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Preliminary assessment of blood mercury contamination in four African crocodile species

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ABSTRACT

Heavy metal contamination in the environment is an increasingly pervasive threat to the long-term persistence of wildlife. As high trophic level consumers, crocodylians are at substantial risk from bioaccumulation of mercury (Hg). Despite that they are generally well-studied and the focal species of many conservation efforts around the world, little is known about Hg contamination levels in most crocodylians. Here we preliminarily evaluate blood Hg contamination in four African species – Central African slender-snouted crocodile (*Mecistops leptorhynchus*), African dwarf crocodile (*Osteolaemus tetraspis*), West African crocodile (*Crocodylus suchus*), and Nile crocodile (*Crocodylus niloticus*) – from a diversity of sites and habitats across 5 different countries representing varying degrees of environmental pollution. All of our sampled crocodiles were Hg contaminated and, worryingly, these African crocodiles generally showed the highest levels of Hg contamination of any crocodylian species examined to date. Of most concern was that Hg concentrations were not only highest in *M. leptorhynchus*, the most threatened amongst our study species, but also in individuals sampled in what are believed to be some of the most remote and pristine natural areas left in Africa – Gabon's national parks. Our results underscore the need to better understand the impact of longstanding petroleum, mining, forestry, and agricultural industries on the entire aquatic food chain throughout much of Africa, including on the threatened species in these habitats and the human populations that depend on them for their subsistence and livelihoods.

1. Introduction

Mercury (Hg) is a major contaminant affecting ecosystems worldwide (Chen et al., 2018). While it is naturally present in the environment, anthropogenic activities such as fossil fuel combustion, deforestation, and mining, have been shown to drastically increase the level of Hg in the environment, particularly in aquatic ecosystems (Pirrone et al., 2010; Hsu-Kim et al., 2018). Under anoxic conditions, Hg can be transformed by aquatic microorganisms to methylmercury (MeHg), the most toxic and bioavailable form of Hg (Campeau and Bartha, 1985; Benoit et al., 2003). In its methylated form, Hg biomagnifies through the food chain and bioaccumulates over time in the tissues of top predators (Eagles-Smith et al., 2018). Mercury has been identified to have deleterious effects on humans and wildlife, causing neurobehavioral and neuronal dysfunction, and further impacting reproduction, embryonic development, hormonal synthesis, metabolic processes, and immune functions (Fingerman et al., 1996; Tan et al., 2009; Tartu et al., 2013; Scheuhammer et al., 2015). Because of this, it is crucial to evaluate Hg contamination in the environment and its impact on biodiversity.

As high trophic level consumers and potentially apex predators within aquatic ecosystems (Somaweera et al., 2020), crocodylians bioaccumulate environmental contaminants that biomagnify through the food webs. As a result, they are excellent species in which to monitor Hg contamination using minimally invasive sampling methods (Lázaro et al., 2015; Lemaire et al., 2021a). Most research to date on Hg in crocodylians has focused on the American alligator (*Alligator mississippiensis*; Yanochko et al., 1997; Elsey et al., 1999; Burger et al., 2000; Rumbold et al., 2002; Campbell et al., 2010; Horai et al., 2014; Nilsen et al., 2017a) and Morelet's crocodile (*Crocodylus moreletii*; Rainwater et al., 2002; Trillanes et al., 2014; Buenfil-Rojas et al., 2015, 2020; Romero-Calderón et al., 2022; Thirion et al., 2022). More recent efforts to understand Hg contamination across the Crocodylia, notably on the spectacled caiman (*Caiman crocodilus*; Schneider et al., 2015; Marrugo-

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Negrete et al., 2019; Lemaire et al., 2021b) and the two dwarf caimans (*Paleosuchus sp.*; Lemaire et al., 2021a,c, 2022), is revealing the possibility that crocodylians across diverse geographies are Hg contaminated. And Hg contamination has been identified as a cause of detrimental effects on crocodylians, including DNA damage and physiological alterations (Marrugo-Negrete et al., 2019; Lemaire et al., 2021b). It is, therefore, crucial to evaluate Hg contamination and its impacts across the Order, especially in contexts where Hg contamination is a likely aggravating factor for their conservation – for example, in the face of anthropogenic activities, like gold mining and petroleum extraction.

Africa is rich in natural and mineral resources, and commercial and artisanal-scale extraction of these resources is expected to intensify across the continent after the Covid19 outbreak (Yoshimura et al., 2021). Gold mining, oil extraction, and agriculture at both industrial and artisanal scales are among the most polluting activities in the region (UNEP, 2019). While it is likely that such extractive activities have led to increased Hg contamination, this is poorly documented across the continent, especially in wildlife species of conservation concern like crocodiles. Africa is home to seven crocodile species, all of which have significant cultural, subsistence, and financial importance to communities and governments across the continent (Thorbjarnarson, 1992; Ross, 1998). However, to the best of our knowledge, only two studies have evaluated Hg concentrations in any African crocodile species (Almli et al. 2005; Du Preez et al. 2018). They evaluated Hg contamination in the eggs, liver, and kidneys of Nile crocodiles (*Crocodylus niloticus*) in Zambia and South Africa and found median concentrations up to 3.7 $\mu g.g^{-1}$ (wet weight) in liver, up to 2.7 $\mu g.g^{-1}$ (wet weight) in kidneys, and up to 0.37 $\mu g.g^{-1}$ (dry wet) in eggshell.

In this study, we evaluated Hg concentrations in the blood, which represent the recent contamination, of four African crocodile species (*Crocodylus niloticus*, *Crocodylus suchus*, *Mecistops leptorhynchus*, and *Osteolaemus tetraspis*) across a diversity of African ecosystems, land management regimes, and economic development contexts. Specifically, we aimed to: 1) evaluate Hg contamination in blood of three of these four African crocodile species for the first time, and 2) preliminarily describe sources of variation in Hg concentration related to species, sex, and/or site characteristics in three of them.

2. Material and method

2.1. Study species

We evaluated Hg levels in four of Africa's seven endemic crocodile species. Between them, they represent all three genera present on the continent, as well as a significant diversity of habitats, life history strategies, and socioeconomic and development contexts, which will certainly impact their exposure risk to Hg.

Two species represent the "true crocodiles" of the genus *Crocodylus*. The Nile crocodile (*C. niloticus*) is one of the largest crocodile species in the world and is widely distributed across East and southern Africa. It is a habitat and dietary generalist, capable of occupying most wetland types throughout its distribution, ranging from estuarine coastal environments (e.g., Gabon and South Africa), to tropical forested wetlands (e.g., Gabon and DR Congo), savannah river and lake systems (throughout), and even arid and desert wetlands (e.g., Namibia and Egypt). Nile crocodiles are considered Least Concern (LC) under IUCN Red List criteria and are generally thought to have stable populations globally (Isberg et al., 2019). The West African crocodile (*C. suchus*) was only recently re-recognized as a relatively cryptic species, highly divergent from the Nile crocodile, to which it is not even a sister species (e.g., Hekkala et al., 2011; Shirley et al., 2015). It is widely distributed across West, Central, and parts of North and East Africa, where it occupies a staggering diversity of habitats, ranging from estuarine coastal environments, tropical forested wetlands, Guinea savannah, and even the Sahara Desert (e.g., Chad and Mauritania). It is generally a medium-bodied crocodile, dietary generalist, and generally not a threat to people, often living near rural communities throughout its distribution. West African crocodiles are currently being evaluated against IUCN Red List criteria, but minimally meet the criteria for Vulnerable and most populations are in decline (Kpera *et al.*, In Prep.).

The other two species in this study are habitat specialists, mostly occupying the freshwater, forested wetlands of the Lower Guinea and Congo Basin biomes of Central Africa. The African dwarf crocodile (*O. tetraspis*) is one of the smallest crocodylian species in the world. It is highly terrestrial and forages mostly on smaller invertebrates, including crustaceans, arachnids, millipedes and snails, as well as other small forest prey items like frogs and fish (Shirley et al., 2017). It is widely distributed throughout forested habitats, including in coastal lagoon mangrove forests and throughout small stream networks within dense forest. Dwarf crocodiles are considered Vulnerable under IUCN Red List criteria, and though some populations are declining, it can be quite abundant within intact habitats and where hunting pressure is not too excessive (Crocodile Specialist Group, 1996). In contrast, the Central African slender-snouted crocodile (*M. leptorhynchus*) is one of the most aquatic crocodylian species in the world, occupying medium- and larger-bodied rivers, lakes, lagoons, and other wetlands throughout the forested wetlands of Central Africa. Like most crocodylians, it will eat whatever it can catch, but is much more specialized in its diet consuming more aquatic prey (e.g., fish, snakes, waterbirds, frogs, etc…) (Shirley et al., 2018). Central African slender-snouted crocodiles are currently being evaluated against IUCN Red List criteria, but minimally meet the criteria for Endangered and most populations are in decline (Shirley *et al.*, In Review).

2.2. Sample collection and preparation

We collected blood samples from wild-caught individuals of the four different crocodylian species from different sites and habitats throughout their ranges between 2006 and 2011 (Fig. 1, Table 1). Specifically, we analyzed *O. tetraspis* (n = 81) samples from 3 different sites, *M. leptorhynchus* (n = 124) samples from 8 different sites, *C. suchus* (n = 56) samples from 6 different sites, and *C. niloticus* (n = 9) samples from a single site (Table 2). To collect the samples, we captured crocodylians using standard methods, including by hand, with tongs, pole snaring, and research darting (Cherkiss et al., 2004; Walsh, 1987; Webb

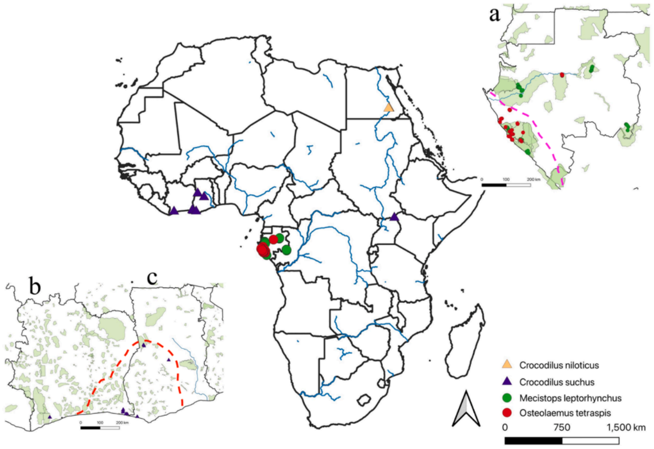

Fig. 1. Geographic location of the study sites in Africa. The inset maps show more detailed sampling locations in Gabon (a), Cote d'Ivoire (b), and Ghana (c) relative to protected areas (light green polygons) in these countries. The purple dashed line in the Gabon inset (a) indicates coastal area where most of the country's on and offshore petroleum concessions are concentrated. Similarly, the red dashed line in the Ghana (c) and Cote d'Ivoire (b) inset indicates the general area of the major gold deposits where much of the artisanal gold extraction happens. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Biometric data (cm) and Hg concentrations (μg.g^{-1} dw) in the blood of four African crocodylian species. Hg values marked with the same letter are not statistically different (Tukey's honesty significant difference test, $p < 0.05$).

Species	n	SVL (cm)		Hg concentration (μg.g^{-1})	
		Mean ± SD	Min – Max	Mean ± SD	Min – Max
Mecistops leptorhynchus	124	48.8 ± 33.4	17.0–152.1	3.400 ± 2.008^{a}	0.570–11.230
Osteolaemus tetraspis	81	48.6 ± 18.5	20.8–92.6	1.655 ± 1.148^{b}	0.390–5.180
Crocodylus suchus	56	50.1 ± 31.9	20.0–139.5	0.528 ± 0.243^{c}	0.207–1.291
Crocodylus niloticus	9	42.4 ± 17.9	15.1–74.6	1.580 ± 0.296^{b}	1.230–2.180

et al., 1983). For each captured individual, we drew a 0.5 ml blood sample from the cervical vessel posterior the occiput and spread it evenly on the lower half of a strip (2.5 cm × 10 cm) of lab grade, standard Whatman filter paper. We allowed the samples to air dry prior to storing them in paper coin envelopes at ambient environmental conditions. We took a standard series of measurements (e.g., head length, snout to vent length (SVL), mass) and determined their sex prior to releasing all captured animals at the site of capture.

Though these samples were originally collected for genetic research (e.g., Hekkala et al., 2011; Shirley, 2014), prior research on both humans and wildlife has demonstrated that dried blood spots (DBS) are appropriate for studies of Hg contamination because the obtained Hg concentrations do not vary from analysis of recently collected wet blood samples (Lehner et al., 2013; Perkins and Basu, 2018; Barst et al., 2020; Santa-Rios et al., 2020). Though this method is fairly underrepresented for contaminant analysis to date, and the use of Whatman filter paper deserve more investigations, it holds extraordinary potential for adding value to DBS samples originally collected for other purposes (e.g., genetics, health, etc.). To use DBS to quantify total mercury, we extracted two standardized 4 mm punches from the card using a biopsy punch. Punches were performed in the center of the DBS. To evaluate the mass of dried blood contained in the card, we weighed standardized 4 mm punches extracted from different cards without any blood to determine and average expected mass of the Whatman filter sample paper prior to soaking with blood (5.62 ± 0.21 mg, 3.8 % of variation, n = 9). Though our procedure closely follows prior Hg analysis using DBS, we diverged in our use of Whatman filter paper which has not been validated so far, thus we additionally analyzed the blank punches for Hg as a form of control (see "Mercury analysis" section below). To evaluate the weight of dried blood in the 4 mm punch, we used the following equation:

$$\textbf{\textit{Mass of dried blood}} = \textbf{\textit{Mass of }} 4\textbf{\textit{mm punch DBS}} - \textbf{\textit{Mean of }} 4\textbf{\textit{mm blank punches mass}}$$

2.3. Mercury analysis

For all samples, we determined Hg concentrations by direct measurement of at least two replicated 4 mm punches from each individual's DBS using an atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254; Altec®). We validated inter-replicate

Table 2
Biometric data (cm) and Hg concentrations (μg.g^{-1} dw) in the blood of four African crocodylian species at each sampling site. All *M. leptorhynchus* and *O. tetraspis* individuals were sampled in Gabon. In the table, NP = national park. For Site Degradation, we qualitatively characterized sites based on our observations whilst in the field sampling as P = Pristine (e.g., habitat mostly or entirely intact, very little contemporary human disturbance), MD – Mildly Degraded (up to moderate habitat loss, some contemporary human presence), or HD = Heavily Degraded (considerable habitat loss, humans extensive in system contemporarily). For Proximity to Hg, we identified the most significant likely contributors of Hg to the habitat, where P = Petroleum, A = Industrial or Intensive Agriculture, and G = Gold mining, and estimated its relative proximity to the site, where 1 = < 50 km, 2 = 50 – 150 km, 3 = 150 – 300 km, and 4 = > 300 km. For both of these columns, the characterizations are applicable to the time of sampling, not after.

Species	Locality	Site Degradation	Proximity to Hg	N	SVL (cm)		Hg (μg.g^{-1})	
					Mean ± SD	Min – Max	Mean ± SD	Min – Max
Mecistops leptorhynchus								
	Ogooué River (middle stretch, Gabon)	MD	P3, A2	24	33.24 ± 9.22	18.1–52.1	3.182 ± 1.629	0.791–7.401
	Bongo River (Loango NP, Gabon)	P	P1	16	43.87 ± 21.14	21.7–101.0	2.793 ± 1.318	0.566–5.891
	Lac Divangui (Gabon)	MD	P1	1	26.2	–	1.823	–
	Lac Evaro (Gabon)	MD	P3, A2	2	46.55 ± 5.02	43.0–50.1	3.064 ± 0.232	2.900–3.227
	Akaka (Loango NP, Gabon)	P	P1	40	71.14 ± 41.35	19.1–152.1	3.029 ± 1.984	0.822–11.228
	Dji Dji River (Ivindo NP, Gabon)	P	G2	19	44.72 ± 35.31	17.0–131.4	4.786 ± 2.293	2.025–10.162
	Mpassa River (Plateau Bateke NP, Gabon)	P	–	16	26.69 ± 11.82	17.1–61.0	3.919 ± 2.440	0.928–8.608
	Nyanga River (Moukalaba-Doudou NP, Gabon)	MD	P2, A1	6	51.37 ± 23.14	31.0–96.7	2.958 ± 1.678	0.739–4.993
Osteolaemus tetraspis								
	Abanda (Fernan Vaz Lagoon area, Gabon)	MD	P1	32	43.1 ± 21.8	20.8–92.6	1.248 ± 0.620	0.535–3.014
	Akaka (Loango NP, Gabon)	P	P1	32	53.5 ± 15.3	21.7–81.8	2.279 ± 1.503	0.390–5.180
	Lope River (Lope NP, Gabon)	P	–	17	49.6 ± 15.1	25.1–76.9	1.245 ± 0.430	0.450–2.328
Crocodylus suchus								
	Abi Lagoon (Cote d'Ivoire)	HD	A1, G3	10	42.8 ± 32.0	20.0–100.2	0.578 + 0.344	0.229–1.291
	Amansuri Wetlands (Ghana)	MD	A1, G2	6	24.5 ± 1.2	22.4–25.7	0.890 ± 0.164	0.667–1.106
	Bui National Park (Ghana)	MD	A1, G2	6	28.9 ± 11.4	22.7–52.0	0.397 ± 0.082	0.285–0.495
	Kidepo Valley NP (Uganda)	P	P4	19	85.3 ± 21.9	47.0–139.5	0.508 ± 0.172	0.253–1.002
	San Pedro River Dam (Cote d'Ivoire)	HD	A1, G2	8	26.3 ± 2.4	22.0–28.9	0.519 ± 0.186	0.289–0.815
	Tiatia Village (Ghana)	HD	A1, G2	7	32.7 ± 7.1	24.0–43.9	0.327 ± 0.080	0.207–0.404
Crocodylus niloticus								
	Lake Nasser (Egypt / Sudan)	P	–	9	42.4 ± 17.9	15.1–74.6	1.580 ± 0.296	1.230–2.180

reproducibility for each sample against a relative standard deviation (RSD) threshold of < 10 %. We analyzed Certified Reference Material (CRM) DOLT-5 (Lobster hepatopancreas from National Research Council of Canada; certified Hg concentration: 0.44 ± 0.18 μg.g^{-1} dry weight (dw)) at the beginning and at the end of the analytical cycle, as well as every 10 samples, to validate measurement consistency across samples. The measured value for the CRM was 0.436 ± 0.005 μg.g^{-1} dw (n = 39), giving a recovery of 99.98 ± 1.22 %. We analyzed the blank punches for Hg concentration and found that they were always under the limit of quantification of the method. Blanks were included at the beginning of analytical runs and the limit of quantification was 0.1 ng.g^{-1}. We express Hg concentrations in μg.g^{-1} dw.

2.4. Statistical analysis

We performed all statistical analyses in R v.3.6.1 (R Development Core Team 2013). Prior to all analysis, we checked data for normality and homogeneity of variances.

For each species individually, we used paired t-tests to compare SVL between adult males and females. Depending on the results, we then used either a paired *t*-test or ANCOVA to compare Hg concentrations between the sexes. Ultimately, we only performed this inter-sex comparison for *M. leptorhynchus* and *O. tetraspis* due to the low number of sampled adults for the other species.

We used one-way ANOVAs to compare SVL between the four species and to compare Hg concentrations within each species. We implemented a post-hoc Tukey's honest significant difference (HSD) test to evaluate the variation of Hg concentration between species.

Also, for each species individually, we used paired t-tests to compare SVL between sites and then used ANCOVAs with site and SVL as explanatory variables predicting Hg concentration differences between sites. Finally, we used linear regressions to evaluate the relationships between Hg concentration and the body size (SVL) for each species and site individually.

3. Results

We detected Hg in all the samples, with the highest average concentration in *M. leptorhynchus* (3.400 ± 2.008 μg.g^{-1}) followed by *O. tetraspis* (1.655 ± 1.148 μg.g^{-1}), *C. niloticus* (1.580 ± 0.296 μg.g^{-1}), and *C. suchus* (0.528 ± 0.243 μg.g^{-1}) (Table 1).

Table 3
Summary statistics of linear regressions relating Hg concentration (μg.g^{-1} dw) to Snout-Vent-Length (SVL in cm) for each sampling location for three African crocodylian species. All *M. leptorhynchus* and *O. tetraspis* individuals were sampled in Gabon. In the table, NP = national park.

Sites	n	R^2	p-value
Mecistops leptorhynchus			
Ogooué River (middle stretch)	24	0.405	**< 0.001**
Bongo River (Loango NP)	16	0.105	0.222
Lac Divangui	1	–	–
Lac Evaro	2	–	–
Akaka (Loango NP)	40	0.352	**< 0.001**
Dji Dji River (Ivindo NP)	19	0.297	**0.016**
Mpassa River (Plateau Bateke NP)	16	0.593	**< 0.001**
Nyanga River (Moukalaba-Doudou NP)	6	0.190	0.387
Osteolaemus tetraspis			
Abanda (Fernan Vaz Lagoon area)	32	0.134	**0.040**
Akaka (Loango NP)	32	0.084	0.109
Lope River (Lope NP)	17	0.075	0.288
Crocodilus suchus			
Abi Lagoon (Cote d'Ivoire)	10	0.016	0.726
Amansuri Wetlands (Ghana)	6	0.034	0.728
Bui National Park (Ghana)	6	0.193	0.384
Kidepo Valley NP (Uganda)	19	0.091	0.211
San Pedro River Dam (Cote d'Ivoire)	8	0.145	0.351
Tiatia Village (Ghana)	7	0.014	0.804

For *M. leptorhynchus*, we did not detect any difference in adult body size (SVL) between the sexes in our sample (*t-test*: t = 2.134, p = 0.056); however, for *O. tetraspis* adult females were smaller than adult males in our sample (*t-test*: t = -2.071, p = 0.046). We did not find any significant difference in Hg concentrations between adult males and females for either species (*M. leptorhynchus*, *t*-test: t = 1.112, p = 0.290; *O. tetraspis*, ANCOVA: $F_{1\text{-}32} = 0.006$, p = 0.939).

We found significant differences in body size (SVL) variation across sample sites for all three species sampled across multiple sites: *M. leptorhynchus* (ANOVA: $F_{1\text{-}116} = 6.233$, p < 0.001), *O. tetraspis* (ANOVA: $F_{2\text{-}78} = 4.396$, p = 0.016), and *C. suchus* (ANOVA: $F_{5\text{-}51} = 25.06$, p < 0.001). Likewise, we found that Hg concentrations were significantly different between sites for all three species: *M. leptorhynchus* (ANCOVA: $F_{5\text{-}115} = 5.218$, p < 0.001), *C. suchus* (ANCOVA: $F_{5\text{-}50} = 7.042$, p < 0.001) and *O. tetraspis* (ANCOVA: $F_{2\text{-}77} = 3.2013$, p = 0.050) (Table 2). However, we only found a significant relationship between Hg concentration and body size (SVL) at 4 sites for *M. leptorhynchus* and 1 site for *O. tetraspis* (Table 3).

4. Discussion

We found that, at least for the localities examined as part of this study, that these four African crocodile species were contaminated at varying concentrations with Hg. Worryingly, *M. lepthorynchus* (3.400 ± 2.008 μg.g^{-1}), *O. tetraspis* (1.655 ± 1.148 μg.g^{-1}), and *C. niloticus* (1.580 ± 0.296 μg.g^{-1}) showed levels of Hg contamination that were particularly high compared to blood Hg concentrations amongst crocodylians analyzed to date – which had average Hg concentrations ranging from 0.07 to 1.38 μg.g^{-1} dw (Table 4). Interestingly, *M. leptorhynchus* in Gabon had the highest Hg blood concentration both on average and as a single individual, with one individual having as much as 11 μg.g^{-1}, of any species of crocodylian measured to date. Here we discuss these findings both within the context of our study systems and for crocodylians globally.

Of particular note, we found high variability in Hg concentrations both between species and sampling sites for all species (Table 2). Several studies have shown that Hg concentration in the blood of vertebrates may represent a relatively recent exposure to Hg related to diet (e.g., Gilmour et al., 2019; Chételat et al., 2020; Seco et al., 2021). It is also correlated with erythrocyte longevity, which ranges from up to two months in birds to four months in mammals, but may be as long as seventeen months in some reptiles (reviewed in Rodnan et al., 1957; Monteiro and Furness, 2001). Because crocodilians are high level consumers, if not apex predators, within their ecosystems, we would expect individual Hg contamination to be closely related to both diet and individual position within the trophic chain. Mercury contamination in the trophic chain depends to some extent on the geological background of the environment but can also be increased by human activities. In this regard, mining, fossil fuel extraction, biomass burning, and agriculture are among the anthropogenic activities driving the largest increases in environmental Hg (Obrist et al., 2018; Crespo-Lopez et al., 2021).

The simultaneous assessment of Hg concentrations in four crocodilians, two of which are syntopic, provides an interesting preliminary opportunity to discuss the role of species ecology in Hg bioaccumulation in this group. Generally speaking, higher concentrations of Hg are to be found in higher trophic level species predominantly consuming prey from aquatic ecosystems (Lavoie et al., 2013; Eagles-Smith et al., 2018). Though *M. leptorhynchus* and *O. tetraspis* occupy the same freshwater wetlands habitats throughout Gabon, including sites sampled and analyzed as part of this study, they have quite divergent feeding ecologies. Slender-snouted crocodiles are predominantly piscivorous, though consume a wide variety of prey accessible from within the wetlands (Shirley et al., 2018 and references therein). In contrast, dwarf crocodiles are largely feeding on terrestrial and aquatic invertebrates

Table 4

Review of Hg concentration (μg.g^{-1} dw) reported in blood of crocodilians. TL stands for Total Length and SVL for Snout-Vent-Length (cm). For Caiman crocodilus sites in Colombia, ^{a}: pristine area; ^{b}: gold mining area. $^{\phi}$Studies did not report SVL values, only TL; using standard allometric relationships, the Caiman crocodilus individuals were ± 31 cm SVL and Melanosuchus niger were ± 78 cm SVL – both generally comparable to other species in the table. *Original data reported in wet weight, transformed to dry weight using a conversion factor of 3.6 following a mean moisture content of 72 % in the blood of caimans from Lemaire et al. (2021a). These transformed values should be viewed cautiously until the universality of the conversion factor can be validated.

Species	Location	N	Tissue	Length (cm)		Hg (μg.g^{-1} dw)		Reference
				Mean ± SD	Min-Max	Mean ± SD	Min-Max	
Mecistops leptorhynchus	Gabon	124	Whole blood	48.8 ± 33.4 (SVL)	17–152.1	3.400 ± 2.008	0.57–11.23	Present study
Osteolaemus tetraspis	Gabon	81	Whole Blood	48.6 ± 18.5 (SVL)	20.8–92.6	1.655 ± 1.148	0.390–5.180	Present study
Crocodylus suchus	Cote d'Ivoire	18	Whole Blood	35.5 ± 24.8 (SVL)	20.0–100.2	0.552 ± 0.278	0.229–1.291	Present study
Crocodylus suchus	Ghana	19	Whole Blood	28.9 ± 8.1 (SVL)	22.4–52.0	0.527 ± 0.277	0.207–1.106	Present study
Crocodylus suchus	Uganda	19	Whole Blood	85.3 ± 21.9 (SVL)	47.0–139.5	0.508 ± 0.172	0.253–1.002	Present study
Crocodylus niloticus	Egypt	9	Whole Blood	42.4 ± 17.9 (SVL)	15.1–74.6	1.580 ± 0.296	1.230–2.180	Present study
Paleosuchus trigonatus	French Guiana	24	Whole Blood	33.3 ± 19.8 (SVL)	10.9–81	0.300 ± 0.178	0.032–0.738	Lemaire et al., 2021a
Paleosuchus palpebrosus	French Guiana	7	Whole blood	38.1 ± 15.3 (SVL)	16.5–62.0	1.376 ± 0.986	0.540–3.415	Lemaire et al., 2021a
Caiman crocodilus	French Guiana	40	Whole blood	32.7 ± 13.6 (SVL)	14.5–103.0	0.605 ± 0.380	0.089–1.532	Lemaire et al., 2021a
Caiman crocodilus	Colombia^a	23	Whole blood	57.5 ± 6.8 (TL) $^{\phi}$	–	0.05 ± 0.02*	–	Marrugo-Negrete et al., 2019
Caiman crocodilus	Colombia^b	23	Whole blood	57.2 ± 3.5 (TL) $^{\phi}$	–	0.23 ± 0.08*	–	Marrugo-Negrete et al., 2019
Melanosuchus niger	French Guiana	72	Whole blood	143.2 ± 61.3 (TL) $^{\phi}$	46–326	1.284 ± 0.672	0.300–3.410	Lemaire et al., 2021d
Alligator mississippiensis	USA / Florida	37	Whole blood	92.1 ± 31.6 (SVL)	43.9–153.5	0.694*	0.280–6.900	Nilsen et al., 2017b

(Pauwels et al., 2007; Shirley et al., 2017). This divergence in prey base is likely one of the most important factors explaining the higher Hg concentration in *M. leptorhynchus* despite both species being exposed to similar environmental conditions. While most invertebrates, like millipedes or even crustaceans, typically have low Hg concentrations, fishing spiders of the genus *Dolomedes* are known to have higher Hg concentrations likely because of their predation on fish (Ortega-Rodriguez et al., 2019). These species are abundant throughout the wetlands also occupied by *M. leptorhynchus* and *O. tetraspis* and are almost certainly widely consumed by juveniles of both species. This may explain, at least in part, the higher concentrations of Hg in *O. tetraspis* than would be expected on the basis of observations in the ecologically homologous *Paleosuchus* spp. (Lemaire et al., 2022).

In addition to likely explaining key interspecific differences, trophic level and foraging habitats are extremely important when considering intraspecific variability in Hg contamination between different study sites, as demonstrated in multiple species (e.g., caiman, Lemaire et al., 2021d; birds, Carravieri et al., 2016; fish, Goutte et al., 2015). Across our sampling sites, the individuals with the highest Hg concentrations were often sampled at sites thought to represent among the most pristine environments in Central Africa (e.g., Ivindo and Loango National Parks in Gabon), even if they are relatively adjacent sites where intense and prolonged oil extraction and varying degrees of subsistence agriculture occur. Higher Hg concentrations in "pristine" environments can often be explained by the relative complexity and intactness of the food webs compared to degraded sites – pristine environments can effectively have more links in the food webs for bioaccumulation of Hg prior to consumption by species like crocodilians resulting in a higher degree of Hg biomagnification (De Almeida Rodrigues et al., 2019). Alternatively, the proximity of these sites, especially Loango National Park (Gabon), to long-term oil extraction concessions may mean they are not as pristine as believed and our results suggest that more in-depth investigation of the spatio-temporal scale and extent to which petroleum concessions drive environmental contamination is warranted. More interestingly, *C. suchus* had the lowest Hg concentrations of all our studied species in spite of its distribution amongst significant artisanal small-scale gold mining (ASGM) activities throughout West Africa, including Ghana and Cote d'Ivoire – countries with high reporting of Hg contamination due to prolific ASGM (World Health Organization, 2015). Aquatic habitats throughout West Africa are generally highly degraded and, thus, *C. suchus* are largely persisting in highly disrupted food webs, potentially limiting the degree of bioaccumulation despite potentially higher levels of exposure. Unfortunately, our preliminary data only allow an extrapolative inference to be made about the relationship between Hg concentrations in crocodilians and human activities, and we recommend that this issue be studied in greater depth, especially in West and Central Africa, which provides an interesting natural laboratory.

Mercury is known to affect physiology, reproduction, and behavior of many wildlife species (Tan et al., 2009; Whitney and Cristol, 2017; Evers, 2018). In seabirds, Hg contamination reduces mating success (Tartu et al., 2013), and hatchling and fledging rates (Hg concentrations in blood of *Diomedea exulans* were 6.2 ± 3.0 μg.g^{-1} dw for males and 10.7 ± 0.5 μg.g^{-1} dw for females, Goutte et al., 2014). In freshwater turtles, Hg exposure has been related to increasing embryonic mortality (Hg concentration in eggs of *Chelydra serpentina* was 3.0 ± 0.19 μg.g^{-1}, Hopkins et al., 2013a) and to a reduction of reproductive females in the population (Hg concentration in the claws of adult *Emys orbicularis* was 2.21 ± 0.06 μg.g-1 dw, Beau et al., 2019). However, little is known to date about the impact of Hg contamination in crocodylians. Nilsen et al. (2017a) showed that Hg contamination negatively impacted body condition in *Alligator mississippiensis,* which had blood Hg concentrations ranging from 0.26 to 6.90 μg.g^{-1} dw. For *Caiman crocodilus*, Marrugo-Negrete et al. (2019) showed erythrocytes DNA damage related to Hg exposure (average 0.23 ± 0.08 μg.g^{-1} dw) and Lemaire et al. (2021b) found that blood Hg concentrations ranging from 0.169 to 1.532 μg.g^{-1} dw were related to disruption of osmoregulation, hepatic function, and endocrine processes. These studies highlight that Hg contamination has toxic effects in crocodylians even at relatively low levels and raise concerns regarding the Hg concentrations obtained in the species studied here. While we did not specifically study the linkage between Hg contamination and deleterious effects in African crocodylians, our results lay the framework for tentative discussion.

For example, in several species, Hg is transferred to the egg during vitellogenesis, including in birds (Ackerman et al., 2016), snakes (Chin et al., 2013; Cusaac et al., 2016), turtles (Hopkins et al., 2013b), and crocodylians (Du Preez et al., 2018; Nilsen et al., 2020; Lemaire et al., 2021c). It has been shown that the quantity of Hg transferred to the egg by the mother is directly related to the body burden of Hg in the reproductive female (Akearok et al., 2010; Nilsen et al., 2020). While it is thought this is a potential detoxification pathway for the female, leading to a reduction of the Hg body burden, it has consequences for the next generation. In birds, Hg exposure during embryonic development reduces reproductive capacity of the next generation (Paris et al., 2018), while in turtles it reduces hatchling success (Hopkins et al., 2013a), and alters the behavior of hatchling snakes (Chin et al., 2013). In crocodylians, maternally transferred Hg resulted in reduced body size of hatchling smooth-fronted caiman (*Paleosuchus trigonatus*) (Lemaire et al., 2021c). We did not find any variation in Hg blood concentration between males and females for the individuals assessed here. While these results are consistent with several other crocodylian Hg studies

(Yanochko et al., 1997; Rumbold et al., 2002; Campbell et al., 2010; Schneider et al., 2012; Lemaire et al., 2021d), the biological explanation is as yet clear and it may simply be that we did not sample any reproductively active females. However, the effects of Hg exposure from maternal transfer during embryonic development are concentration-dependent, and the high Hg concentrations found in both sexually mature females and juveniles in our study species is worrying and highlights the potential long-term consequences for reproductive success and population growth and sustainability in these species. In particular, further assessment of Hg contamination in *M. leptorhynchus* and *O. tetraspis* in and around the oil extraction concessions of Gabon, including maternal transfer and reproductive stability implications, should be further assessed and monitored.

Crocodylians are widely consumed as bushmeat throughout Africa. In particular, dwarf crocodiles (*Osteolaemus* spp.) are among the most widely consumed bushmeat species throughout Cameroon (Wilcox and Nambu, 2007; Smolensky, 2015; Dipita et al., 2022), Congo (Behra, 1987; Thorbjarnarson and Eaton, 2004; Eaton, 2006; Eaton et al., 2010; Mbete et al., 2011; Shirley et al., 2019), Democratic Republic of Congo (Batumike et al., 2021), Equatorial Guinea (Juste et al., 1995; Fa et al., 2002), Gabon (Behra, 1987; Thorbjarnarson and Eaton, 2004; Eaton, 2006; Abernethy and Ndong Obiang, 2010), and Nigeria (Dore, 1996; Akani et al., 1998), where tens to hundreds of thousands of individuals are hunted and consumed annually. These diminutive species are also regularly transported for consumption outside of their natural range, including to destinations as close as Bioko Island (Equatorial Guinea; Cronin et al., 2015) and as far away as western Europe (e.g., Chaber et al., 2019). Similarly, *M. leptorhynchus* is readily consumed locally and nationally throughout its entire range (e.g., Shirley et al., 2018 and references therein). While humans globally are exposed to Hg in the environment, Hg ingestion through the consumption of various food items (e.g., tuna and other fish) is the main exposure risk for people. Mercury toxicity through food consumption can result in significant neurological and behavioral effects in humans (Yang et al., 2020). As a result, the World Health Organization (WHO) has released safety guidelines for the consumption of fish recommending consumption of foods only at 0.5 $\mu g.g^{-1}$ of Hg or below. Though we did not measure Hg concentrations in commonly consumed crocodylian body parts (e.g., muscle tissue), previous work on *Caiman crocodilus*, *Melanoscuchus niger* and *Alligator mississippiensis* has shown that Hg concentration is higher in muscles than in blood (Eggins et al., 2015; Nilsen et al., 2017b). Though few people strictly depend on crocodile bushmeat for their protein, the volume of harvest and consumption of these species is sufficient to justify a more in-depth study of their potential consequences on human health. Further, the demographics most likely to be consuming crocodylians tend to be fishermen, their families, and others living in predominantly fishing communities suggesting that they may be bioaccumulating Hg at rates not dissimilar to the crocodiles. As previously recommended, further investigation of Hg concentrations in crocodylian and human prey (e.g., fish) throughout the rural areas of West and Central Africa is warranted.

5. Conclusion

Mercury contamination is understudied in crocodylians, including the extent to which it is an issue across the diversity of the Crocodylia, the habitats and geographies they occupy, and the specific and long-term ramifications of such contamination. However, all attempted studies to date have found that crocodylians are impacted by Hg contamination, almost regardless of species and study site. Our results reinforce this issue, with data from three crocodylian species that have never been studied, including from localities ranging from those thought to be heavily impacted by humans (most of West Africa) to those thought to be among the most pristine, unimpacted environments in the world today (most of Gabon). Worryingly, individuals sampled in these latter sites and habitats have the highest individual Hg contamination levels for any species of crocodylian anywhere in the world. In consideration of the conservation status of these species, as well as their importance as protein and economic resources to local communities throughout their distributions, our preliminary results underscore the importance of further studies on Hg contamination in African crocodylians. Such future work should not only continue to map patterns and process of Hg contamination in these bioindicator species, but dig deep into the bioaccumulation pathways, the long-term ramifications for population dynamics, and the potential health consequences for people consuming crocodylians and their shared prey (e.g., fish and other species).

CRediT authorship contribution statement

Jérémy Lemaire: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Paco Bustamante:** Methodology, Funding acquisition. **Matthew H. Shirley:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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