SRAS Final Report

Parasitic Ecology and Ecotoxicology of *Caiman latirostris* (Daudin, 1802) in the Capibaribe River Basin, Northeast Brazil (CSG Project No. 24/15)

Carlos Fernando Rodrigues de Souza Neto
Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil

Abstract

The broad-snouted caiman (*Caiman latirostris*) is an apex predator in aquatic food webs, widely distributed throughout Brazil, where it plays an important ecological role in maintaining the structure and functioning of aquatic ecosystems. Although classified as a species of Least Concern (LC), its populations are increasingly exposed to anthropogenic impacts resulting from habitat loss, pollution, and urbanization. However, studies integrating ecotoxicology and parasitology in free-ranging populations inhabiting anthropized tropical estuaries remain scarce. In this context, this study investigated the parasitic ecology and ecotoxicological aspects of *C. latirostris* in a remnant of Atlantic Forest and mangrove within the eutrophic Capibaribe River estuary (Recife, northeastern Brazil) under a One Health approach. Two field surveys were conducted during the dry and rainy seasons of 2024, resulting in the capture of 22 individuals, including 19 adults and three subadults, comprising 14 males and eight females. The animals were necropsied for the collection of helminths and biological samples. Fecal samples were examined using Sheather's flotation technique for the detection of endoparasites, blood smears were analyzed for hemoparasites, and scale, muscle, liver, and kidney samples were collected for the determination of 13 essential and potentially toxic trace elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Hg, and Pb) by inductively coupled plasma mass spectrometry (ICP-MS). Of the 22 individuals examined, 11 (50.0%) were infected exclusively by nematodes of the genus *Micropleura* (Nematoda: Dracunculoidea) located in the coelomic cavity (mean abundance of infection = 1.23 ± 0.24; mean intensity of infection = 2.45 ± 1.37; range = 1–4). No hemoparasites or parasite forms were detected in fecal samples, indicating low parasite richness in the studied population. Among the analyzed elements, Fe, Zn, Al, and Cu showed the highest concentrations across all tissues, with the kidney being the primary organ of bioaccumulation. Significant sex-related differences were observed for some elements, with females exhibiting higher hepatic Zn and renal Mg concentrations, whereas males showed higher muscular concentrations of Cu, Cd, and Pb. Regarding age class, subadults exhibited significantly higher concentrations of Mg, Zn, Se, and Cd in the liver; Co, Cu, As, Se, and Cd in the kidney; and Al, Se, and Hg in the muscle, whereas adults showed higher concentrations of Mg and Zn in scales. Hepatic melanomacrophages were observed in all individuals, and 54.5% exhibited a high frequency of these phagocytic cells, a pattern consistent with chronic exposure to contaminants and other environmental stressors. This study provides the first record of *Micropleura* sp. infecting *C. latirostris*, expanding the known host range of this nematode in South America. The low parasite richness suggests that host-related intrinsic factors may exert a greater influence on parasite community structure than local environmental stressors. The presence of heavy metals represents a potential risk not only to the health and conservation of caimans but also to the estuarine biota, including human populations that consume fish and shellfish harvested from this river. The species shows considerable potential as a sentinel organism for environmental monitoring and for supporting biodiversity conservation and environmental management actions within the One Health framework.

Introduction

The broad-snouted caiman, *C. latirostris* (Daudin, 1802), is a medium-sized crocodilian, being a long-lived and opportunistic semi-aquatic predator with a generalist diet (Platt et al., 2006), regulating food webs and facilitating nutrient transport among different habitats (Somaweera et al., 2020). It is found in vegetated habitats and lentic water bodies, such as floodplains, wetlands, mangroves, reservoirs, and estuaries (Verdade & Piña, 2005). Approximately 70% of its global population is concentrated within Brazilian territory, particularly in areas with high human population density (Coutinho et al., 2013). The species is classified as Least Concern (LC) at both the global (IUCN, 2024) and national levels in Brazil (ICMBio, 2024). In Brazil, populations are under intense anthropogenic pressure due to the drainage of water bodies, deforestation, habitat reduction, urban expansion, illegal hunting, pollution, and contamination by pesticides (Verdade, 1997 and 2001; Filogônio et al., 2010; Mascarenhas-Junior et al., 2021).

Human activities have caused substantial impacts on biodiversity (Daszak et al., 2001; WWF, 2024; Silva et al., 2025), also affecting parasite–host relationships (Sures et al., 2023). In Environmental Parasitology studies, parasites are considered bioindicators of the health of both ecosystems and their hosts (Hatcher et al., 2012; Sures et al., 2017, 2023), particularly in aquatic environments (Lafferty, 1997; Marcogliese & Pietrock, 2011; Sures et al., 2017, 2023; Fonseca et al., 2021). Within the One Health framework, the health of humans, non-human animals, photosynthetic organisms, and the environment should be considered a priority (Mettenleiter et al., 2023), particularly in anthropogenically impacted aquatic ecosystems, such as rivers and their estuaries (Fonseca et al., 2021, 2023).

Studies on the parasitic fauna of *C. latirostris* are scarce and are restricted to the taxonomic identification and occurrence records of intestinal and blood protists, nematodes, digeneans, and acanthocephalans (Vicente et al., 1993; Batista et al., 2011, 2012; Tellez, 2014; Werneck & Leandro, 2021; Werneck et al., 2023). There are no studies on the parasitic fauna of this crocodilian in eutrophic estuarine environments, where the influence of factors such as salinity, temperature, and contaminants may interfere with parasitism and, consequently, host susceptibility (Rogowski & Stockwell, 2006; Charruau et al., 2017; Grabner et al., 2023; Farias et al., 2024; Gorza et al., 2025). The use of parasites as bioindicators of health (Lafferty, 1997; Marcogliese & Pietrock, 2011; Sures et al., 2017; Fonseca et al., 2021) may provide support for the development of health promotion and conservation actions and policies within the One Health framework.

The dynamics of estuarine environments are strongly influenced by hydrodynamic factors, such as salinity, temperature, nutrient availability, and tides (McLusky & Elliott, 2004; Zinardi-Lamardo et al., 2016). The continuous input of organic matter and nutrients creates a favorable environment for the survival of various organisms, including caimans, as well as fish, crustaceans, and other organisms that constitute the diet of this crocodilian (Mallin & Paerl, 1994).

Characteristics such as longevity, prey diversity, and prolonged residence in water bodies (Campbell et al., 2004; Schneider et al., 2012, 2015; Nilsen et al., 2017) make crocodilians vulnerable to the accumulation of high concentrations of contaminants (Verdade & Piña, 2005; Poletta et al., 2008; Stoker, 2011; Schneider et al., 2012, 2015; Nilsen et al., 2017). These animals may be exposed throughout all life stages, either through their diet or through the water and sediments of the habitats they occupy (Poletta et al., 2008; Schneider et al., 2013; Buah-Kwofie et al., 2018), although embryos are considered the most sensitive to contaminants (Poletta et al., 2008). In *C. latirostris*, alterations in embryogenesis, as well as reduced hatching success and egg mass loss, have already been reported following exposure to organic contaminants, such as herbicides and pesticides (Poletta et al., 2008). In Pernambuco, in the Capibaribe River basin, bioaccumulation studies have also been conducted on *C. latirostris* inhabiting lentic environments within the Atlantic Forest (Santos et al., 2021, 2024).

Studies demonstrating the impact of anthropogenic disturbance and contamination by essential and toxic elements on *C. latirostris* populations are important for the implementation of conservation strategies for this species and the biota associated with continental aquatic ecosystems, as well as for providing information on the health risks to the human population (Aragão & Araújo, 2006) that consumes food resources originating from polluted and contaminated water bodies, such as the Capibaribe River, which ranks seventh among the ten most polluted rivers in Brazil (Associação Brasileira de Engenharia Sanitária e Ambiental, 2022). The Capibaribe estuary is heavily impacted by urbanization, pollutant discharge, and vegetation removal, while also being used for fishing, tourism, recreation (CPRH, 2020; APAC, 2021), and supplying drinking water to approximately 2 million inhabitants (Silva et al., 2025). In this context, health and conservation actions and policies for this ecosystem are required based on the principles of the One Health approach, which, through transdisciplinary and intersectoral actions and policies, promotes the well-being and health of humans, animals, photosynthetic organisms, and ecosystems in an interconnected and interdependent manner (Mettenleiter et al., 2023).

Accordingly, the objectives of this study in natural populations of *C. latirostris* were to: (I) identify the parasitic fauna of *C. latirostris* in a hypereutrophic urban estuary, within Atlantic Forest and mangrove habitats; and (II) evaluate the exposure of *C. latirostris* to environmental contaminants through the quantification and analysis of essential and toxic trace elements, testing the following hypotheses: (a) the liver and kidney bioaccumulate higher concentrations of metals; (b) metal concentrations differ between males and females; and (c) among size classes.

Materials and Methods

Study area

The Capibaribe River is approximately 280 km long, flowing through 42 municipalities in the state of Pernambuco, northeastern Brazil (Figure 1a,b), where it receives large amounts of domestic effluents (lower course), as well as agricultural and industrial effluents (middle and upper courses) (CPRH, 2024). The Capibaribe River supplies drinking water to approximately 2 million inhabitants (Silva et al., 2025), and is also used for fishing, recreation, and tourism (CPRH, 2024). Currently, the river ranks seventh among the ten most polluted rivers in Brazil (SoCientifica, 2022).

The study was conducted in the river estuary, in the city of Recife (8°02′30.34″ S; 34°55′13.7″ W), within an area of approximately 16 km (Figure 1c), encompassing Atlantic Forest and mangrove habitats (CPRH, 2024). The estuary is characterized by strong tidal influence and intense urban activity, with areas of vegetation suppression and discharge of domestic effluents, and is classified as hypereutrophic (CPRH, 2024).

Capture of caimans, collection, and processing of biological samples

This study was authorized by the Biodiversity Authorization and Information System (SISBIO) of the Chico Mendes Institute for Biodiversity Conservation (ICMBio) (SISBIO #63030-11), the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN #AFOEC7C), and the Animal Use Ethics Committee (CEUA) of the Federal Rural University of Pernambuco (CEUA/UFRPE #860200622).

Individuals of *C. latirostris* were captured in January and October 2024 through active nocturnal surveys using a motorized boat (Bayliss, 1987; Silveira et al., 1997). The animals were physically restrained for biometric measurements and biological sample collection (Jesus Filho & Freitas-Filho, 2021). The geographic location of each individual was recorded using a handheld GPS receiver. Following capture, sex was determined by cloacal palpation (Webb et al., 1984), and biometric measurements were recorded using a measuring tape and calipers. Based on snout–vent length (SVL, cm), individuals were classified into the following size classes, adapted from Leiva et al. (2018): juvenile < 25 cm; subadult 25–67.9 cm; adult > 68 cm. Body mass was recorded using spring scales with a capacity of up to 100 kg.

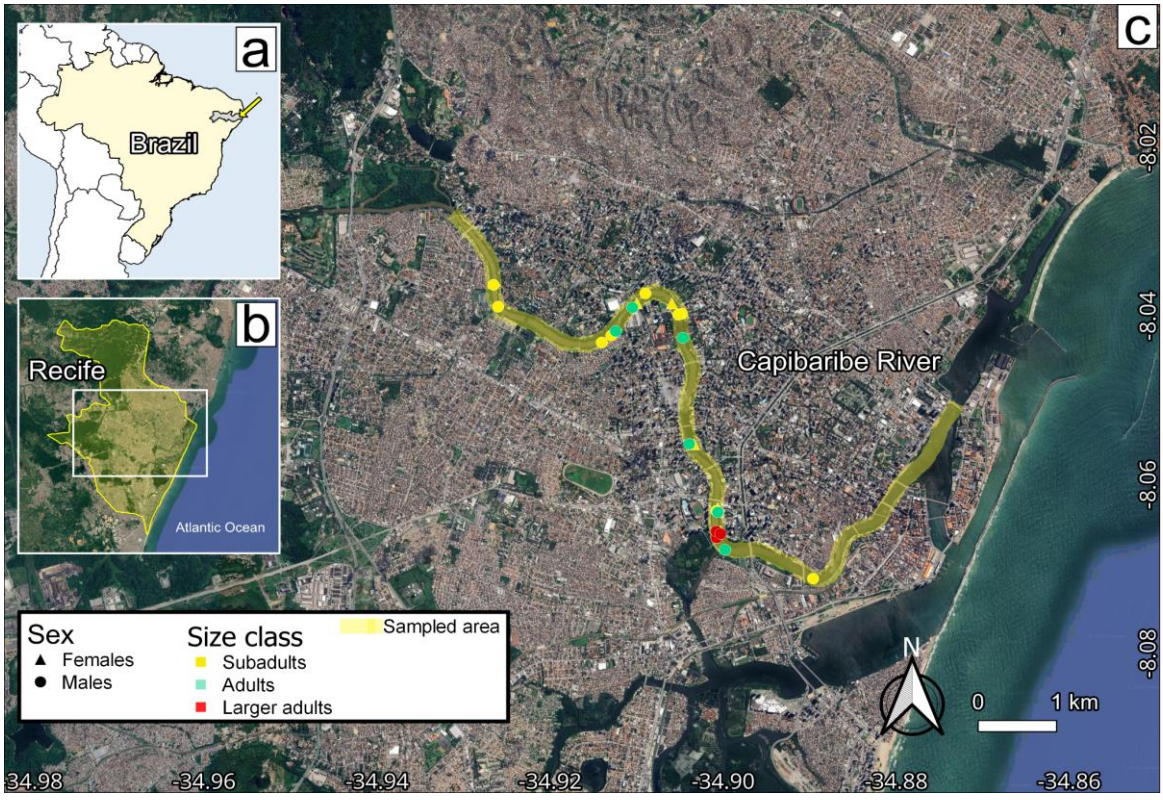


Figure 1. Study area showing the capture sites of broad-snouted caimans (*Caiman latirostris*) in the state of Pernambuco (a) Shows the study area relative to northeastern Brazil. (b) Monitoring was conducted in the urban area of the city of Recife, (c) The 16-km stretch of the Capibaribe River.

Parasite analysis

For blood collection, the animals were positioned in sternal recumbency according to the criteria of Myburgh et al. (2014), and blood was collected from the post-occipital region using conventional hypodermic syringes of varying gauges. Immediately after collection, the blood was stored for subsequent hematological analysis. Blood smears were prepared and stained with Panótico Rápido for the detection of hemoparasites.

Subsequently, the specimens were euthanized following the recommendations of the National Council for the Control of Animal Experimentation, using a combination of xylazine (1 mg/kg) and ketamine (20 mg/kg), and, after muscle relaxation was achieved, a lethal dose of potassium chloride (60 mg/kg) was administered into the occipital sinus (Vieira et al., 2013).

Fecal samples evacuated *in vivo* and/or collected directly from the intestine after euthanasia were obtained and processed using the modified Sheather flotation technique (with a hypersaturated sugar solution, specific gravity 1.3) (Santos et al., 2015). The oral cavity, gastrointestinal tract, lungs, liver, gallbladder, coelomic cavity, kidneys, and urinary bladder were examined for parasites. The collected nematodes were preserved in 70% ethanol and subsequently cleared in Amann's lactophenol and mounted on temporary slides (Amato & Amato, 2010). Photomicrographs under light and electron microscopy were obtained using the Toup View 3.7.6701® software. Parasites were identified according to Vicente et al. (1993) and Anderson et al. (2009).

Tissue samples presenting macroscopic alterations, whether associated with parasites or not, were collected and preserved in 10% neutral buffered formalin for subsequent processing using conventional histological techniques. Liver and kidney samples were microscopically evaluated using Hematoxylin and Eosin (H&E) and Sudan Black B (SB-B) staining. For the H&E technique, liver and kidney fragments were fixed in 10% buffered formalin for 48 h, trimmed and routinely processed in alcohol and xylene, embedded in paraffin, sectioned at 4 µm, and stained. In these sections, the degree of hepatocyte vacuolization, indicative of lipid deposition, was evaluated. Regarding distribution, vacuolization was classified as focal, multifocal, or diffuse. Regarding intensity, this lesion was graded as absent (−), mild (+), moderate (++), or severe (+++). The same classification and grading system was adopted for the occurrence of melanomacrophages and inflammation. The presence of microorganisms in these tissues was also investigated.

For the SB-B technique, fragments of the same organs were frozen at −80°C and subsequently cryosectioned, followed by staining with Sudan Black B (SB-B) and counterstaining with Harris hematoxylin (HH). The occurrence of lipid positivity was investigated, and the intensity of staining was graded as absent (−), mild (+), moderate (++), or severe (+++). Parasitological indices of prevalence, mean abundance of infection, and mean intensity of infection were calculated according to Bush et al. (1997).

Elemental analysis

Scale, muscle, liver, and kidney samples were collected for the determination of elemental concentrations of Al, Cr, Fe, Mg, Co, Cu, Zn, As, Se, Cd, Hg and Pb.

Reagents and standards: All solutions were prepared using concentrated nitric acid (~65%, m m^{-1}; Synth, Brazil), which was sub-distilled (DST-1000, Savillex, USA). Deionized water (18.2 MΩ cm resistivity; Master System All, Gehaka, Brazil) was used for all analytical procedures and cleaning steps. Sample digestion was performed using an acid digestion block (EasyDigest, Analab, Wantzenau, France). Calibration standards consisted of a 10 mg L^{-1} Hg standard solution (Perkin Elmer, USA) and a 10 mg L^{-1} multi-element stock solution (Inorganic Ventures, USA). Calibration curves were prepared using the following concentration ranges: 50, 100, 200, 500, 1000, 2000, and 5000 µg L^{-1} for Fe and Zn; 0.1, 2.5, 10, 25, 50, 100, and 200 µg L^{-1} for Al, Cr, Mn, Co, Ni, Cu, As, Se, Cd, and Pb; and 0.1, 0.2, 0.5, 1, and 2 µg L^{-1} for Hg. A mixed Rh and Ge solution (25 µg L^{-1}) was used as the internal standard. The certified reference materials SRM 1643f (National Institute of Standards and Technology, USA) and DORM-4 (National Research Council Canada) were used for quality control. Elemental determinations were performed using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7900, Hachioji, Japan).

Acid block digestion: Scale samples were initially washed with Milli-Q water in an ultrasonic bath for 5 min. Muscle, scale, liver, and kidney samples were subsequently freeze-dried for 72 h, ground, and weighed. Approximately 200 mg of each sample was transferred into 50 mL Falcon tubes, followed by the addition of 1,000 µL of sub-distilled HNO_3. The tubes were sealed and subjected to acid digestion according to the following protocol: (i) pre-digestion for 48 h at room temperature (25°C); (ii) manual shaking for homogenization; and (iii) heating at 95°C for 4 h. This digestion procedure was adapted from the method described by Paniz et al. (2018).

Method validation and evaluation of digestion efficiency: Calibration curve quality was evaluated using the certified reference material SRM 1643f, whereas method accuracy was assessed by recovery rates, which ranged from 92% to 112%. Digestion efficiency was evaluated using the certified reference material DORM-4, which underwent the same digestion procedure applied to the biological samples. Recovery rates for DORM-4 also ranged from 92% to 112%. The limit of detection (LOD) was calculated from the mean concentration (MC) and standard deviation (SD) obtained from ten analytical blanks prepared together with the samples. The instrumental limit of detection (iLOD) was calculated as: $iLOD = MC + 3 \times SD$. The instrumental limit of quantification (iLOQ) was calculated as: $iLOQ = 3.3 \times iLOD$, following the recommendations of Eurachem (2020). After digestion, the resulting solutions were diluted to a final volume of 50 mL with deionized water. Analytical blanks, prepared in triplicate, underwent all sample preparation steps, together with the certified reference materials.

Statistical analysis: For all quantitative variables, data were initially evaluated to determine whether parametric or non-parametric statistical approaches were appropriate. Homogeneity of variances was assessed using Bartlett's test, whereas normality was evaluated using the Shapiro–Wilk test. Differences among group means were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test when the assumption of homogeneity of variances was met. When this assumption was violated, differences were evaluated using the Kruskal–Wallis test followed by Fisher's LSD post hoc test. For comparisons between two groups, Student's *t*-test was applied when data met the assumption of normality. Otherwise, the non-parametric Mann–Whitney test was used. Thus, differences between means or medians were evaluated according to the distributional assumptions of the data. All statistical tests adopted a significance level of 5%. Statistical analyses were performed using R software.

Results

Parasite analysis

Eleven specimens (50%) were parasitized (8 males and 3 females) by nematodes of the genus *Micropleura* Linstow, 1906 (Nematoda, Dracunculoidea), collected from the coelomic cavity (Figure 2). A total of 27 nematodes were recovered from the parasitized hosts (mean abundance of 1.23 ± 0.24 and mean intensity of infection of 2.45 ± 1.37; range: 1–4). The highest intensity of infection (four nematodes) was recorded in animals from mesohaline areas. Of the 11 parasitized animals, 10 (90.9%) were subadults (7 males and 3 females), and one was an adult male (9.1%). Neither age class ($p = 0.310$) nor sex ($p = 1$) influenced parasitism.

Only female nematodes were recovered from the parasitized animals. These females (Figure 2B,C) were identified as *Micropleura* sp. based on the presence of morphological characteristics typical of the genus (Travassos, 1933; Moravec, 2004; Moravec et al., 2006). Species of this genus are distinguished primarily by the morphological characteristics of the males, such as the morphology of the spicules and caudal papillae (Moravec, 2006). Therefore, due to the absence of male nematodes, it was not possible to confirm the identification of the collected nematodes at the species level.

A body fragment from two females belonging to different infrapopulations was used for DNA extraction targeting the 18S rDNA (Floyd et al., 2005), 28S rDNA (De Ley et al., 2005), and COI mtDNA (Folmer et al., 1994) markers. However, no suitable sequences were obtained for phylogenetic analyses.

No other species of metazoan parasites or developmental stages of hemoparasitic protists were detected in the blood smears.

None of the animals presented anatomo-histopathological lesions associated with the parasites detected, nor was the presence of bacteria, fungi, or parasitic structures identified in the tissues. However, some animals exhibited whitish livers (Figure 3A). Histopathological analysis of the livers of the 22 animals studied revealed the presence of melanomacrophages, which was classified as moderate (54.5%), mild (27.3%), and severe (18.2%). In addition, 14 (63.6%) animals presented moderate (71.4%) or severe (28.6%) hepatic lipidosis (Figure 3B,C).

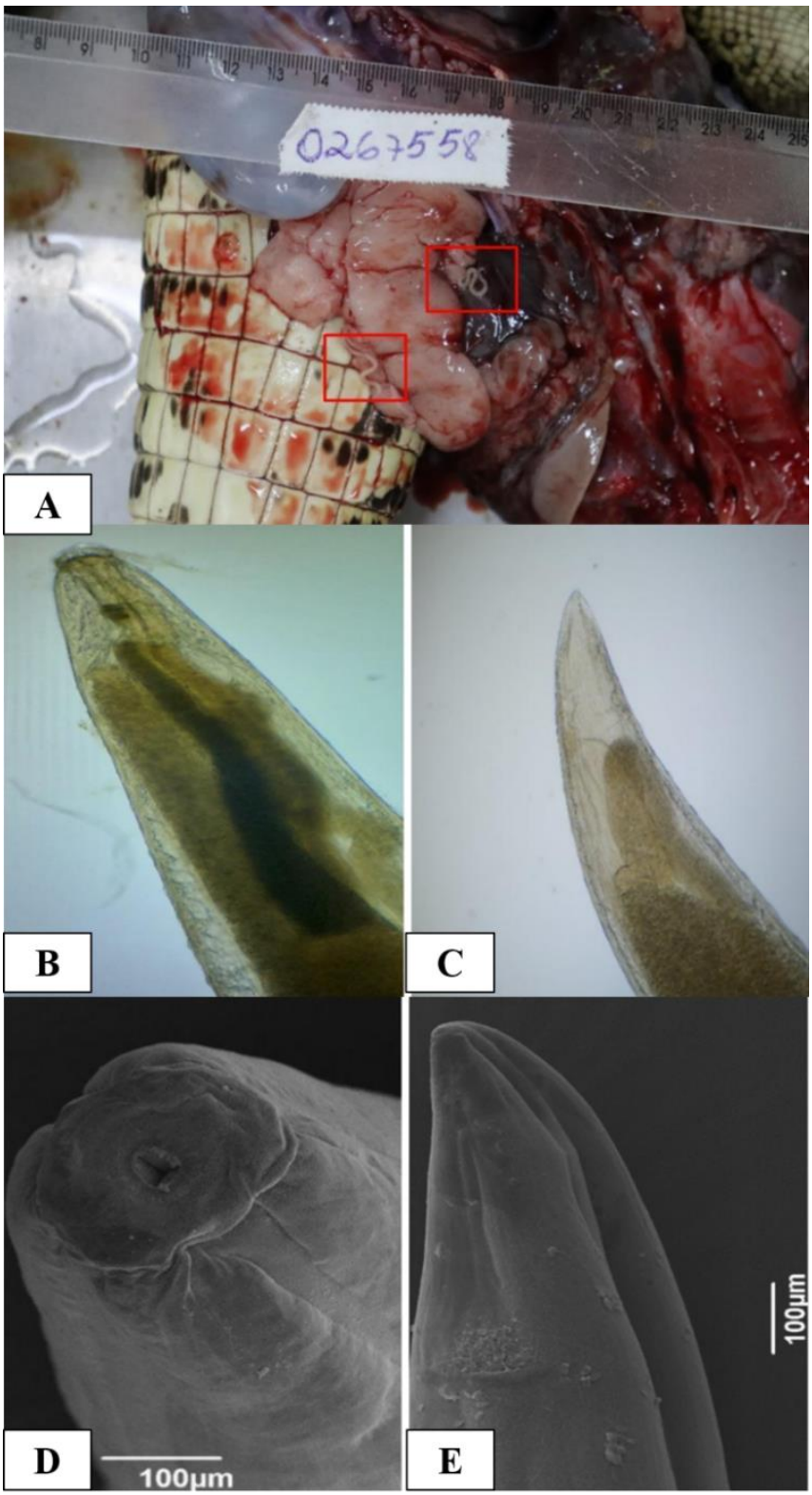


Figure 2. (A) Specimens of *Micropleura cf. vazi* (red boxes) in the coelomic cavity of *Caiman latirostris*; (B) female of *Micropleura cf. vazi*, anterior extremity and (C) posterior extremity; (D) scanning electron micrographs of a female, anterior extremity and (E) posterior extremity.

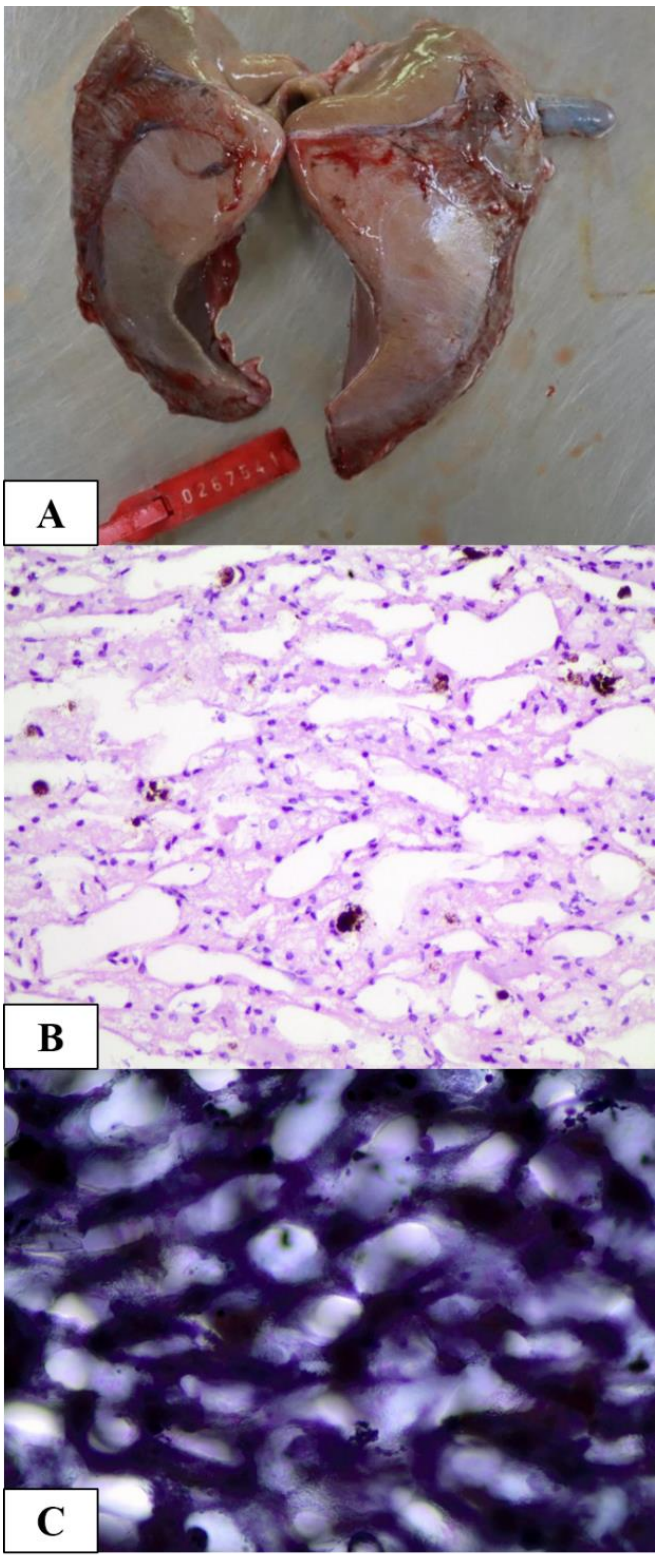


Figure 3. (A) Whitish liver of a *Caiman latirostris* specimen; (B) liver showing a moderate presence of melanomacrophages; and (C) liver with severe lipidosis.

Elemental analysis

During two expeditions conducted in January and October 2024, 15.91 km and 13.01 km were surveyed, respectively, totaling 16 hours of sampling effort and resulting in the capture of 22 individuals (11 per expedition). Among these, 19 were classified as adults and three as subadults, comprising 14 males and eight females. The mean body mass was 7,165 ± 6,616 g, and the mean snout–vent length (SVL) was 57.86 ± 14.73 cm.

A total of 88 samples of scales (n = 22), kidney (n = 22), muscle (n = 22), and liver (n = 22) were analyzed for the determination of 13 elements (Al, Cr, Fe, Mg, Co, Cu, Zn, As, Se, Cd, Ni, Hg, and Pb). Nickel (Ni) concentrations were below the detection limit in all samples, preventing its quantification; therefore, this element was excluded from the statistical analyses.

Biological matrices

Overall, Fe, Zn, Al, and Cu exhibited the highest concentrations compared with the other elements across all four analyzed biological matrices. When evaluating the main elements with the highest mean concentrations in these samples, the kidney showed the following pattern: Fe (12.25) > Zn (11.01) > Al (9.84) > Cu (9.15), followed by the liver: Fe (13.05) > Zn (10.72) > Cu (9.93) > Al (9.44), scales: Fe (12.05) > Al (9.87) > Zn (9.81) > Cu (7.55), and muscle: Zn (10.44) > Fe (10.23) > Al (9.24) > Cu (6.67) (Figure 4).

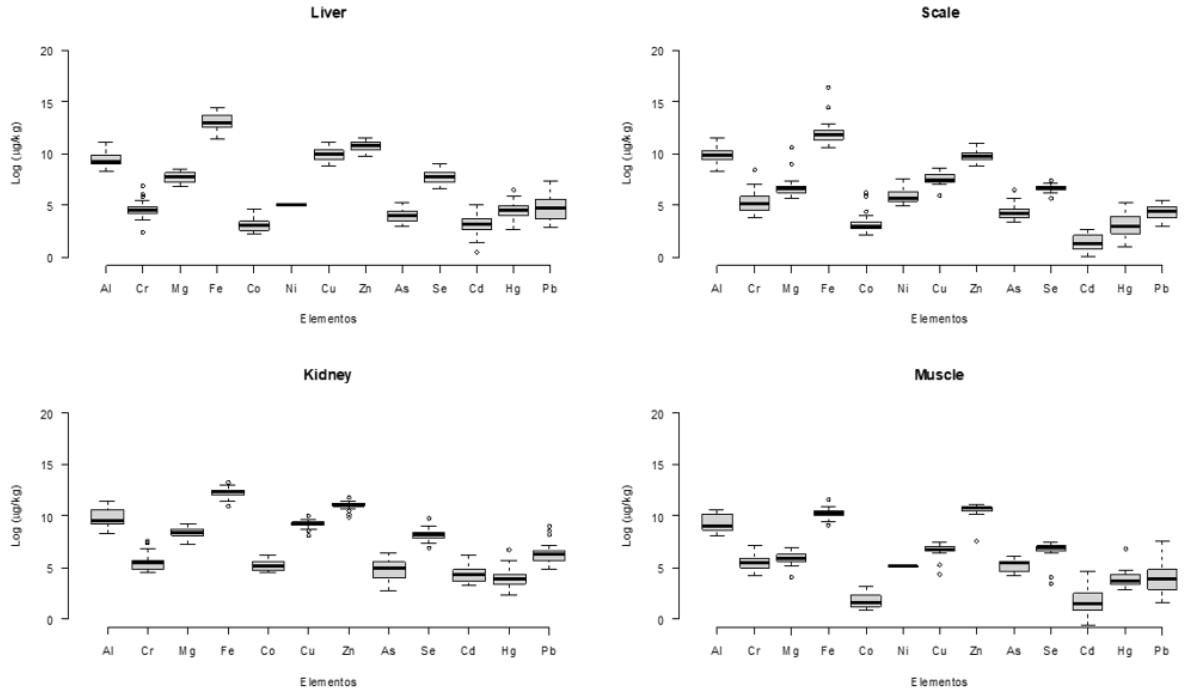
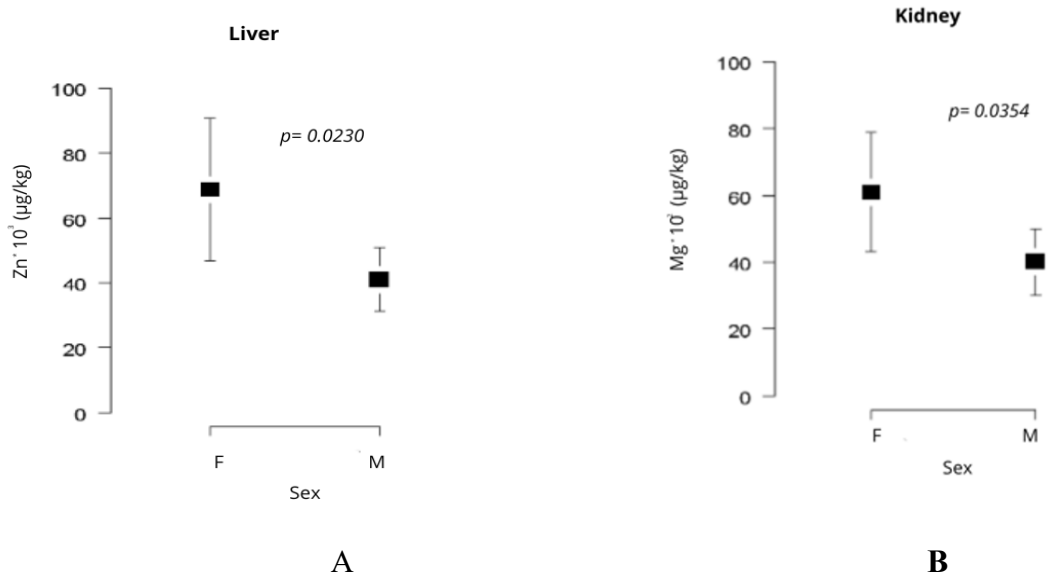


Figure 4. Mean concentrations of the detected elements: aluminum (Al), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), cadmium (Cd), arsenic (As), and zinc (Zn) in liver, kidney, scale, and muscle samples of *Caiman latirostris* captured in the urban estuary of the Capibaribe River, Brazil.

Differences between sexes

Significant differences between sexes were observed for specific elements and biological matrices. In the liver matrix, Zn showed higher mean values in females (mean ± SD) (68,685.61 ± 26,314.19 µg/kg) compared to males (41,058.08 ± 17,354.80 µg/kg) ($p = 0.0230$) (Figure 5A). Additionally, in the kidney matrix, females also exhibited higher mean concentrations of Mg (females: 6,105.95 ± 2,139.91 µg/kg; males: 4,015.45 ± 1,706.89 µg/kg) ($p = 0.0354$) (Figure 5B).



A B

Figure 5. Females exhibited higher mean Zn concentrations (p = 0.0230) in the (A) liver and higher mean Mg concentrations (p = 0.0354) in the (B) kidney of *Caiman latirostris* captured in the urban estuary of the Capibaribe River, Brazil.

Conversely, in the muscle matrix, males presented higher concentrations of Cu, Cd, and Pb compared to females. For Cu, median values were 961.30 µg/kg in males and 762.01 µg/kg in females, with mean ± standard deviation values of 1,046.93 ± 448.47 µg/kg and 699.04 ± 214.88 µg/kg, respectively (p = 0.0422). For Cd, median values were 8.24 µg/kg for males and 3.43 µg/kg for females, with mean ± standard deviation values of 21.87 ± 32.14 µg/kg and 3.06 ± 1.95 µg/kg, respectively (p = 0.0292). Finally, for Pb, males presented a median of 111.25 µg/kg and females 18.22 µg/kg, with mean ± standard deviation values of 381.70 ± 614.88 µg/kg and 24.64 ± 19.67 µg/kg, respectively (p = 0.0038) (Figure 6).

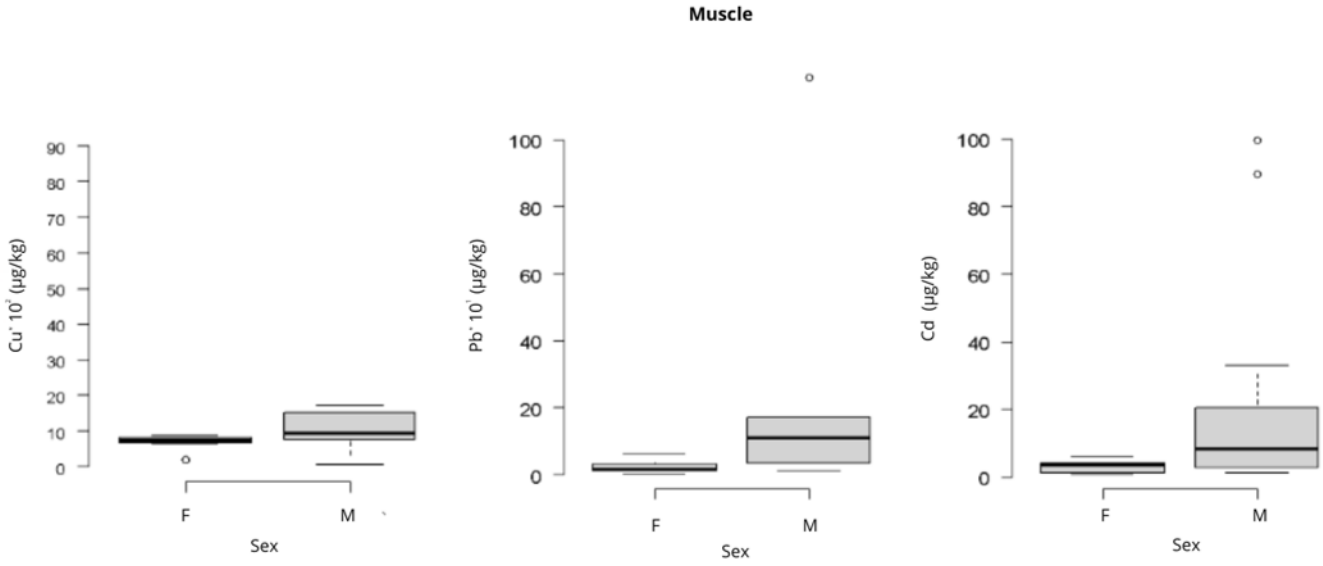


Figure 6. Median concentrations of Cu, Cd, and Pb in the muscle of female and male *Caiman latirostris* captured in the urban estuary of the Capibaribe River, Brazil, with males exhibiting higher concentrations (Cu: p = 0.0422; Cd: p = 0.0292; and Pb: p = 0.0038).

Size class

Statistically significant differences were observed between size classes. Subadult individuals exhibited higher median concentrations (µg/kg) of Mg (2782.81 vs. 1549.85; p = 0.0098), Zn (59077.39 vs. 35152.06; p = 0.0095), Se (2565.31 vs. 1429.55; p = 0.0184), and Cd (26.95 vs. 14.49; p = 0.0310) in the liver (Figure 7). In the kidney, higher median concentrations were observed for Co (201.40 vs. 142.25; p = 0.0093), Cu (11204.90 vs. 9062.71; p = 0.0477), As (159.39 vs. 55.35; p = 0.0583), Se (3711.87 vs. 2773.20; p = 0.0193), and Cd (95.45 vs. 32.56; p = 0.0477) (Figure 8). In the muscle, higher median concentrations of Al (9468.42 vs. 5632.24; p = 0.0310) and Se (1148.67 vs. 614.70; p = 0.0009) were detected (Figure 9).

In contrast, adults exhibited higher median concentrations of Mg (873.26 vs. 589.94 µg/kg; p < 0.0001) and Zn (30514.35 vs. 15854.26 µg/kg; p = 0.0246) in the scales (Figure 10).

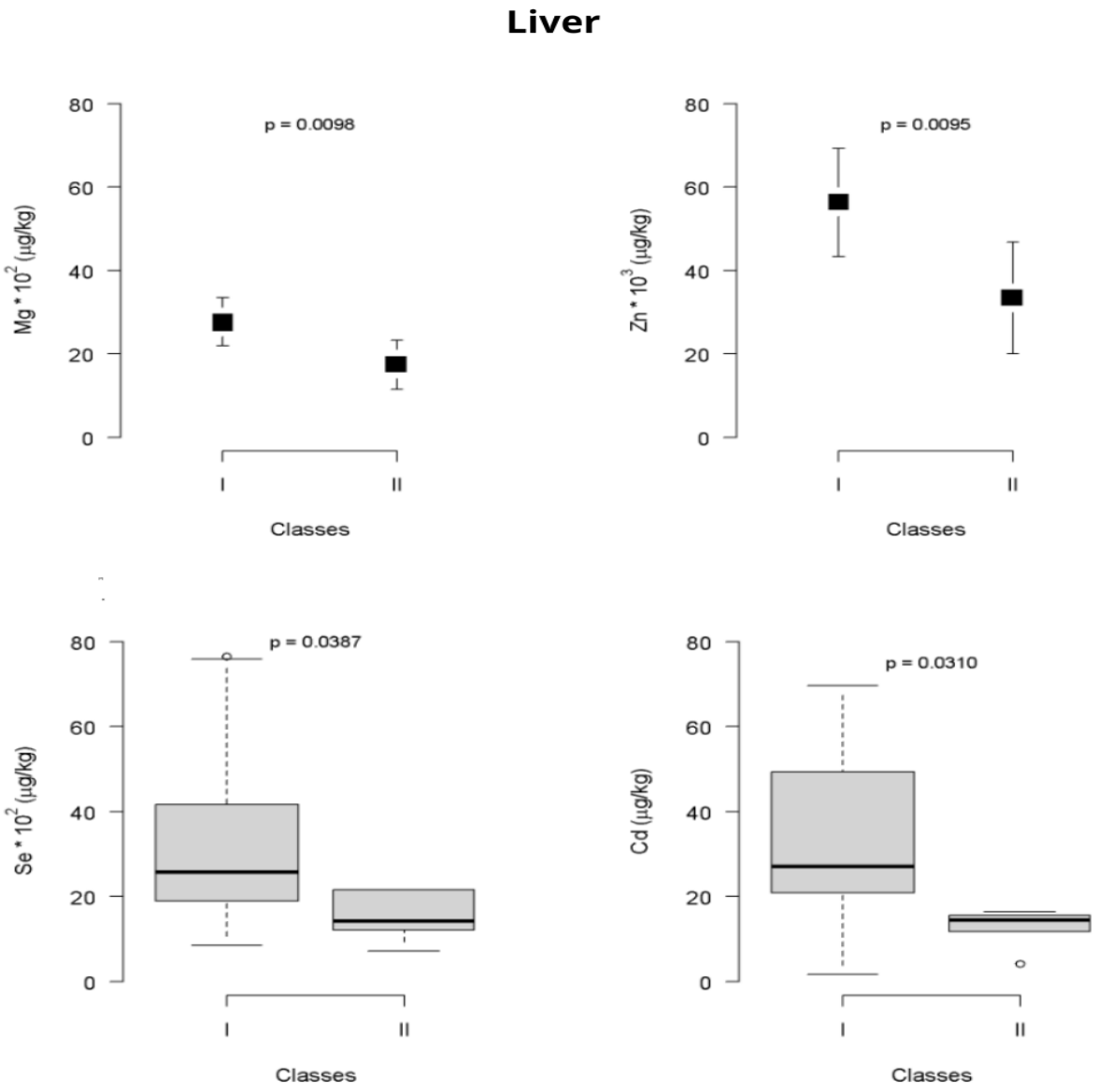


Figure 7. Mean concentrations of Mg and Zn, and median concentrations of Se and Cd in the liver of subadult (Class I) and adult (Class II) caimans captured in the urban estuary of the Capibaribe River, Brazil.

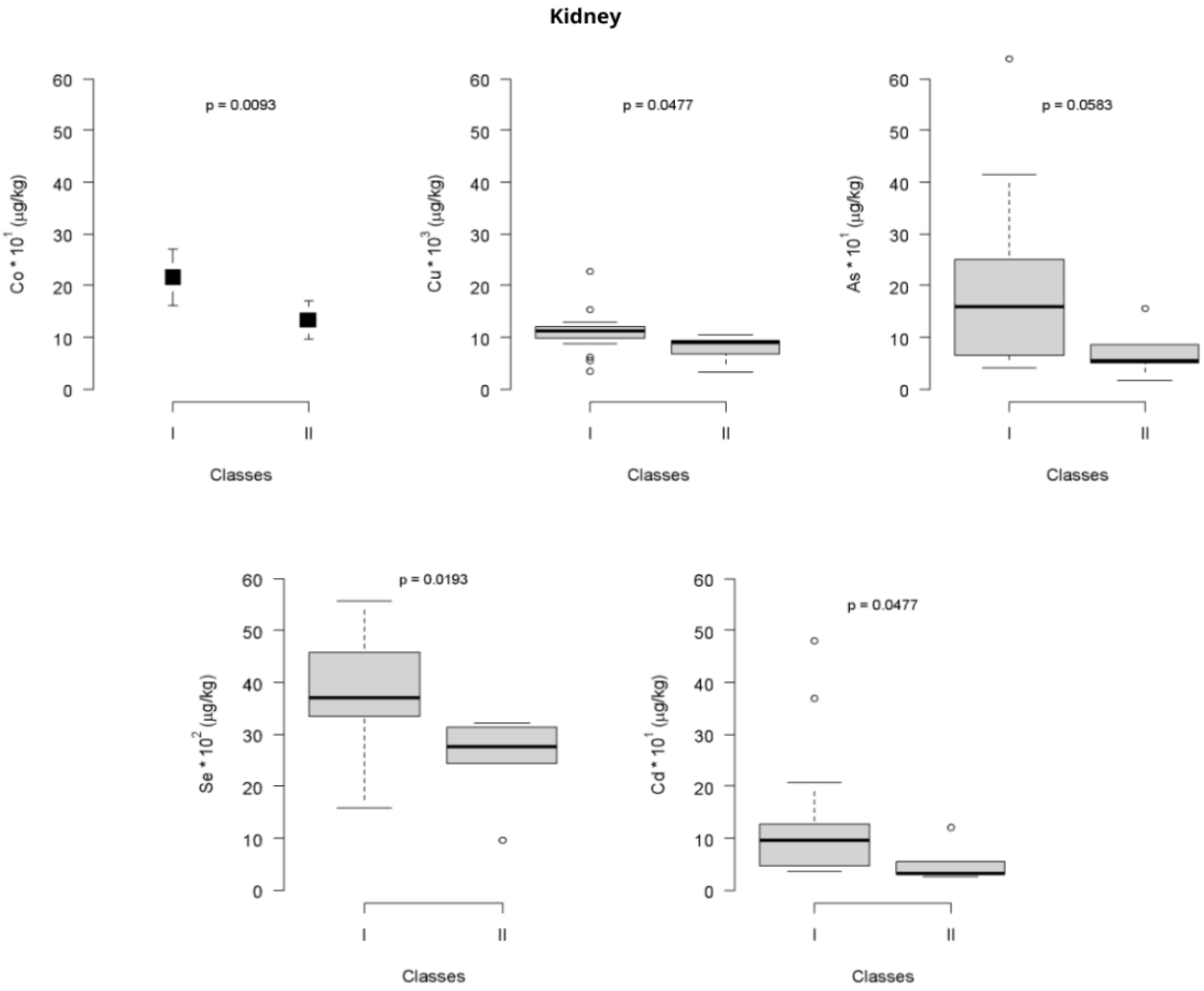


Figure 8. Mean Co concentrations and median Cu, As, Se, and Cd concentrations in the kidney of subadult (Class I) and adult (Class II) caimans captured in the urban estuary of the Capibaribe River, Brazil.

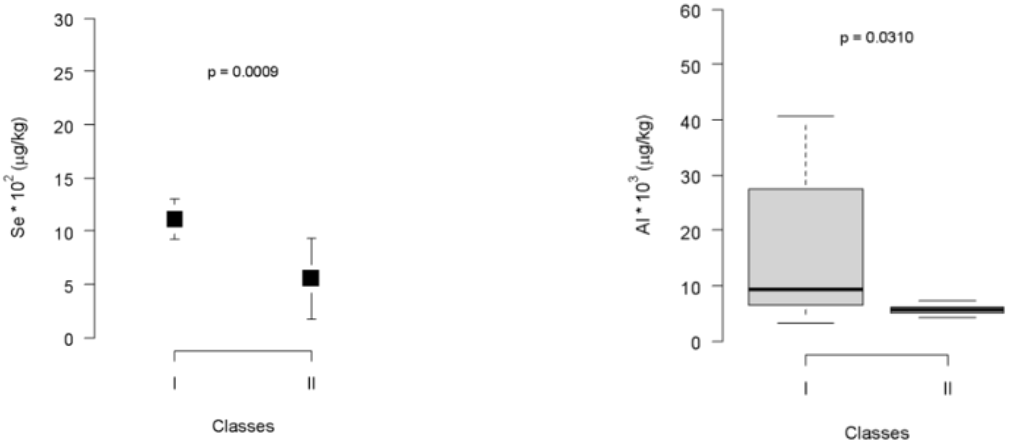


Figure 9. Concentrations of Al, Se, and Hg in subadult (Class I) and adult (Class II) caimans captured in the urban estuary of the Capibaribe River, Brazil.

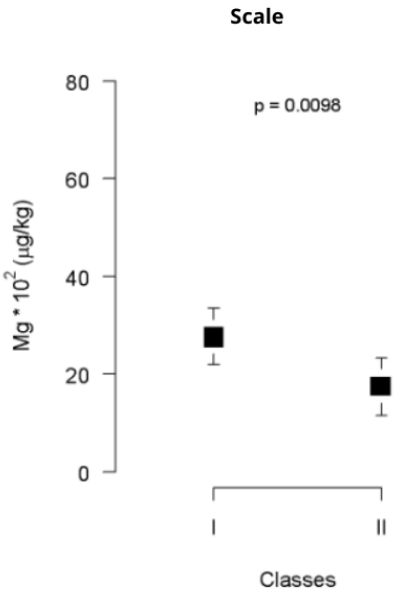


Figure 10. Mean Mg concentration in scales of subadult (Class I) and adult (Class II) caimans captured in the urban estuary of the Capibaribe River, Brazil.

Discussion

Due to the absence of male nematodes and the impossibility of obtaining suitable sequences for phylogenetic analyses, it was not possible to confirm the identification of the nematodes at the species level. Despite this, this is the first record of a *Micropleura* species parasitizing *C. latirostris*, thus expanding the known host range of these nematodes in South America.

Nematodes of the genus *Micropleura* are parasites of body cavities and serous membranes of crocodilians and turtles in South America, Africa, and Oceania (Goldberg et al., 1991; Anderson, 2000; Moravec et al., 2004, 2006). Currently, five valid species are known for this genus (Moravec, 2006; Junker and Mutafchiev, 2017), of which only *M. vazi* occurs in Brazil, parasitizing *C. crocodilus* in Atlantic Forest and Cerrado areas (Travassos, 1933; Vicente et al., 1993; Werneck and Leandro, 2021) and *C. yacare* in areas of the Pantanal (Catto and Amato, 1994; Gorza et al., 2025). In other countries, *M. vazi* has also been recorded in *C. yacare* in Paraguay (Goldberg et al., 1991), Venezuela (Moravec and Prouza, 2003), and Bolivia (Mollericona et al., 2021).

The prevalence of *Micropleura* sp. recorded in the present study is consistent with helminthological studies involving *C. crocodilus* parasitized by this nematode in the Cerrado and Atlantic Forest, although these studies analyzed a reduced number of hosts (Travassos, 1933; Vicente et al., 1993; Werneck and Leandro, 2021). When compared with parasitism by *M. vazi* in *C. yacare* in the Pantanal, the prevalence of this parasite in the current study is considerably lower than that recorded by Catto and Amato (1994), who analyzed parasitism in 64 host specimens (prevalence of 78%). However, another study reporting parasitism by *M. vazi* in *C. yacare* in the Pantanal observed an overall prevalence of 7.8% in 13 host specimens analyzed. In studies with natural populations of *C. yacare* in streams, ponds, and lakes of an Indigenous territory in Bolivia, a prevalence of 10.6% of *M. vazi* was reported, occurring only in hosts inhabiting ponds (Mollericona et al., 2021).

Crocodilians are hosts to a wide diversity of helminths, protists, and pentastomids (Catto and Amato, 1994; Batista et al., 2012; Mollericona et al., 2021); however, studies on parasites of free-ranging *C. latirostris* in Brazil are fragmented and relatively scarce, revealing a low-richness parasite fauna that includes two species of protists, one intestinal and one blood parasite, *Isospora jacarei* and *Hepatozoon caimani*, respectively (Tellez, 2013), and five species of helminths, including nematodes and digeneans. The low helminth richness

observed in *C. latirostris* in this study corroborates previous studies with this crocodilian, in which only one helminth species was also recorded in the analyzed hosts.

From an ecological perspective, low parasite richness may be related to biotic factors, such as age-associated immunity, since adult hosts may exhibit lower parasite richness and intensity (Poulin, 2007; Valenzuela-Sánchez et al., 2021), a pattern also observed in the present study. Host home range size and population density should also be considered, as they may act as density-dependent factors in parasite communities, influencing, for example, parasite aggregation (Poulin, 2007, 2013). The mean home range of *C. latirostris* in anthropized environments is 96.6 ± 183.9 ha, indicating that this species does not exhibit a migratory pattern or extensive territorial displacement; males tend to be more territorial, whereas females exhibit greater dispersal, mainly for egg laying (Batista et al., 2012; Marques et al., 2020).

There was no influence of age class or sex on parasitism, although most parasitized animals were subadults captured in mesohaline and oligohaline areas. *Caiman latirostris* does not possess salt glands, which are responsible for osmoregulation in saline environments (Taplin et al., 1985), and relies on the impermeability of its scales and urea excretion to survive in these environments (Grigg et al., 1998). Smaller animals tend to remain in freshwater environments, whereas subadults and adults exhibit greater mobility among environments with different salinities (Grigg et al., 1998).

Environmental factors may also influence the structure of parasite communities. Although some parasites can tolerate high water salinity — as is the case of *Paratrichosoma recurvum*, a monoxenous ectoparasitic nematode of *Crocodylus acutus* in the Mexican Caribbean islands (Charruau et al., 2017) — others may not tolerate salinity variations. In the present study, the highest infection intensity was recorded in animals from mesohaline areas. Farias et al. (2024) highlighted the need to expand studies on the influence of salinity on parasite communities and their association with ecotoxicological data. Sures et al. (2023) also point out this need from the perspective of Environmental Parasitology.

Parasites are health bioindicators for both their hosts and ecosystems, especially in aquatic environments, as they are as sensitive to environmental changes as the hosts themselves (Fonseca et al., 2021; Sures et al., 2023). In a study with populations of the Geoffroy's side-necked turtle *Phrynops geoffroanus* (Schweigger, 1812) (Testudines, Chelidae), also conducted in the estuary where the present study was carried out, six species of monoxenous and heteroxenous helminths were recorded (four digeneans, two nematodes, and one monogenean) (Fonseca et al., 2021). Contrary to what was recorded by Fonseca et al. (2021) in *P. geoffroanus*, the parasite richness of *C. latirostris* in the Capibaribe River estuary was very low, indicating that host-related factors may have influenced this result.

In studies conducted with gastrointestinal parasites of free-ranging *C. latirostris* in Brazil, digenean trematodes, parasites that use mollusks as primary intermediate hosts, constitute the most diverse group (Table 2). Some of these studies were conducted with hosts from less anthropized water bodies than the Capibaribe River estuary, such as the studies by Pinto and Barbosa (1972), in the state of São Paulo, and Werneck et al. (2023), in the state of Bahia (Table 2). The Capibaribe River ranks seventh among the most polluted rivers in Brazil (SoCientifica, 2022). However, neither eutrophication nor the salinity of the analyzed estuary appears to interfere with the biological cycle of *M. vazi*. This may occur because copepods, which act as intermediate hosts of nematodes of the superfamily Dracunculoidea, are organisms resistant to salinity and eutrophication in this tropical hypereutrophic estuary (Guenther et al., 2019; Choi et al., 2023).

The comparative analysis among tissues demonstrated that the kidney and liver presented the highest mean concentrations of microminerals, corroborating their central role in the processes of detoxification and elimination of toxic substances (Cortés, 2016; Siroski et al., 2023). A similar pattern was observed by Buenfil-Rojas et al. (2026) in Crocodylus moreletii, with greater metal accumulation in these organs.

Scales presented intermediate concentrations, reflecting their function as a historical record of exposure and a secondary detoxification pathway through keratinization (Nilsen et al., 2017), which justifies their widespread use in ecotoxicological studies with crocodilians (Buenfil-Rojas et al., 2020; Santos et al., 2021; Thirion et al., 2022). In contrast, muscle tissue presented the lowest mean concentrations, in agreement with the results reported by Salazar-Pammo et al. (2021) for C. yacare.

In the present study, differences in mineral concentrations between sexes were observed, with females presenting higher concentrations of Zn in the liver and Mg and Cu in the kidney. This pattern may be related to the higher metabolic demand during the reproductive cycle, since the liver plays a central role in the storage and mobilization of metals essential prior to oviposition (Jose et al., 2018). Zn is essential for vitellogenesis

and embryonic development, making its maternal transfer fundamental for reproductive success (Eisler, 2000; Grillitsch & Schiesari, 2015).

The higher Mg concentrations in the kidneys of females may also be associated with its reabsorption and mobilization for eggshell formation (Elejalde-Cadena et al., 2025). In A. mississippiensis, the kidney is recognized as an important site of metal accumulation due to its high vascularization (Nilsen et al., 2017). In C. latirostris, exposure to metal contaminants has already been associated with alterations in embryogenesis, reduced hatching rates, and egg mass loss (Poletta et al., 2008), demonstrating the potential impact of these elements on the reproductive success of the species.

Regarding age class, significant differences were observed ($p < 0.05$), with subadult individuals presenting higher concentrations of Mg, Zn, Se, and Cd in the liver; Co, Cu, As, Se, and Cd in the kidney; and Al, Se, and Hg in the muscle. Similar results were described by Schneider et al. (2013) for A. mississippiensis, in which juveniles presented higher concentrations of Zn and Cu compared to adults. This pattern may be explained by the higher metabolic rate and intestinal assimilation efficiency during growth, which favor the absorption of nutrients and trace elements (Schneider et al., 2013; Tracy et al., 2015), in addition to the foraging behavior in shallow waters, where the consumption of benthic macroinvertebrates associated with contaminated sediments may intensify bioaccumulation (Nilsen et al., 2017; Buenfil-Rojas et al., 2020).

In contrast, adult individuals presented higher concentrations of Mg and Zn in scales, corroborating the results of Barbosa (2023) for C. latirostris in the Metropolitan Region of Recife. This pattern reinforces that keratinized tissues, such as scales, reflect chronic exposure to contaminants (Nilsen et al., 2017), whereas the liver and kidney are more indicative of recent exposure. Thus, the higher concentrations observed in the scales of adult individuals likely reflect their longer lifespan and, consequently, the more prolonged period of exposure to environmental contaminants (Buah-Kwofie et al., 2018; Santos et al., 2021; Barbosa, 2023).

Conclusion

The low parasitic richness observed in Caiman latirostris highlights the need to expand knowledge about the effects of biotic and abiotic factors on the parasite fauna of natural crocodilian populations in eutrophic environments in the Neotropical region. These results reinforce the importance of integrating health and conservation strategies for continental aquatic ecosystems and associated biota from the perspective of the One Health approach.

The bioaccumulation patterns observed demonstrate marked differences among the analyzed tissues, with higher concentrations of several elements in the kidney, consistent with the physiological role of this organ in the processes of contaminant storage and elimination. The differences between males and females suggest that physiological factors, possibly related to the reproductive cycle, also influence the accumulation dynamics of certain trace elements. Together, these results reinforce the potential of C. latirostris as a sentinel species for biomonitoring trace element contamination in tropical estuaries subjected to urbanization and intense anthropogenic influence.

The combined use of ecotoxicological and parasitological indicators expands the understanding of the effects of environmental contamination on organisms at the top of the food chain and represents a promising approach for integrated environmental quality assessments. From the perspective of One Health, future studies employing these approaches may provide more robust scientific bases for biomonitoring programs, support conservation strategies, and assist in the sustainable management of Neotropical estuarine ecosystems.

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