzed as samples. Results of Hg, Pb and Se quantification were in agreement with the certified values, and the standard deviations were low, proving good repeatability of the methods. The results for CRMs displayed recoveries of the elements ranging from 92.1 ± 17.5% (n = 8) for Pb, 110.7 ± 4.7% for Se (n = 8). Hg, Pb and Se results are further expressed in $\mu g.g^{-1}$ dw.

2.5. Statistical analyses

All analyses were performed using the Software R v.3.6.1. (*R development Core Team*). The data was first checked for normality and homogeneity of variances. Analyses on trace elements were performed on log-transformed values. The selenium:mercury (Se:Hg) molar ratio was calculated using the Hg and Se concentrations (in $\mu g.g^{-1}$ dw) divided by the molecular weight of each element, respectively 200.59 for Hg and 78.96 for Se, following the equation:

$$Se : Hg\ molar\ ratio = \frac{[Se]/78.96}{[Hg]/200.59}$$

The relation between trace elements, biometric measurements, and biochemistry parameters were assessed by linear regression.

Corticosterone concentrations of *Caiman crocodilus* were not influenced significantly by the time of handling ($F_{2,18} = 0.274$, $p = 0.787$) and relations between trace elements and corticosterone concentrations were assessed with linear regressions.

3. Results and discussion

Non-essential trace elements affected physiological parameters, in particular osmoregulatory, hepatic, endocrine and renal functions. Below we discuss the physiological mechanisms which appear to be potentially affected by these contaminants.

3.1. Mercury

Hg concentrations ranged from 0.168 to 1.532 $\mu g\ g^{-1}$ dw (Table 1) and were negatively correlated with natremia ($R^2 = 0.345$, $p = 0.005$, Fig. 1, Table 2) and, to a lesser extent with chloremia ($R^2 = 0.437$, $p = 0.053$). This suggests that higher Hg concentrations negatively influenced ionic regulation which is related to renal functions in caimans.

We found a negative relationship between Hg and alkaline phosphatase (ALP) concentrations ($R^2 = 0.601$, $p = 0.024$, Fig. 1, Table 2). Elevation of Hg concentrations is linked to a diminution of ALP in the blood of *Caiman crocodilus*, suggesting an alteration of hepatic function.

Our results show a negative relationship between corticosterone levels and Hg concentrations ($R^2 = 0.276$, $p = 0.021$, Table 2), which suggests that elevation of Hg concentrations may disrupt endocrine processes in caimans by a diminution of corticosterone production (HPA axis).

Table 1

Morphometrics, biochemistry parameters and trace element concentrations of the Spectacled Caiman (*Caiman crocodilus*) from French Guiana.

	Caiman crocodilus		
	N	Mean ± SD	Min - Max
Morphometric parameters			
Snout-Vent-Length (SVL)[a]	23	35.9 ± 7.7	20.2–48.5
Weight (W)[b]	23	1443 ± 950	250–3950
Metal concentrations			
Hg[c]	21	0.676 ± 0.414	0.168–1.532
Se[c]	21	1.35 ± 0.30	0.76–1.92
Se:Hg	21	7.90 ± 5.85	1.45–21.54
Pb[c]	21	0.13 ± 0.06	0.04–0.28
Biochemistry parameters			
Total calcium (Ca^{2+})[d]	23	10.6 ± 0.9	9.1–12.5
Potassium (K^+)[e]	22	4.6 ± 0.7	3.4–6.1
Sodium (Na^+)[e]	23	139.1 + 7.2	126–152
Chlorine (Cl^-)[e]	10	103.9 ± 6.0	93–112
Phosphorus (P)[d]	14	52 ± 11	35–74
Uric acid (UA)[d]	14	14 ± 8.0	6.0–33
Glucose (GLU)[d]	23	662 ± 152	330–960
Total bilirubin (TBIL)[d]	10	2.5 ± 1.0	20–30
Total protein (TP)[f]	23	49 ± 8.0	35–72
Creatine kinase (CK)[g]	13	3245 ± 1420	1192–6148
Aspartate aminotransferase (AST)[g]	23	133.4 ± 35.0	88–229
Alanine aminotransferase (ALT)[g]	10	43.0 ± 10.9	27–66
Alkaline phosphatase (ALP)[g]	8	28.6 ± 9.6	16–46
Total carbon dioxide ($tC0_2$)[h]	10	15.8 ± 4.6	8–24
Corticosterone[i]	21	27.72 ± 14.01	7.98–53.61

[a] in cm.

[b] in g.

[c] in $\mu g.g^{-1}$.

[d] in $mg.L^{-1}$.

[e] in $mmol.L^{-1}$.

[f] in $g.L^{-1}$.

[g] in $U.L^{-1}$.

[h] in $mosm.L^{-1}$.

[i] in $ng.mL^{-1}$.

Although Hg toxicity is well documented in several taxa (Scheuhammer et al., 2007; Morcillo et al., 2017; Evers, 2018; Zheng et al., 2019), its effects remain poorly studied in reptiles (Schneider et al., 2013). Mercury can act as an inhibitor of the Na^+/K^+-ATPase (Kramer et al., 1986; Magour, 1986; Chuu et al., 2007), and can lead to a disruption of osmoregulation in taxa such as fishes and crustaceans (Lock et al., 1981; Bianchini and Gilles, 1996; Handayani et al., 2020). In Alligatorids, osmoregulation principally occurs in the kidneys (Mazzotti and Dunson, 1989; Grigg et al., 1998) and our results suggest that renal function may be negatively affected by Hg. Freshwater species need to maintain osmolality in a hyposmotic environment, as the regulation of the hydromineral balance is crucial for survival (Schmidt-Nielsen, 1983). Hyponatremia has been shown to cause neurological dysfunction, muscle damage and death (Patterson, 2011; Gankam Kengue and Decaux, 2018; Martemyanov and Poddubnaya, 2020, Arieff, 1986, 2006). Therefore, consequences of chronic hyponatremia in Hg-contaminated freshwater crocodilians needs to be assessed.

The negative relationship between Hg and ALP suggests that some liver functions in *Caiman crocodilus* are disrupted due to Hg contamination, as previously found in fish (Sastry and Sharma, 1980) and rodents (El-Shenawy and Hassan, 2008). ALP is a liver cytoplasmic enzyme involved in the hepatocytic functions, and perturbations of its activity occur via mechanisms such as ATPase disruption (El-Shenawy and Hassan, 2008). Potential effects of decreased ALP, and/or altered liver functions linked to Hg contamination in caimans deserve further investigation.

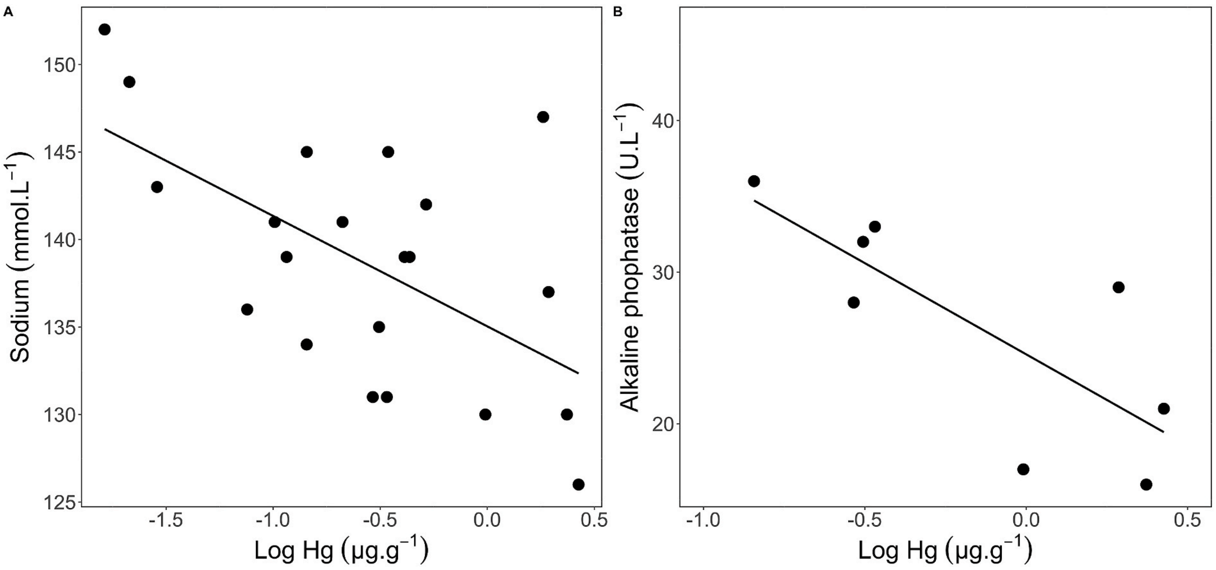

Fig. 1. Relationships between Hg concentration (Log µg.g^{-1} dw) and (**A**) sodium concentration (mmol.L^{-1}, $R^2 = 0.345$, $p = 0.005$) and (**B**) alkaline phosphatase concentration (U.L^{-1}, $R^2 = 0.601$, $p = 0.024$) in the blood of the Spectacled Caiman (*Caiman crocodilus*) from French Guiana.

Table 2
Summary statistics of linear regression between morphometrics, metal concentrations and biochemistry parameters in the Spectacled Caiman (*Caiman crocodilus*) in French Guiana. Bold denotes significant relationship ($p < 0.05$) and arrows indicate a positive or negative correlation.

Parameters	Hg			Se			Se:Hg			Pb		
Caiman crocodilus	n	R^2	p	n	R^2	p	n	R^2	P	n	R^2	p
Snout-vent length (SVL)	21	0.143	0.091	20	0.091	0.198	20	0.161	0.080	21	0.290	**0.012 ↑**
Weight (W)	21	0.163	0.070	20	0.130	0.118	20	0.197	0.050	21	0.129	0.110
Total calcium (Ca^{2+})	21	0.112	0.138	20	0.017	0.587	20	0.058	0.307	21	0.122	0.121
Potassium (K^+)	20	0.045	0.370	19	0.074	0.259	19	0.058	0.319	20	0.186	0.057
Sodium (Na^+)	21	0.345	**0.005 ↓**	20	0.184	0.059	20	0.378	**0.004 ↑**	21	0.001	0.897
Chlorine (Cl^-)	9	0.437	0.053	9	0.109	0.386	9	0.395	0.070	9	0.041	0.602
Phosphorus (P)	13	0.091	0.315	12	0.030	0.592	12	0.062	0.436	13	0.099	0.295
Uric acid (UA)	13	0.009	0.757	12	0.161	0.197	12	0.079	0.376	13	0.032	0.562
Glucose (GLU)	21	0.014	0.612	20	0.073	0.251	20	0.031	0.455	21	0.239	**0.025 ↓**
Total bilirubin (TBIL)	9	0.209	0.217	9	0.082	0.455	9	0.200	0.228	9	0.096	0.418
Total protein (TP)	21	0.008	0.700	20	0.024	0.514	20	0.015	0.611	21	0.006	0.740
Creatine kinase (CK)	13	0.000	0.972	12	0.014	0.716	12	0.005	0.828	13	0.003	0.866
Aspartate aminotransferase (AST)	21	0.003	0.824	20	0.078	0.232	20	0.013	0.629	21	0.162	0.070
Alanine aminotransferase (ALT)	9	0.001	0.929	9	0.002	0.910	9	0.002	0.921	9	0.740	**0.003 ↑**
Alkaline phosphatase (ALP)	8	0.601	**0.024 ↓**	8	0.035	0.655	8	0.477	0.058	8	0.003	0.894
Total carbon dioxide (tCO_2)	9	0.142	0.318	9	0.028	0.667	9	0.125	0.351	9	0.175	0.263
Corticosterone	19	0.276	**0.021 ↓**	18	0.090	0.228	18	0.272	**0.026 ↑**	19	0.064	0.295

Mercury accumulates in the pituitary gland and the thyroid and alters the endocrine system in vertebrates (Colborn et al., 1993; Tan et al., 2009; Meyer et al., 2014; Tartu et al., 2013). Consistent with these studies, the negative relationship between corticosterone levels and Hg concentrations suggests that Hg may disrupt endocrine processes in caimans as already shown in other taxa (Moore et al., 2014; Meillère et al., 2016; Soto et al., 2019). Disruption of corticosterone levels has consequences on metabolism, behaviour and reproduction (Denardo and Licht, 1993; Guillette et al., 1995; Scott et al., 2019). Effects of environmental contaminants (e.g., pesticides, trace elements) on the endocrine system of crocodilians have been already reported (Guillette et al., 1994; Arukwe et al., 2016; Finger et al., 2018), while endocrine disruption associated to Hg contamination is yet unknown but demands future evaluation.

3.2. Selenium and Se:Hg molar ratio

Se concentrations ranged from 0.76 to 1.92 µg g^{-1} dw (Table 1) and was not related to any parameters (all $p > 0.05$, Table 2), suggesting that Se concentrations were not high enough to induce any effects.

Our results show that natremia (Na^+: $R^2 = 0.378$, $p = 0.004$, Fig. 2, Table 2) and corticosterone levels ($R^2 = 0.272$, $p = 0.026$, Table 2) increased with the Se:Hg molar ratio. These results show that Na^+ and corticosterone levels are positively influenced by an excess of Se against Hg, suggesting a potential positive effect of Se.

Selenium is an essential trace element involved in antioxidant defense and thyroid metabolism which can be toxic in high concentrations (Behne et al., 2000; Ramauge et al., 1996; Rayman, 2000). In the American alligator, *Alligator mississippiensis,* chronic Se exposure affects stress parameters such as corticosterone levels (Finger et al., 2019). Our results suggest that Se concentrations were not high enough to trigger toxic effects. However, the relationships found between Se:Hg molar ration and natremia and corticosterone levels suggests positive effects of this ratio. As already discussed, Hg concentrations show a negative relationship with Na^+ and corticosterone concentrations. Because Se reduces Hg toxicity in many taxa (Beijer and Jernelov, 1978; Freidman et al., 1978; Ohi et al., 1980; Culvin-Aralar and Furness, 1991; Suzuki, 1997; Ralston et al., 2006; Ralston and Raymond, 2010), we suggest that

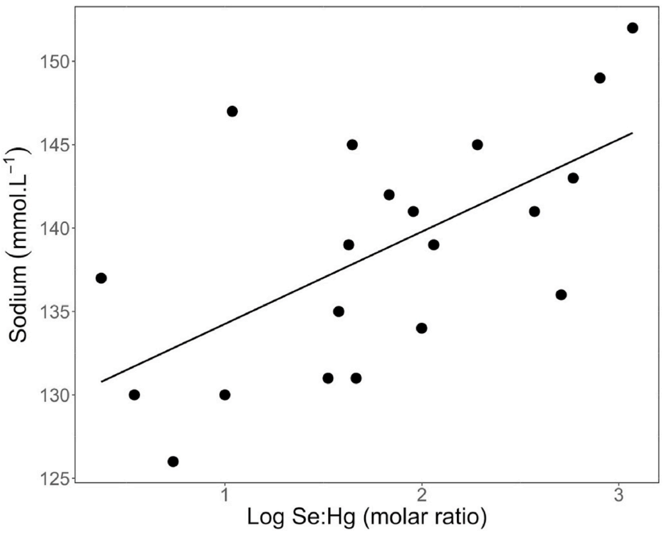

Fig. 2. Relationship between Se:Hg molar ratio and sodium concentration (mmol.L^{-1}, R^2 = 0.378, p = 0.004) in the blood of the Spectacled Caiman (*Caiman crocodilus*) from French Guiana.

Se:Hg molar ratio has a role of protection against Hg toxicity in *Caiman crocodilus*. This hypothesis is reinforced by the marginal relationship detected between Se concentration and Na^+ values. Our results emphasize the need for future studies on this potential protective effect of the Se:Hg molar ratio in crocodilians.

3.3. Lead

Pb concentrations ranged from 0.04 to 0.28 μg g^{-1} dw (Table 1) and were negatively related to glucose levels ($R^2 = 0.239$, $p = 0.025$, Fig. 3, Table 2), suggesting that Pb affects mechanisms related to the regulation of glucose. Our results show a positive relationship between Pb concentration and alanine aminotransferase concentration (ALT) ($R^2 = 0.740$, $p = 0.003$, Fig. 3, Table 2) suggesting that the organism increases the production of ALT in response to elevated Pb concentration in the blood.

The detrimental effects of lead contamination are well studied and affects vascular, nervous, renal, hepatic, immune, endocrine and reproductive systems (Eisler, 1988; Pattee and Pain, 2003; Grillitsch and Schiesari, 2010). The biokinetics of Pb in the blood of crocodilians is shorter than in other vertebrates, with a half-life of 3 days, in comparison to 13 days in birds and mammals (Anders et al., 1982; Castellino and Aloj, 1964; Hammerton et al., 2003). No clinical signs of Pb toxicity were found in crocodilian studies yet, suggesting resistance of the taxon to this contaminant (Cook et al., 1998; Camus et al., 1998; Hammerton et al., 2003; Lance et al., 2006; Warner et al., 2016). However, our results show a negative relationship between Pb and glucose level suggesting that Pb affects the endocrine systems of *Caiman crocodilus* and alters its liver function. Glucose is regulated by the liver and complex interactions with the hypothalamus, pituitary and adrenal glands (Lin and Accili, 2011; Cady et al., 2017). Our results are consistent with studies in marine turtles exposed to Pb (Komoroske et al., 2011). Our findings are strengthened by the positive relationship we additionally found between Pb and ALT, an indicator of hepatocellular damages (Kew, 2000; Maheswari et al., 2008). While our findings show some toxic effects of Pb on liver functions in crocodilians, it deserves further investigations.

4. Conclusion

The present study provides the first evidence that Hg and Pb affect physiological parameters in *Caiman crocodilus*. Mercury was related to disruptions of sodium, alkaline phosphatase, and corticosterone levels, which suggests a negative effect on osmoregulation, hepatic functions and endocrine processes. Lead was related to disruption of glucose and alanine aminotransferase levels, suggesting hepatocellular damages. Although the Hg and Pb concentrations of the present study are commonly found in crocodilians, the relationship between contaminant levels and blood parameters are of concern. Interestingly, results that investigate the Se:Hg molar ratio suggest a protective effect of Se against Hg toxicity in caimans. This study is a starting point for further evaluation of trace element consequences on physiological mechanisms in caimans, particularly those more vulnerable to exposure. Indeed, our sampled individuals were relatively small and thus probably young individuals which suggest that physiological alterations linked to non-essential trace elements can occur early in the life of crocodilians.

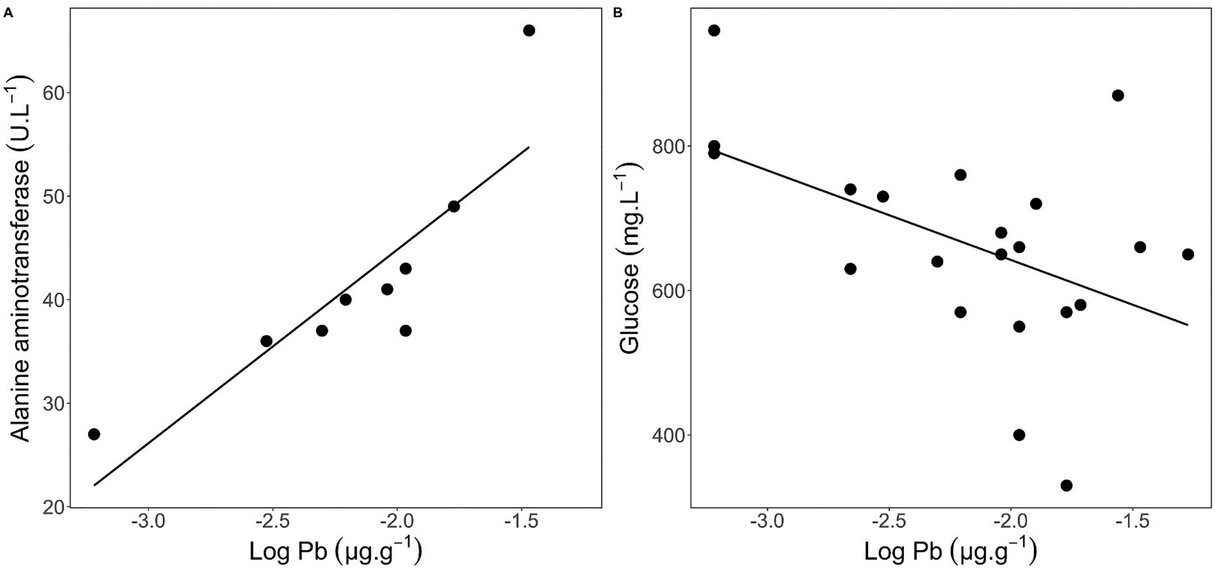

Fig. 3. Relationships between Pb concentration (Log μg.g^{-1} dw) and (**A**) Alanine aminotransferase (U.L^{-1}, R^2 = 0.740, p = 0.003) and (**B**) Glucose (mg.L^{-1}, R^2 = 0.239, p = 0.025) in the blood of the Spectacled Caiman (*Caiman crocodilus*) from French Guiana.

Credit author statement

Jérémy Lemaire: Conceptualization, Investigation, Formal analysis, Software, Funding acquisition, Writing – Original Draft, Writing – Review and Editing. **Paco Bustamante**: Conceptualization, Investigation, Funding acquisition, Writing – Original Draft, Writing – Review and Editing, Supervision. **Rosanna Mangione**: Conceptualization, Investigation, Writing – Original Draft, Writing – Review and Editing. **Olivier Marquis**: Conceptualization, Investigation, Funding acquisition, Writing – Original Draft, Writing – Review and Editing, Supervision. **Carine Churlaud**: Investigation. **Maud Brault-Favrou**: Investigation. **Charline Parenteau**: Investigation. **François Brischoux**: Conceptualization, Funding acquisition, Writing – Original Draft, Writing – Review and Editing, Supervision.

Declaration of competing interest

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.

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