

Soil Pb and Cd accumulation and age-dependent biochemical responses in *Crocodylus moreletii*: Evidence of chronic contamination in a protected urban lagoon

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Abstract

Laguna de las Ilusiones (LI) is an urbanized, hydrologically restricted lagoon in Tabasco, Mexico, subject to sustained anthropogenic pressure. This study evaluates lead (Pb) and cadmium (Cd) concentrations in soil and water and their association with biochemical biomarkers in a wild population of *C. moreletii*. Environmental samples were analyzed by atomic absorption spectrophotometry, and blood samples were collected to assess cholinesterases (AChE, BuChE) and hepatic enzymes (AST, ALT, GGT), using a captive population as reference. Soil Pb and Cd concentrations were significantly higher than in water and correlated with organic matter and pH. Wild crocodiles showed significantly elevated AChE, BuChE, AST, and ALT activities relative to the reference group ($p < 0.05$), independent of sex but varying across age classes, suggesting ontogenic modulation of physiological responses. Although direct causal relationships cannot be established, the co-occurrence of soil metal enrichment and altered enzymatic profiles indicates chronic environmental stress, underscoring the value of sentinel species biomarkers for long-term contaminant monitoring in tropical urban wetlands.

INTRODUCTION

The intensification of anthropogenic activities over the last century has resulted in a significant increase of persistent contaminants in freshwater ecosystems, among them, lead (Pb) and cadmium (Cd) are recognized as priority toxic metals due to their persistence, non-biodegradability, and bioaccumulation capacity (**Doadrio-Villarrejo, 2006; Acosta de Armas & Montilla-Peña, 2011**). Although both elements occur naturally, urban runoff, industrial emissions, hydrocarbon extraction, wastewater discharge, and atmospheric deposition have enhanced their mobilization and environmental bioavailability in freshwater environments (**Ramírez, 2002**), particularly in tropical developing regions where rapid urban expansion frequently outpaces environmental management. Urban lagoons represent particularly vulnerable systems due to restricted hydrological exchange that promotes long-term retention of contaminants in bottom soils and their subsequent incorporation into food webs. Soils function both as sinks and potential secondary sources of metals, depending on physicochemical conditions such as pH, redox potential, organic matter content, and dissolved oxygen dynamics (**Eggleton & Thomas, 2004**). Pb and Cd may precipitate or adsorb organic and mineral particles in alkaline systems, favoring their accumulation in the benthic compartment while maintaining the potential for remobilization under fluctuating environmental conditions. Consequently, organisms inhabiting such systems may experience chronic, low-level exposure through trophic transfer and soil interaction.

Biochemical biomarkers are sensitive tools for the detection of physiological sublethal alterations before the onset of overt pathology. Enzymes associated with hepatic metabolism and detoxification, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP), are widely used in vertebrates as indicators of hepatocellular membrane integrity and metabolic disturbance (**Ozer et al., 2008**). Cholinesterases, including acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), are traditionally employed as neurotoxicity biomarkers but are also responsive to systemic oxidative and inflammatory stress (**Richetti**

et al., 2011; Lionetto et al., 2013). However, the interpretation of enzymatic biomarkers in reptiles requires caution. Reptiles differ substantially from endothermic vertebrates in terms of physiological characteristics and contaminant retention times that influence both baseline biochemical values and toxicokinetic responses (**Campbell, 2006**). In crocodilians, available reference intervals remain limited compared to other animals, and physiological variation associated with age, season, and reproductive status can be considerable (**Hamilton et al., 2016; Brandt et al., 2022**).

Crocodilians are particularly suitable sentinel species for monitoring long-term contamination. As apex predators, they integrate contaminant signals across multiple trophic levels, providing insight into the dynamics of ecosystem-level pollution (**Buenfil-Rojas et al., 2022**). Their longevity, high trophic position, and strong site fidelity allow for the integration of contaminant exposure across spatial and temporal scales (**Hale, 2029; Lemaire, 2023**). Morelet's crocodile (*Crocodylus moreletii*) is widely distributed in southeastern Mexico and Central America, occupying urban and semi-urban wetlands where contaminant pressures can be substantial. Metal accumulation and endocrine disruption have been documented in crocodilians exposed to environmental pollutants (**Rainwater et al., 2007; 2008; Trillanes et al., 2014; Buenfil-Rojas et al., 2022**). However, enzymatic biomarker responses in wild populations remain poorly characterized, particularly with respect to ontogenetic variation.

Laguna de las Ilusiones (LI), an urban and hydrologically restricted lagoon located in Villahermosa, Tabasco, Mexico that represents a system subjected to sustained anthropogenic influence, including hydrocarbon-related activities, urban runoff, and wastewater inputs (**Mendez, 2000**). Despite its designation as a protected ecological reserve, the lagoon exhibits conditions conducive to contaminant retention. Given the ecological relevance of *C. moreletii* within this system, evaluating sublethal physiological responses in relation to environmental metal burdens is critical for understanding potential ecosystem-level impacts. In this context, the present study aimed to (1) quantify Pb and Cd concentrations in water and soil from LI and (2) evaluate associated enzymatic biomarkers of hepatic and systemic stress in a free-ranging population of *C. moreletii*, considering sex and age-related variation and comparison with a captive reference population maintained under controlled conditions. This study assesses the suitability of this species as a sentinel species for monitoring sublethal heavy metal exposure in urban tropical wetlands by integrating environmental chemical characterization with physiological biomarkers.

MATERIALS AND METHODS

Study Sites and Environmental Characterization

Laguna de las Ilusiones (LI), a 259.7-hectare urbanized lagoon, and the Investigation Center for Endangered Species (CICEA), a 2-hectare facility dedicated to the conservation of *C. moreletii*, both in Villahermosa, Tabasco (**Gobierno del Estado de Tabasco, 2000**). Villahermosa (17°59'N, 92°55'W; ~10 m a.s.l.) is characterized by a humid tropical climate with a mean annual temperature of 27°C and high seasonal precipitation (~ 2,550 mm). The landscape is dominated by fluvial systems and lagoons under

intense anthropogenic pressure. Primary activities include hydrocarbon extraction (Petróleos Mexicanos, PEMEX) and agro-industrial runoff. Rapid urban expansion contributes to significant contamination in local wetlands (**Mendez, 2000; Huang et al., 2017; Islam et al., 2015**).

Environmental samples

In 2010, 40 samples (20 soil and 20 surface water) were obtained from LI. Temperature, pH, and dissolved oxygen (DO) were measured *in situ* using an oximeter (YSI-54) and a digital potentiometer (HANNA Basic HI 98107). Water samples were stored in nitric acid-rinsed high-density polyethylene containers, and soil samples were collected from the topsoil layer and sealed in acid-rinsed plastic bags. All samples were transported to the laboratory at 4°C.

- **Water analysis:** Water samples were filtered (0.45 µm pore cellulose acetate membrane), acidified with nitric acid (2%), and analyzed in a graphite furnace atomic absorption spectrometer (GFAAS) at 217 nm for Pb and 228.8 nm for Cd, with duplicate sample analyses Method Nos. 7420 and 7421 (**EPA, 2001**).
- **Soil analysis:** Samples were dried, sifted (2mm sieve), and ground (5 g). Samples were analyzed for organic matter (NOM-147-SEMARNAT/SSA 1-2004) and digested according to the Environmental Protection Agency's (EPA) Method 3051A. For Pb and Cd concentrations (ISO 5961:1994 and ISO 11047:1998), we used the Standardized Flame Atomic Absorption Spectrometry method (Varian Spectra A 220 model) (**USDA, 2000**).

Blood sampling and analysis

A total of 36 organisms (10 yearlings, 4 juveniles (2 females and 2 males), and 22 adults (12 females and 10 males) were captured in LI, and similarly in CICEA during the mating and hatching season. We assigned age based on total length and weight and sexed the organisms via cloacal palpation. We obtained blood by puncturing the occipital sinus with plastic syringes previously washed with EDTA-K2, and we maintained the samples in heparinized Vacutainers at 4°C.

Gama-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), aspartate (AST), and alanine (ALT) transaminases were assessed using Reflotron analysis (**Deneke et al., 1985**). At the same time, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were measured by Ellman's method (**Ellman et al., 1961**). Finally, we measured albumin with the green bromocresol method (**Rodkey, 1964**) and total protein with the Biuret method (**Savory et al., 1968**).

Statistical analysis

The statistical significance of environmental parameters (Pb and Cd concentrations) and biological markers (enzymatic activity) was evaluated using one-way ANOVA, with differences assessed across sex, age classes, and study sites. Additionally, a multivariate analysis of variance (MANOVA) (Kruskal-

Wallis and MKruskal-Wallis respectively for non-normal data) was performed to determine the significance of interactions among the environmental and physiological variables. All statistical analyses were conducted using GraphPad Prism (version X.X; GraphPad Software, USA), with an alpha level of 0.05.

RESULTS

3.1 Spatial distribution of metals and environmental conditions

Spatial mapping revealed heterogeneous distribution of soil Pb and Cd concentrations across LI (Map 1). Elevated Pb concentrations (81,500–130,000 ppm) were primarily detected in the central lagoon sector, whereas the highest Cd levels (24,412–58,587 ppm) were concentrated near recreational and high-traffic areas. All sites exceeded the international soil quality guideline for Cd (3,500 ppm), and 60% exceeded the Pb threshold (91,300 ppm) (Table 2). Descriptive statistics for physicochemical parameters are summarized in Table 1. Water temperature was consistently high (31.6–34.6°C), and pH values were uniformly alkaline (mean $\approx$ 9.0). Dissolved oxygen ranged from hypoxic (1.8 mg L⁻¹) to supersaturated conditions (17 mg L⁻¹), indicating localized redox variability despite hydrological isolation. Soil organic matter (OM) ranged from 0.65% to 11.87%, with 55% of sites classified as enriched.

Metal concentrations in water were comparatively lower than in soils (Table 2), although several sites met or exceeded national regulatory limits (NOM-001-SEMARNAT-2021). Site-specific physicochemical and metal concentration data are provided in Supplementary Tables 1 and 2. Multivariate analyses identified significant associations between environmental variables and metal concentrations (Table 3). Soil Pb concentrations were significantly influenced by OM content ($F = 28.01$, $p < 0.001$) and temperature ($F = 156.33$, $p = 0.006$), whereas soil Cd was significantly associated with OM ($F = 19.38$, $p < 0.001$). In water samples, Pb concentrations were significantly related to pH and temperature ($p < 0.01$), while Cd concentrations were significantly associated with OM and pH ($p < 0.01$).

Cross-compartment analyses revealed significant interactions between soil and water matrices (Table 3), particularly between soil Cd and aqueous Pb ($F = 10.28$, $p = 0.002$), suggesting active geochemical coupling between benthic and water column compartments.

3.2 Plasma protein biomarkers

Plasma protein concentrations varied according to site and age class (Fig. 3). Three-way ANOVA results are summarized in Table 4. Albumin levels were significantly influenced by site ($F = 5.95$, $p < 0.023$) and age ($F = 10.80$, $p < 0.001$), with individuals from LI consistently exhibiting lower albumin concentrations relative to the CICEA reference population. No significant site $\times$ age interaction was detected. Total protein concentration was significantly associated with age ($F = 6.94$, $p < 0.005$), but not with site or sex (Table 4). Juveniles and yearlings generally exhibited higher total protein concentrations than adults, regardless of site.

3.3 Cholinesterase activity

Cholinesterase activity exhibited marked age-dependent patterns (Fig. 2; Table 4). AChE activity was significantly influenced by site ($F = 8.85$, $p < 0.007$), sex ($F = 6.05$, $p < 0.022$), and age ($F = 176.00$, $p < 0.001$), with significant site $\times$ age interactions ($F = 22.49$, $p < 0.001$). Yearlings from LI exhibited the highest AChE activity, whereas CICEA yearlings displayed comparatively lower levels. BuChE activity was primarily influenced by age ($F = 44.46$, $p < 0.001$), with a significant site $\times$ age interaction ($F = 4.35$, $p < 0.026$), but no independent site or sex effects (Table 4). Across all groups, AChE activity exceeded BuChE activity. Interpretation of juvenile data ($n = 2$ per group) should be approached cautiously, although observed trends were consistent with patterns in older cohorts.

3.4 Hepatic enzyme activity

Hepatic enzyme activity differed significantly between sites (Fig. 3; Table 4). AST and ALT activities were significantly influenced by site ($p < 0.001$ for both) and age ($p < 0.01$), with significant site $\times$ age interactions ($p < 0.05$). Individuals from LI exhibited consistently elevated AST and ALT activities comparative to the CICEA population. In contrast, ALP and GGT activities were not significantly associated with site, sex, age, or their interactions (full model results provided in Supplementary Table 4). Overall, biochemical variation was primarily driven by habitat and ontogenetic stage, whereas sex effects were limited to AChE activity.

DISCUSSION

The present findings indicate that LI functions as a soil-dominated sink for Pb and Cd, with metal enrichment strongly associated with organic matter content and alkaline conditions. Such distribution patterns are consistent with the well-established affinity of divalent metals for humic substances and fine particulate fractions in freshwater systems (**Eggleton and Thomas, 2004**). As a hydrologically isolated urban lagoon, LI receives contaminants through multiple pathways, including urban runoff, atmospheric deposition, and untreated wastewater discharges (**Galvao & Corey, 1987; EPA, 2001**), generating sustained anthropogenic pressure. Collectively, these inputs reinforce chronic environmental loading and explain the observed persistent accumulation in bottom soils. The positive association between organic matter (OM) and soil metal concentrations further supports the role of OM as a key regulator of metal sequestration and long-term persistence in tropical freshwater systems (**Doadrio-Villarreal, 2006; Núñez-Chavez, 2007; Acosta de Armas & Montilla-Peña, 2011**).

The alkaline pH (~ 9.3) recorded in LI likely enhances metal retention through adsorption and precipitation mechanisms, including the formation of Pb and Cd hydroxycarbonates and oxyhydroxides (**Rodríguez-Mellado & Marín-Galván, 1999; USDA, 2000; Li et al., 2013; Islam et al., 2015**), thereby promoting deposition within the benthic compartment. Although such geochemical conditions may temporarily reduce dissolved concentrations, they simultaneously create a persistent soilary reservoir susceptible to remobilization. Diurnal shifts between photosynthetic oxygenation and nocturnal hypoxia may alter metal speciation and binding with sulfur-containing ligands, facilitating periodic fluctuations in

bioavailability (**Rodríguez-Mellado & Marín-Galván, 1999; Benoff et al., 2002; Li et al., 2013; Huang et al., 2017**). Collectively, these dynamics support the interpretation of LI as a metal-retaining system capable of sustaining chronic low-level exposure.

Long-lived apex predators may experience cumulative exposure primarily through trophic transfer rather than direct waterborne uptake despite comparatively lower aqueous concentrations. Crocodilians integrate contaminant burdens over extended temporal scales due to their longevity and site fidelity (**Guillette et al., 2000; Selcer, 2005; Rainwater et al., 2007**). Therefore, even moderate but sustained environmental burdens may be biologically meaningful. Although tissue metal concentrations were not quantified in this study, established toxicological pathways for Pb and Cd provide biological plausibility for the enzymatic modulation observed, including disruption of enzymatic processes, oxidative stress, and altered protein synthesis (**Ramírez, 2002; Benoff et al., 2002**).

The enzymatic profiles of LI crocodiles differed consistently from those of the captive reference population. Elevated AST and ALT activities are commonly interpreted as indicators of altered membrane permeability or hepatocellular stress rather than definitive structural damage (**Ozer et al., 2008; Bogan & Mitchell, 2014**). Concurrent reductions in albumin and total protein levels may indicate broader systemic involvement, potentially associated with altered hepatic function or renal compromise (**Honeyfield et al., 2008**). However, given the physiological variability of reptiles and the limited availability of species-specific baseline values (**Campbell, 2006**), these biomarkers should be interpreted cautiously as indicators of physiological modulation rather than definitive evidence of pathology. In this context, the observed biochemical patterns are consistent with environmentally associated stress and potential hepatic impairment, rather than conclusive evidence of chronic liver damage.

Cholinesterase responses further suggest systemic biochemical modulation. Although AChE inhibition is traditionally associated with neurotoxicity, altered cholinesterase activity has also been reported under oxidative stress and heavy metal exposure scenarios (**Lionetto et al., 2013**). The age-dependent pattern observed in LI indicates that ontogenetic stage may influence metabolic sensitivity, detoxification capacity, or exposure dynamics. Differences in growth rate, diet, and habitat use across life stages may contribute to variability in contaminant assimilation and physiological responses. The age-dependent patterns identified here highlight the importance of incorporating ontogenetic structure into ecotoxicological assessments in long-lived reptiles, a factor that remains underexplored in crocodilian biomonitoring studies.

Enzymatic variation was more strongly associated with site and age than with sex, underscoring the role of environmental context and ontogenetic stage as primary drivers of physiological modulation. Similar age-related variability in biochemical parameters has been described in crocodilian species, including *Alligator mississippiensis* (**Schoeb et al., 2002; Bogan & Mitchell, 2014**). Increased cholinesterase activity in certain age classes may reflect compensatory responses to prolonged oxidative or inflammatory stress (**López-Hernández et al., 2015**), whereas reduced activity in younger individuals could indicate heightened developmental vulnerability.

Although soil metal enrichment and enzymatic alterations co-occur in this system, the absence of tissue-specific metal quantification precludes direct attribution of the observed biochemical responses to Pb and Cd exposure. Furthermore, organisms in urban aquatic systems are rarely exposed to single contaminants, rather, they are exposed to complex mixtures that may include hydrocarbons and other trace elements. Therefore, the observed biochemical responses likely reflect cumulative or interactive effects of multiple environmental stressors rather than isolated metal toxicity. Additional exposure to organochlorines and organometallic compounds previously reported in tropical urban systems (**Oliveira et al., 2015**) may further contribute to mixture-driven effects that diverge from single-metal toxicity profiles.

The convergence of elevated soil metal loads and measurable physiological modulation in a long-lived apex predator highlights the chronic contamination status of this protected urban lagoon from an ecosystem perspective. Crocodilians function as integrative biomonitors due to their trophic position, longevity, and site fidelity (**Manolis et al., 2002; Tellez & Merchant, 2015; Dos Santos et al., 2021; Lemaire, 2023**). The age-dependent enzymatic patterns identified in this study emphasize that population-level evaluations of contaminant impact should explicitly account for ontogenetic structure. Overall, the integration of soil geochemistry and biochemical biomarkers provides complementary insight into the dynamics of chronic contaminants in tropical urban wetlands. Future investigations incorporating tissue metal quantification, histopathology, and mixture-oriented approaches will be essential to refine exposure pathways and clarify long-term implications on the health of the crocodile population.

Limitations

This study presents several limitations that should be considered when interpreting the results. Environmental and biological sampling was conducted in 2010, and although these data provide valuable baseline information on contaminant dynamics in LI, current environmental conditions may have changed due to continued urbanization and management practices. In addition, metal concentrations were quantified in environmental matrices but not in crocodile tissues, limiting the ability to establish direct causal relationships between exposure and physiological response. The use of a captive population as a reference group introduces potential confounding factors (differences in diet, activity, and stress), which may influence biochemical parameters independently of contaminant exposure. Moreover, the limited sample size in certain age classes, particularly juveniles, reduces statistical power and constrains interpretation of ontogenetic patterns. Finally, the focus on Pb and Cd does not capture the complexity of contaminant mixtures characteristic of urban systems. Consequently, the findings should be interpreted as indicative of environmentally associated physiological modulation rather than definitive evidence of metal-specific toxicity.

Despite these limitations, integrating environmental geochemistry with physiological biomarkers offers valuable insight into chronic exposure dynamics in tropical urban wetlands. The age-dependent responses observed reinforce the value of crocodilians as sentinel species. Future work should

incorporate tissue-specific contaminant burdens, histopathological analyses, and mixture-based approaches to clarify exposure pathways and population-level implications.

CONCLUSIONS

LI functions as a geochemically regulated, soil-dominated system in which organic matter content and alkaline conditions favor the accumulation of Pb and Cd. The observed differences in enzymatic profiles between crocodiles from the urban lagoon and the reference population are consistent with environmentally associated physiological stress and potential hepatic modulation. Biochemical responses varied more strongly with site and ontogenetic stage rather than sex, highlighting the importance of incorporating demographic structure into ecotoxicological assessment of long-lived reptiles. However, given the absence of tissue-specific contaminant data and the complexity of exposure to multiple stressors in urban systems, these findings should be interpreted as indicative rather than definitive evidence of metal-specific toxicity. Future research integrating tissue burdens, histopathology, and mixture-oriented contaminant profiling will be essential for clarifying mechanistic pathways and population-level consequences.

Declarations

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

FUNDING AND CONFLICT OF INTEREST

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Tables

Table 1. Environmental parameters and metal concentrations in water and sediments from Laguna de las Ilusiones (n = 20 sites).

Variable	Matrix	Mean $\pm$ SD	Range
Temperature ($^{\circ}\text{C}$)	Water	33.23 ± 1.02	31.6 – 34.6
pH	Water	9.28 ± 0.35	8.9 – 10.0
Dissolved Oxygen (mg L^{-1})	Water	7.78 ± 3.38	1.8 – 17.0
Organic Matter (%)	Sediment	4.90 ± 3.55	0.65 – 11.87
Pb (mg kg^{-1})	Sediment	$92,018.75 \pm 23,479.70$	81,500 – 130,000
Cd (mg kg^{-1})	Sediment	$36,744.65 \pm 8,593.30$	24,412 – 58,587
Pb (mg L^{-1})	Water	1.72 ± 2.13	0.30 – 9.44
Cd (mg L^{-1})	Water	0.063 ± 0.042	0.029 – 0.206

Table 2. Spearman correlations (ρ) between environmental parameters and Pb and Cd concentrations.

Variable	Pb (Soil)	Cd (Soil)	Pb (Water)	Cd (Water)	df
pH	ns	ns	0.72*	0.65*	9
Dissolved Oxygen	ns	ns	ns	ns	18
Organic Matter	0.81*	0.74*	ns	0.58*	19
Temperature	0.69*	ns	0.66*	ns	17

*** $p < 0.05$**

ns = not significant

df= degrees of freedom

Table 3. Interaction of Pb and Cd concentrations in water and soil samples (χ^2).

Cross compartment relationship			
	df	F	p
Soil Pb + Water Cd	16	1.45	0.428
Soil Pb + Water Pb	16	1.39	0.445
Soil Cd + Water Cd	12	8.67	0.004
Soil Cd + Water Pb	12	10.28	0.002
Water Pb + Water Cd	16	8.81	0.049
Soil Pb + Soil Cd	12	17.01	0.999

df= Degrees of freedom

Table 4. Effects of site, sex, and age on biochemical biomarkers in *Crocodylus moreletii* (three-way ANOVA).

Biomarker	Factor	df	F	p-value
Albumin	Site	1	5.95	0.023
	Sex	1	0.86	0.362
	Age	2	10.8	<0.001
Total Protein	Site	1	0.68	0.418
	Sex	1	0.07	0.789
	Age	2	6.94	0.004
AChE	Site	1	8.85	0.007
	Sex	1	6.05	0.022
	Age	2	176	<0.001
BuChE	Site	1	0.82	0.377
	Sex	1	0.22	0.741
	Age	2	44.46	<0.001
AST	Site	1	23.15	<0.001
	Sex	1	0.6	0.445
	Age	2	9.09	0.001
ALT	Site	1	26.73	<0.001
	Sex	1	0.44	0.515
	Age	2	7.19	0.003
GGT	Site	1	0.11	0.74
	Sex	1	0.11	0.74
	Age	2	0.18	0.836
ALP	Site	1	0.01	0.938
	Sex	1	0.08	0.785
	Age	2	2	0.157

Maps

Map 1 is available in the Supplementary Files section.

Figures

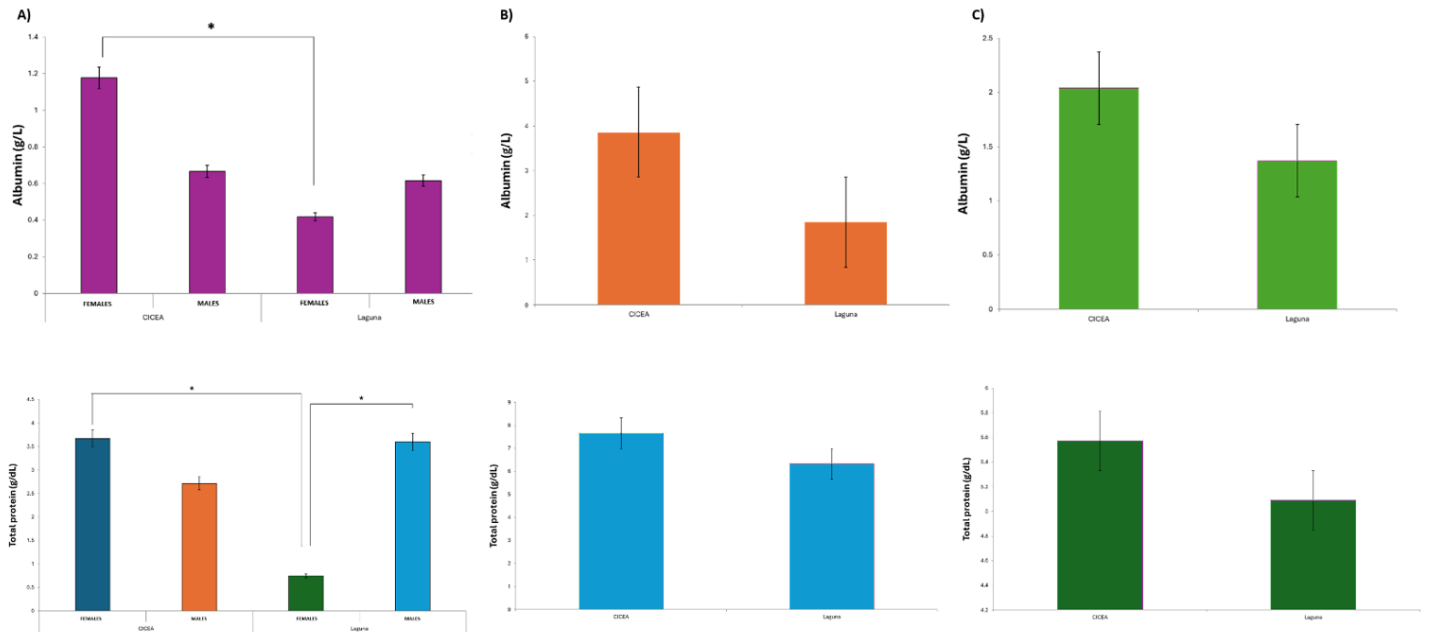


Figure 1

Plasma albumin and total protein concentrations in *Crocodylus moreletii* from Laguna de las Ilusiones (LI) and the captive reference population (CICEA). (A) Adults (n = 5 per group), (B) yearlings (n = 5 per group), and (C) juveniles (n = 2 per group). Values represent mean $\pm$ SD. Technical replicates were performed in quadruplicate. Significant differences between sites are indicated (*p < 0.05; three-way ANOVA). Results for juveniles should be interpreted with caution due to the limited sample size.

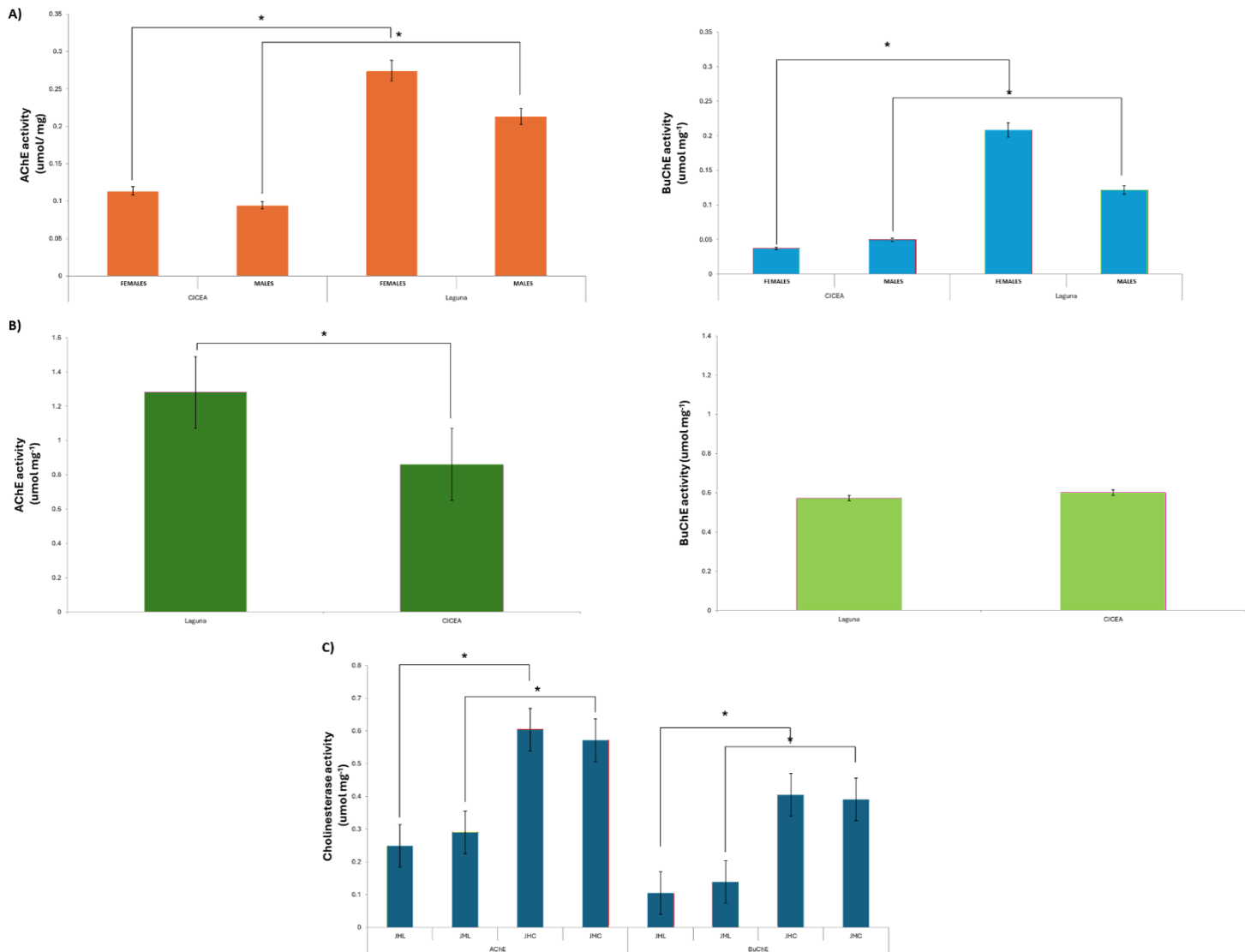


Figure 2

Specific activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in plasma of *Crocodylus moreletii* from Laguna de la Ilusiones (LI) and the captive reference population (CICEA). (A) Adults (n = 5 per group), (B) yearlings (n = 5 per group), and (C) juveniles (n = 2 per group). Values are presented as mean $\pm$ SD. Technical replicates were performed in quadruplicate. Significant site effects of site are indicated (* $p < 0.05$). Results for juveniles should be interpreted with caution due to the limited sample size.

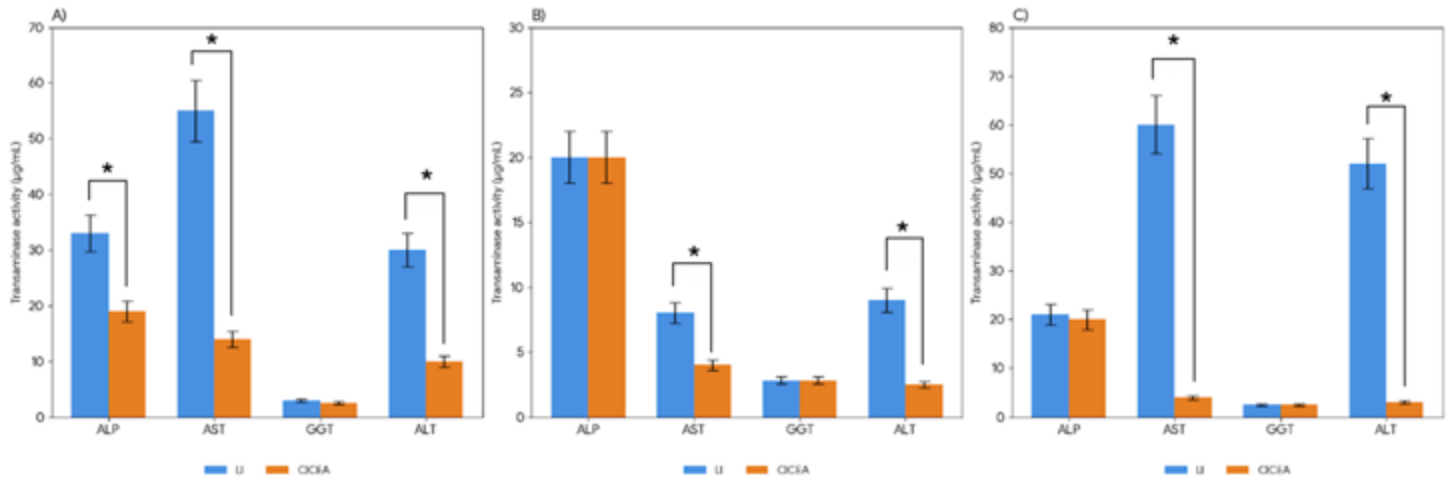


Figure 3

Hepatic enzyme activity (ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: alanine aminotransferase) in plasma of *Crocodylus moreletii* from Laguna de las Ilusiones and the captive reference population (CICEA). (A) Adults (n = 5 per group), (B) yearlings (n = 5 per group), and (C) juveniles (n = 2 per group). Activities are presented as mean ± SD. Technical replicates were performed in quadruplicate. Significant site effects are indicated (*p < 0.05; three-way ANOVA). Results for juveniles should be interpreted with caution due to the limited sample size.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Map1.png](#)