Body condition and blood biochemistry of free-range *Caiman latirostris* in Northeast Brazilian Atlantic Forest

Gabriela Mota Gama[1], Luiza Figueiredo Passos[2], Adriano Pereira Paglia[1,3], Marcos Eduardo Coutinho[1,4]

Affiliation(s)

1. Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Fauna Silvestre, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
2. Liverpool John Moores Univ, Sch Biol & Environm Sci, James Parsons Bldg, Liverpool L3 3AF, Merseyside, England
3. Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
4. Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios (RAN), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)

* Corresponding author

Email: gabrielamgama@gmail.com

Co-author details

Luiza Figueiredo Passos - l.figueiredopassos@ljmu.ac.uk

Adriano Pereira Paglia - apaglia@ufmg.br

Marcos Eduardo Coutinho - marcos.coutinho@icmbio.gov.br

Abstract

The Atlantic Forest broad-snouted caiman (*Caiman latirostris*) inhabits regions within one of the world's most ecologically diverse ecosystems, yet few studies have explored the relationship between body condition, blood biochemistry, and environmental factors in the wild. Our study investigated the effects of sex, ontogeny, habitat, and environmental variables on the body condition and blood biochemistry of free-ranging caimans from the state of Alagoas, Northeast Brazil. From 2020 to 2022, we captured 75 caimans across three sites in different seasons. Results revealed sex-specific responses to seasonal and inter-annual weather changes, with females showing higher body condition in the wet season, while males peaked in the dry season. Elevated glucose, total protein, albumin, triglycerides, and fructosamine were linked to higher body condition and larger individuals, while elevated aspartate aminotransferase to low body condition. Seasonal rainfall influenced blood parameters, with the dry season associated with higher creatinine, calcium, and alanine aminotransferase levels, and the wet season with higher total protein, sodium, and potassium. Differences in glucose, alkaline phosphatase, and gamma-glutamyl transferase across sites pointed to physiological effects of human activities. Blood biochemical values varied widely, with some exceeding reported species ranges. These findings highlight the need to interpret physiological data within the context of local habitat and environmental conditions. Conservation strategies should go beyond species presence and habitat preservation, incorporating pollution control. Our study advances understanding of *Caiman latirostris* ecophysiology, offering valuable insights for the conservation and management of crocodilian populations in both wild and captive environments.

Keywords

Caiman latirostris; crocodylia; crocodilian; reptile; body condition; blood biochemistry; Alagoas; Atlantic Forest; Brazil

52 **Research Highlights**

53 1. Body condition and blood biochemistry were influenced by seasonal changes in rainfall.
54 2. Body condition showed sex-specific differences between rainfall seasons.
55 3. Blood biochemistry differences across sites indicate influence of human activities in caiman
56 health

57

58 ## 1. Introduction

59 Body condition is an individual attribute intimately related to life history traits such as reproduction,
60 survival, and growth (Brommer, 2000; McNamara & Houston, 1996). Individuals with higher body
61 condition are associated with greater reproductive and hatching success, and faster growth rates due
62 to their larger energy reserves (Labocha et al., 2014; McNamara & Houston, 1996). Indices of body
63 condition typically rely on the relationship of morphometric variables such as body mass and length
64 (Labocha et al., 2014). Body condition indices in crocodilians can change over short periods due to
65 seasonal patterns of growth (Hutton, 1987), dietary changes (Coutinho, 2000; Hutton, 1987), and
66 fluctuations in environmental conditions such as water and rainfall levels, and temperature (Campbell
67 et al., 2008; Coutinho, 2000; Fujisaki et al., 2009; Labarre et al., 2020; Mazzotti et al., 2009).
68 Additionally, body condition varies according to sex and size in response to distinct energy investment
69 for reproduction (Barão-Nóbrega et al., 2018) and dietary shifts during different life stages (Borteiro
70 et al., 2009; Coutinho, 2000; Magnusson et al., 1987). Assessing body condition indices in ecological
71 studies with crocodilians can provide vital information about the health status of monitored populations
72 as well as the ecological health of monitored ecosystems (Balaguera-Reina et al., 2022; Mazzotti et
73 al., 2009; Somaweera et al., 2020).

74 Integrating the analysis of body condition indices and blood biochemical parameters deepens
75 understanding about the relationship between environmental and habitat conditions with
76 biological/physiological responses, ultimately affecting species life history traits. Blood testing has
77 been used to assess crocodilians' physiological responses to factors including salinity gradients,

seasonality, diet, stressors, contaminants, and reproduction, across wild and captive populations (Barão-Nóbrega et al., 2018; Campbell et al., 2008; Coulson & Hernandez, 1983; Coutinho, 2000; Grigg et al., 1998; Moleón et al., 2023; Murray et al., 2013; Santos et al., 2024). Key parameters associated with energetic metabolism, electrolyte balance, and renal and hepatic function reflect an individual's nutritional condition and metabolic responses to various factors including diet, diseases or anthropogenic activities. Parameters related to lipid, protein and carbohydrate metabolism – such as glucose and triglycerides – reflect nutritional conditions, as these are the main source of energy for crocodilians for short and longer-term use (Coulson & Hernandez, 1953, 1983; Dessauer, 1970). Total protein levels indicate nutrition status as well as liver and kidney function. Parameters related to electrolytes balance – phosphorus, calcium, sodium, potassium and chloride – provide insights on osmoregulation, dehydration, and are essential for various physiological processes, including muscle function. Levels of renal function indicators like creatinine and urea provide insights into kidney health, as they accumulate in the blood when the kidneys are not functioning properly. Hepatic function markers like alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transferase reflect the health and functioning of the liver, and respond to many pathological conditions.

Given the relevance of body condition and blood biochemical parameters in assessing the physiological responses of crocodilians, examining these aspects in a species highly adapted to diverse South American ecosystems, such as the broad-snouted caiman (*Caiman latirostris*) is a unique opportunity. *Caiman latirostris* is a crocodilian species widely distributed along rivers, mangroves, and wetlands in south-eastern South America, in Argentina, Bolivia, Paraguay, Uruguay and Brazil (Coutinho et al., 2013; Siroski et al., 2020). In Brazil, the Atlantic Forest *C. latirostris* populations are within the most human populated regions in one of the world's most ecologically diverse ecosystems (Myers et al., 2000). Despite of anthropogenic disturbances in the region, the species persists in both natural and human-altered habitats, including hydroelectric dams and eutrophicated lakes (Bassetti, 2016; Coutinho et al., 2013; Marques et al., 2016; Nóbrega, 2017; Passos et al., 2014). In Northeast Brazil's State of Alagoas – characterized by extensive aquatic

ecosystems surrounded by an agricultural landscape – the species coexists with traditional fishing practices that provide protein and income for local families (Gutberlet et al., 2007). Recently, the Brazilian caiman population has been downlisted from CITES Appendix I to II, opening opportunities for future sustainable management (CITES, 2023).

Despite the ecological and socioeconomic importance of *C. latirostris*, and its resilience to human influence, there is still much to learn about its physiological responses in the wild and how the species cope with changing environments. Few studies have been conducted regarding *C. latirostris* body condition and blood biochemical parameters in the wild, considering environmental factors, local habitat conditions, as well as sex and size effects (Bassetti, 2016; Grigg et al., 1998; Nóbrega, 2017; Nóbrega et al., 2022). Most research on *C. latirostris* blood biochemistry has been conducted in captive settings under controlled situations, mainly in higher latitudes like Argentina and Southeastern Brazil with distinct environmental conditions from Northeast Atlantic Forest's climate (Barboza et al., 2008; Coppo et al., 2006; Moleón et al., 2023; Nóbrega et al., 2022; Troiano et al., 1997; Zayas et al., 2011). In this Atlantic Forest region, the climate is mostly hot and humid throughout the year, with low daily thermal amplitude, with dry summer instead of a wet summer often found elsewhere (Barros et al., 2012). These distinctions are particularly relevant because crocodilian physiology and attributes of life histories are closely linked to environmental conditions, and responses can change along the species distribution. Filling these information gaps across the species' distribution is crucial, considering the wide range of environmental conditions, for more accurate health assessments for both wild and captive populations.

This research integrated the assessment of a body condition index (residuals index) and the analysis of 17 blood biochemical parameters related to nutritional and health status of free-range *C. latirostris* across three study sites located in the Alagoas Atlantic Forest. The main goal was to investigate the effects of sex, ontogeny, and environmental and local habitat variables on body condition and on blood biochemical parameters. Specifically, this research aimed to (I) determine how sex and varying environmental conditions influence shifts in the body condition index of caimans; as well as (II) to elucidate the complex relationships between physiological changes in the 17 blood biochemical

parameters values, considering sex, body condition, ontogeny, and environmental and local habitat variables. We hypothesized that (i) body condition varies according to sex, environmental and local habitat conditions; and that (ii) changes in blood biochemical values will be related to various factors including sex, body condition, ontogeny, and environmental and local habitat conditions.

2. Material and methods

Study sites

The study area includes three sites in the northeast Atlantic Forest in the State of Alagoas, Brazil: Marituba do Peixe Environmental Protected Area (APA Marituba) (-10.313142°; -36.369745°), Lagoa do Jequiá Marine Extractive Reserve (RESEX Jequiá) (-10.004828°; -36.022862°), and Guaxuma lagoon (-10.036140°, -36.071365°) (Figure 1). The region is characterized by extensive aquatic ecosystems of coastal lagoons and wetlands surrounded by Atlantic Forest, with established fishing communities that constitute a relevant socioeconomic context for the conservation and management of *C. latirostris*. The climate exhibits marked seasonality, with wet seasons occurring from April to August (autumn-winter) and dry seasons from September to March (spring-summer) (Barros et al., 2012; Carvalho et al., 2017). According to Koppen's system, the climate is classified as As' type (Tropical savanna, with dry summer), characterized by a markedly dry season. Highest precipitations are registered in May, while November records the lowest values. Total rainfall during the wet season varies from 1,000 mm to 1,500 mm. The air temperature typically ranges from 21 °C to 31 °C, with an annual mean temperature of 25-26 °C (Barros et al., 2012).

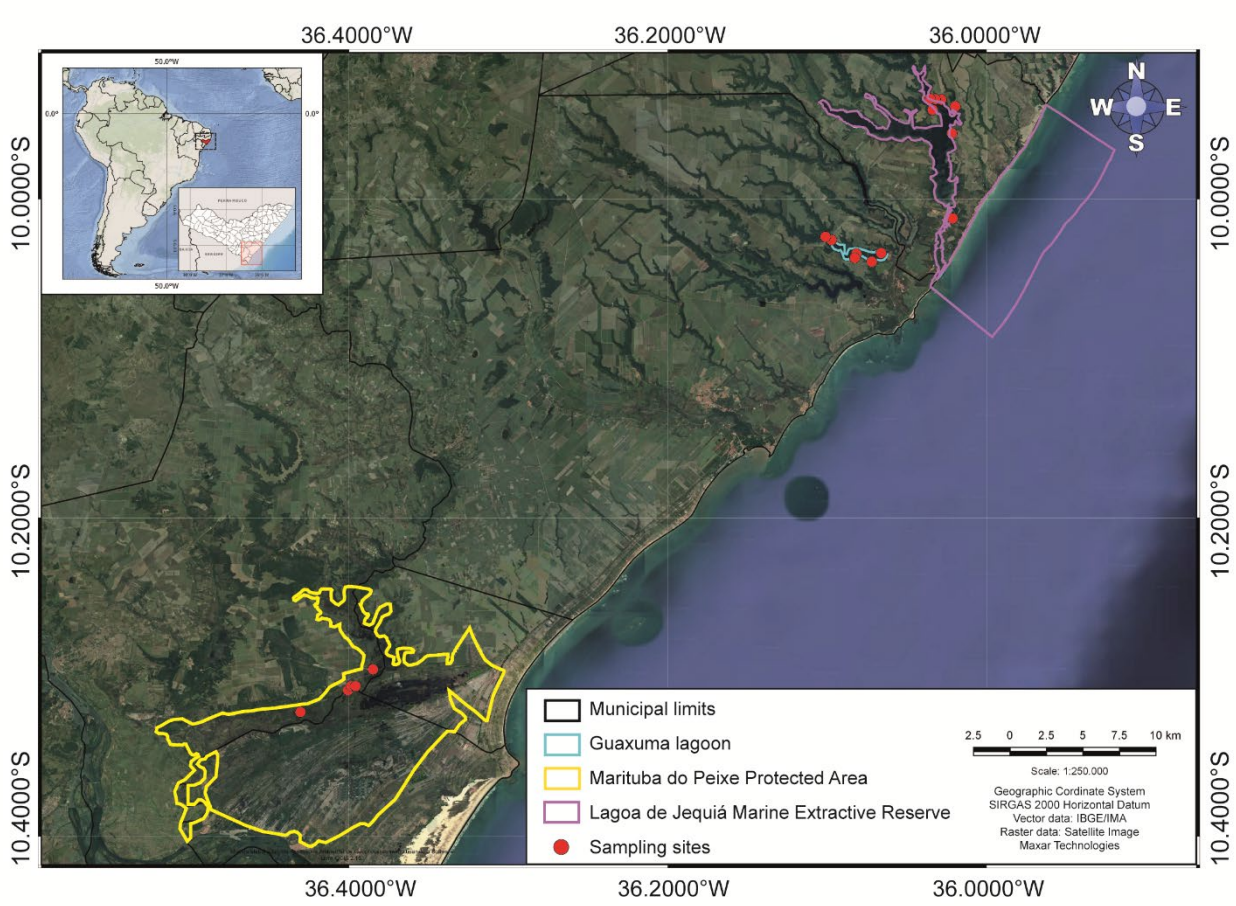


Figure 1. Map of the studied sites located in the State of Alagoas, Northeast Brazil, in the coastal portion of the Atlantic Forest. Red dots indicate the localities where broad-snouted caimans *Caiman latirostris* were captured between 2020 and 2022.

APA Marituba is a state-managed sustainable use reserve (equivalent to category V in IUCN classification of protected areas), encompassing 18,600 hectares in the lower São Francisco River basin. The landscape includes 27% of seasonally flooded habitats, open grasslands, and humid and white-sand Atlantic Forest (Alagoas, 2006). Local fishing activity in APA Marituba has been severely depleted due to environmental impacts such as resources overexploitation, water dam effects, and pollutants from intensive agriculture and sugarcane industries. Fires and drainage for agriculture and cattle raising have also affected the floodplains. Surveys in 2001 and 2007 highlighted the need for caiman conservation plans in APA Marituba (Filogonio et al., 2010; Verdade, 2001). RESEX Jequiá is a federal-managed extractive reserve (IUCN category VI) with 10,200 hectares of coastal and marine areas. In addition to the marine area, which extends 5 km into the ocean, there are mangroves

and the Jequiá lagoon, which forms part of the Jequiá River hydrographic basin. Artisanal fishing is the main activity of the 12 communities within the reserve. RESEX Jequiá is relatively less affected by factors such as environmental degradation, fishing pressure, and agriculture-related impacts. Guaxuma lagoon is a smaller coastal lagoon with 220 hectares that supports intensive fish farming production, but not formally designated as a protected area. APA Marituba and RESEX Jequiá are recognized officially under Brazilian laws to safeguard local livelihoods artisanal fishery communities through sustainable resource management.

Fieldwork

Field studies occurred between September 2020 and December 2022, encompassing nine expeditions covering the three study sites. The fieldwork was carried on a three-month interval between each expedition, aiming at covering year climatic variations.

Most of the caimans were captured during night surveys using baited funnel traps installed along the shorelines. The funnel traps were set in the evening in the first day of fieldwork and left in place during a 3-day period in each site. Traps were checked at specific intervals, in early evening and early morning, and baited again if necessary. Two small caimans were captured by hand and one was captured with a locking steel snare cable mounted to a pole. Once captured, blood samples were promptly obtained to minimize the influence of temperature changes and capture stress on blood parameters regardless of capture method. Air, water and cloacal temperatures were immediately recorded with a thermometer with a fixed probe (Hanna HI955502). A maximum of 6 ml of blood sample was collected from the post-occipital vein using a 10 ml plastic syringe without heparin addition. The total volume of blood was readily transferred to two distinct tubes and stored on ice: a 2 ml sodium fluoride tube, for glucose analysis; and a 10 ml serum separating tube with clot activator for the other blood parameters (described below). Individuals were sexed, measured to their snout-to-vent length (SVL) and total length, and weighted. Caimans were marked by inserting microchips at the left lateral side of the tail, and by cutting tail scutes. After measurements, caimans were released

at their site of capture. At the field lab, blood samples were subjected to a centrifugation process for 10 minutes at 6,000 rpm using a mini centrifuge Sprout Plus (Heathrow Scientific, USA). The resulting plasma (from sodium fluoride tube), and serum (tube with clot activator) were stored and frozen in 2 ml microtubes for analysis after each expedition.

Blood samples and biochemistry

A total of 17 blood biochemical parameters related to nutritional and health status were measured. These included indicators of energetic metabolism – glucose, total proteins, albumin, cholesterol, triglycerides and fructosamine; electrolyte balance – phosphorus, ionized calcium, sodium, potassium and chloride; renal function – creatinine and urea; and hepatic markers – alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT). These parameters are common indicators of nutritional condition and of many pathological states, and were compiled from different studies with *C. latirostris* in both wild and captivity (Barboza et al., 2008; Bassetti, 2016; Coppo et al., 2006; Grigg et al., 1998; Moleón et al., 2023; Nóbrega et al., 2022; Troiano et al., 1997; Zayas et al., 2011).

An automated biochemical analyser (SX160, Sinnowa Healthcare) was used for measuring 13 parameters – glucose, total proteins, albumin, cholesterol, triglycerides, fructosamine, urea, creatinine, phosphorus, and serum activity of ALP, ALT, AST and GGT. These analyses were done with biochemistry test kits (Bioclin, Quibasa Química Básica, Belo Horizonte, Minas Gerais, Brazil), according to the manufacturer's instructions. The analysis of ionized calcium, sodium, potassium and chloride was performed using an automated electrolyte analyser (Max Ion 800, Meizhou Cornley Hi Tech Co) with a calibration solution kit (Pack (A/B/FLUSH) Max Ion - Medmax). It should be noted that not all blood parameters could be determined for all individuals due to lipemia or hemolysis of samples.

Index of body condition

A body condition index was calculated for each individual in the sampled population by the residual values from allometric relationship between the natural logarithm (ln) of body mass on ln of snout-vent length (SVL) (Jakob et al., 1996). The use of SVL is recommended over total length to avoid measurement errors induced by tail-tip amputations (Hutton, 1987). The residuals of this linear regression provide a measure of an individual's deviation from the average for that group. Based on the residual values, whether negative or positive in relation to the average, individuals were classified as under (below average – negative residuals) or overweight (above average – positive residuals) for the group (Campbell et al., 2008). This resulted in two ways to interpret the index of body condition: as a continuous variable, and as a categorical variable with two levels (under/overweighted animals) (Table 1).

Data analysis

We defined a set of 12 explanatory variables (Table 1) to evaluate the effects of sex and environmental conditions on body condition and to investigate the relationships between physiological changes in the 17 blood biochemical values, considering sex, body condition, ontogeny, and habitat.

Table 1– Description of the 12 explanatory variables used to investigate changes in body condition index of sampled population, and on blood biochemical parameters.

n	Category	Variable	Description	Variable type	Analysis
1	Local	Study sites	Guaxuma, Jequiá and Marituba	Categorical (3 levels)	Body condition and blood biochemistry
2	Environment	Rainfall levels	Dry (total monthly rainfall < 60 mm) or Rainy month (total monthly rainfall > 60 mm), according to Koppen's system As type for the study area	Categorical (2 levels)	Body condition and blood biochemistry
3	Environment	Rainfall season	Dry (September to March) or Wet (April to August)	Categorical (2 levels)	Body condition and blood biochemistry
4	Environment	Total monthly rainfall 1	Total monthly rainfall for a single month (mm)	Continuous	Body condition and blood biochemistry
5	Environment	Total monthly rainfall 2	Total monthly rainfall from the sum of two months (mm)	Continuous	Body condition and blood biochemistry
6	Environment	Total monthly rainfall 3	Total monthly rainfall from the sum of three months (mm)	Continuous	Body condition and blood biochemistry
7	Environment	Water temperature	Water temperature in °C	Continuous	Body condition and blood biochemistry

8	Sex	Sex	Male or Female	Categorical (2 levels)	Body condition and blood biochemistry
9	Body condition index	Body condition index (Continuous BC)	Residual values of the linear regression of the natural logarithm (ln) of body mass on ln of snout-vent length (SVL) calculated for individuals in the sampled population	Continuous	Blood biochemistry
10	Body condition index	Body condition index (Categorical BC)	Under or over-weight, considering the residual values of the linear regression of ln of body mass on ln of snout-vent length (SVL), whether negative or positive	Categorical (2 levels)	Blood biochemistry
11	Ontogeny	Snout-vent length (SVL)	Size in centimeters from the tip of the snout to the most posterior opening of the cloacal slit (vent)	Continuous	Blood biochemistry
12	Ontogeny	Size class	Class I/Hatchlings: < 25 cm SVL; Class II/Juveniles: 25 to 67.9 cm SVL; Class III/Adults: 68 to 99.9 cm SVL	Categorical (3 levels)	Blood biochemistry

The monthly rainfall variables included lagged data by summing the totals for one, two- and three-months preceding captures, in order to capture any effect of cumulative rainfall in response variables. Body condition and physiological changes in blood biochemistry are generally a time-lagged response to previous feeding opportunities (Fujisaki et al., 2009), related to factors like species life histories, resource availability, rainfall and water levels, and temperature, among others. Incorporating previous rainfall data, allows inferring associations between physiological responses and preceding rainfall levels. This approach improves predictive accuracy for body condition and blood biochemistry changes as current states result from past nutritional intake. Furthermore, quantitative variables were categorized to facilitate analysis, visualization, interpretation of the data and comparison with other studies by calculating descriptive statistics (e.g., mean, median) for each category, providing different insights when interpreting results. For instance, the body condition index was processed as a continuous variable (Continuous BC) and as a categorical variable with two levels (underweight/overweight, denoted by Categorical BC); and snout-vent length as a continuous variable of size, and also sorted into defined size classes, as detailed in Table 1.

In order to evaluate the effects of sex, habitat and environmental conditions on the index of body condition (Continuous BC) eight independent variables were included in the model: study sites; sex; rainfall levels; rainfall season; total monthly rainfall for the previous month, two months prior, and three months prior, and water temperature. We assessed the relationship between Continuous BC and predictors using two-way ANOVA to test for independent effects of sex and explanatory variables as well as their interaction. Model was defined as follows: Continuous BC ~ sex + explanatory variable

257 + sex*explanatory variable). We also tested for inter-annual differences in Continuous BC separately
258 for males and females, with one-way ANOVA, for 2020, 2021 and 2022 (Continuous BC ~ year).

259 To examine the relationships between blood biochemical parameters and the effects of sex, body
260 condition, ontogeny, environmental and habitat variables, we employed the full set of 12 explanatory
261 variables. We initially compared blood values in different study sites using ANOVA. This comparison
262 revealed variations in glucose levels, ALP levels, and GGT levels among habitats. Subsequently, we
263 employed two distinct statistical approaches to assess the effects of explanatory variables on blood
264 biochemical parameters. One approach involved running generalized linear mixed models (GLMMs),
265 with lmer function in R (lme4 package), considering study site as a covariate for parameters
266 significantly influenced by local conditions at the study sites; while for other parameters, differences
267 were examined using student's t-Test, ANOVA or linear regressions with lm function in R depending
268 on the type of predictor variable. T-tests were used to assess differences among sex, rainfall levels,
269 rainfall season, and Categorical BC; one-way ANOVA was conducted to explore differences between
270 size classes; and linear regression was applied to analyze relationships between blood parameters
271 in relation to total monthly rainfall 1, Continuous BC and snout-vent length (SVL). Multicollinearity
272 among total monthly rainfall data was tested, resulting in strong correlation between variables
273 (Spearman's correlation; $r \leq |0.7|$). Due to this correlation, only total monthly rainfall 1 was used
274 to assess relationships between blood parameters.

275 We provided mean, standard deviation, median, maximum, and minimum ranges for each blood
276 parameter in the entire dataset. We also described group-specific values for blood components when
277 relationships were statically significant ($p<0.05$). Variables were assessed for normality and
278 homoscedasticity before conducting all statistical analyses using R (R Core Team, 2023).

279

280 **3. Results**

A total of 75 blood samples were collected from free-range *C. latirostris* (14 females, and 61 males). A description of basic morphometric and demographic data (SVL, body mass, size structure and sex ratio) from sampled population for each study site is provided in Table 2. Body condition index (Continuous BC) values were normally distributed, ranging from -0.27449 to 0.24575. Relationship between SVL and body mass of sampled individuals for each study site demonstrated that there were no differences among the structure of the sample ($F_{(2,72)=}$1,46, p=0,23) (Figure 2). Air temperature ranged from 24.7 – 34.0 cm [Mean (SD): 29.09 (2.28)], water temperature from 25.9 – 32.8.0 cm [Mean (SD): 29.74 (1.41)], and cloacal temperature from 24.3 – 33.0 cm [Mean (SD): 29.31 (1.89)] (Figure 3). Blood parameters data are summarized in Table 3.

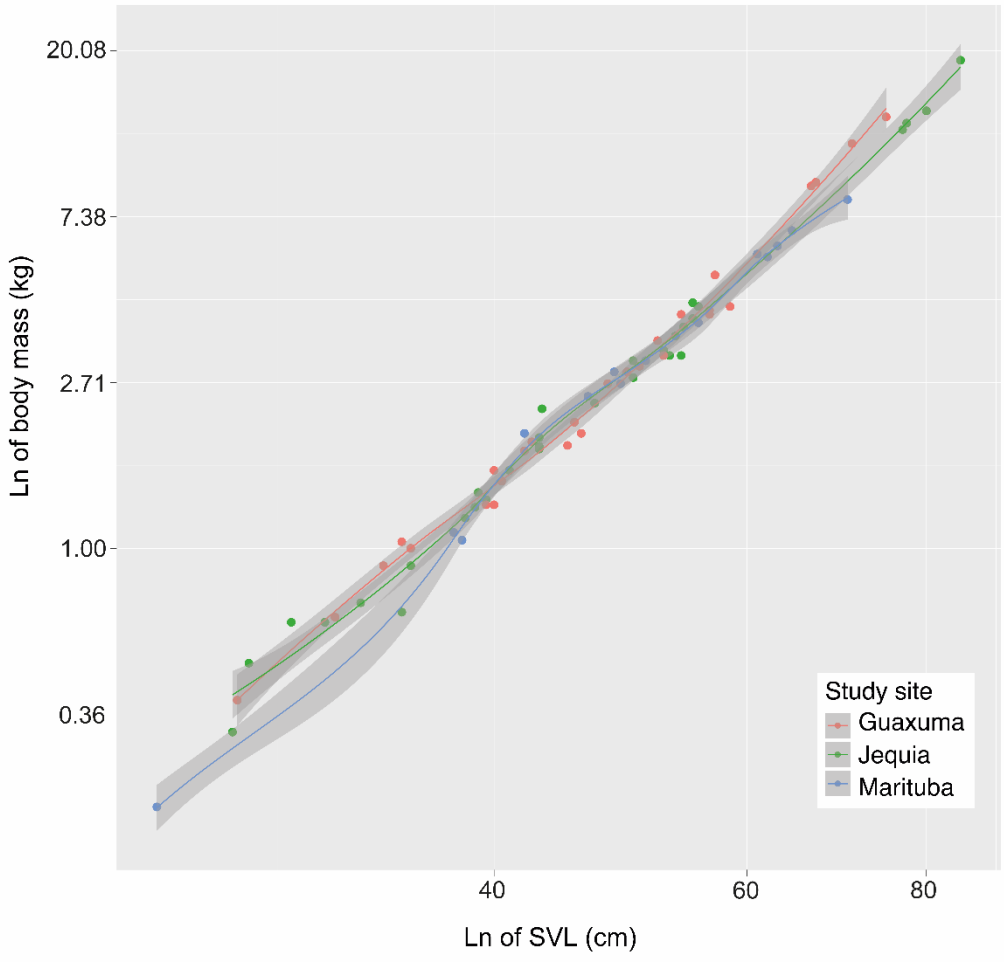


Figure 2. Relationship between SVL and body mass of sampled individuals of *C. latirostris* for Guaxuma lagoon, RESEX Jequiá and APA Marituba. There were no differences among sample structure among study sites.

Table 2. Description of SVL, body mass, size structure and sex ratio from sampled population of *C. latirostris* for Guaxuma lagoon, RESEX Jequiá and APA Marituba.

Parameter	APA Marituba (n=14)		RESEX Jequiá (n=32)		Guaxuma lagoon (n=29)		Total (n=75)	
	Mean (SD)	**Median [Min-Max]**	**Mean (SD)**	**Median [Min-Max]**	**Mean (SD)**	**Median [Min-Max]**	**Mean (SD)**	**Median [Min-Max]**
SVL (cm)	50.9 (12.9)	50 [23.3-70.5]	47.9 (15.1)	45.1 [26.3-84.5]	48.1 (12.1)	46 [26.5 - 75]	48.5 (13.5)	48 [23.3-84.5]
Body mass (kg)	3.7 (2.4)	3 [0.2-8.2]	3.7 (4.5)	2.4 [0.3-19]	3.5 (3.3)	2.1 [0.4 - 13.5]	3.6 (3.7)	2.7 [0.2-19]
Size structure	Class I - 1 (7.1%); Class II - 12 (85.7%); Class III - 1 (7.1%)		Class I - 0 (0.0%); Class II - 28 (87.5%); Class III 4 (12.5%)		Class I - 0 (0.0%); Class II - 27 (93.1%); Class III - 2 (6.9%)		Class I - 1 (1.3%); Class II - 67 (89.3%); Class III - 7 (9.3%)	
Sex ratio (Female: Male)	1.33: 1		1: 7		1: 13.5		1: 4.35	

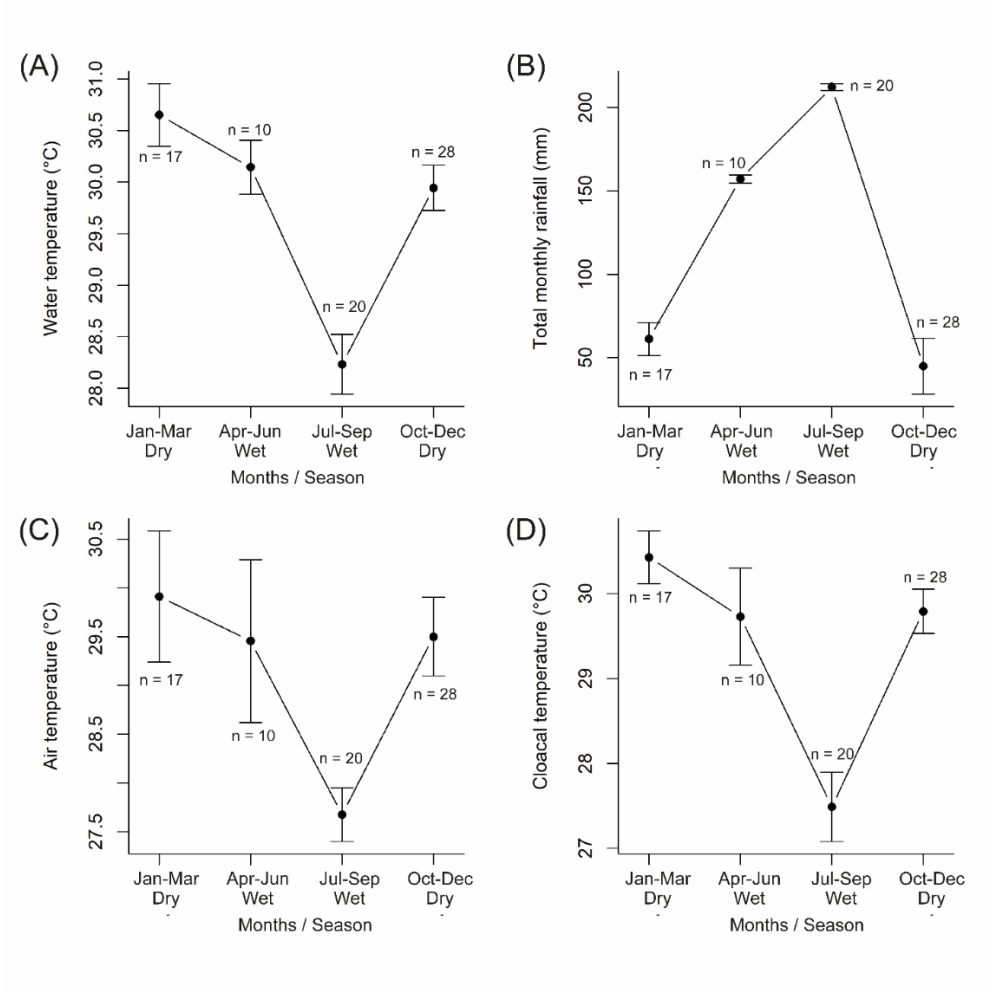


Figure 3. Mean values (points) and standard errors (vertical bars) of trimestral variation during the sampling period (September 2020 and December 2022) in (A) water temperature, (B) total monthly rainfall, (C) air temperature, and (D) cloacal temperature.

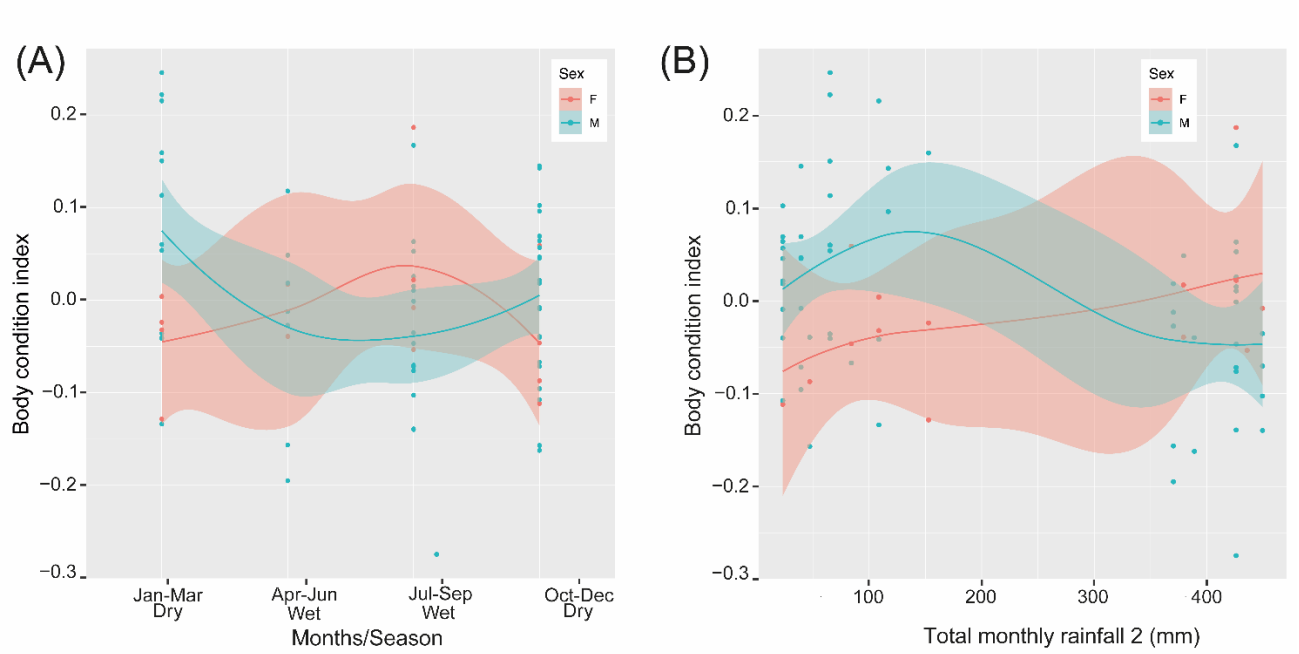
Table 3. Blood biochemical parameters data of free-range *Caiman latirostris* sampled in the northeast Atlantic Forest in the State of Alagoas, Brazil, from September 2020 to December 2022, and statistical results for correlation with sex, body condition, ontogeny, and environmental and local habitat variables. Bold/shaded values indicates statistically significant correlations.

Analyte	n	Mean (SD)	Median [Min-Max]	Study sites	Rainfall levels	Rainfall season	Total monthly rainfall 1	Water temperature	Sex	Body condition (Categ.)	Body condition (Quant.)	Snout-vent length (SVL)	Size class
Glucose (mg/dL)	49	112.4 (58.7)	93 [37 - 327]	**0.04**	0.52	0.37	0.94	0.53	0.9	**0.05**	**0.05**	**0.01**	**0.01**
Total protein (g/dL)	52	5.2 (2.1)	4.8 [0.1 - 10.5]	0.17	**0.03**	**0.01**	0.17	**0.01**	0.19	**0.04**	0.19	**<0.01**	0.07
Albumin (g/dL)	52	1.5 (0.6)	1.5 [0.2 - 3.2]	0.36	0.38	0.29	0.49	0.21	0.24	**<0.01**	**<0.01**	**<0.01**	**0.01**
Cholesterol (mg/dL)	52	148.4 (56.6)	138.5 [58 - 380]	0.45	0.51	0.21	0.64	0.68	0.68	0.33	0.97	0.75	0.66
Triglyceride (mg/dL)	74	166.8 (217.3)	95 [7 - 1120]	0.81	0.45	0.4	0.26	0.4	0.45	**0.01**	0.08	0.12	0.33
Fructosamine (mmol/L)	52	223.2 (75.4)	217.1 [69.1 - 469.2]	0.26	0.39	0.11	0.36	0.73	0.29	**<0.01**	**0.01**	**<0.01**	0.07
Creatinine (mg/dL)	75	0.5 (0.5)	0.3 [0.01 - 2.9]	0.16	**0.03**	0.1	0.07	**0.01**	0.08	0.28	0.95	0.72	0.8
Urea (mg/dL)	74	3.9 (2.9)	3 [1 - 17]	0.14	0.11	0.18	0.24	0.43	**0.03**	0.22	0.06	0.58	0.47
Phosphorus (mmol/L)	74	5.9 (3.1)	5.2 [1.0 - 14.8]	0.46	0.16	0.43	0.15	0.73	0.34	**0.01**	**0.01**	0.15	0.31
Calcium (iCa) (mmol/L)	51	3.8 (1.2)	3.6 [2 - 7]	0.26	0.07	0.14	**0.03**	**<0.01**	0.5	0.51	0.5	0.35	0.64
Sodium (mmol/L)	51	140 (11.6)	141.2 [103 - 160.5]	0.72	**0.02**	**0.02**	0.09	0.73	0.26	0.15	0.34	0.12	0.14
Potassium (mmol/L)	51	3.9 (1.1)	3.9 [2 - 7]	0.75	**0.01**	**<0.01**	0.15	0.66	0.29	0.51	0.84	0.19	0.21
Chloride (mmol/L)	51	105.4 (12.2)	106 [76.8 - 166.1]	0.32	0.41	0.45	0.63	0.66	0.4	0.28	0.08	0.94	0.44
Alkaline phosphatase (FAL) (ui/L)	52	30.5 (22.5)	27 [2.6 - 131]	**0.04**	0.5	0.13	0.62	0.91	0.38	0.87	0.64	0.32	0.25
Alanine Aminotransferase (ALT) (ui/L)	74	63.9 (64.4)	45 [6 - 415]	0.65	**0.03**	**0.01**	**0.02**	**<0.01**	0.37	0.35	0.84	0.52	0.61
Aspartate aminotransferase (AST) (ui/L)	74	163 (91.2)	149 [36 - 659]	0.75	0.06	0.06	0.88	0.63	0.503	**0.03**	**0.01**	0.39	0.37
Gamma glutamyl transferase (GGT) (ui/L)	73	8.2 (11)	5 [1 - 66]	**0.03**	0.57	0.94	0.54	0.78	0.77	0.5	0.42	0.11	0.11

The effects of sex, study sites and climate on body condition

We identified specific relationships between body condition and sex, habitats and environmental variables. When testing for independent effects, there were no differences in Continuous BC in response to most of the predictor's variables, except for rainfall levels (dry month /rainy month) (Figure S1). Continuous BC differed between distinct rainfall levels ($F_{rainfall(1,71)}$=4.49, p=0.03), with higher values observed during the dry months [Mean (SD): 0.02 (0.09)] compared to the rainy months [Mean (SD): -0.02 (0.10)].

Furthermore, we detected an interaction between sex and rainfall season (dry/wet season) on body condition ($F_{sex*season\ (1,71)}$=5.05, p=0.02). In the dry season, males had higher body condition than females, while in the wet season, females showed greater body condition than males (Figure 4). Additionally, variation in body condition was explained by the interaction between sex and total monthly rainfall for one month ($F_{sex*rainfall1}$(1,70)=4,61, p=0,03), for two months ($F_{sex*rainfall2(1,70)}$=6.09, p=0.01), and three months ($F_{sex*rainfall3(1,70)}$=5.88, p=0.01) preceding blood collection. Increasing monthly rainfall may contribute to the improvement of female body condition, whereas the same can negatively affect males (Figure 4). Inter-annual changes in body condition were observed for males ($F_{(2,58)}$=2.43 p=0.05), but not for females ($F_{(2,11)}$=0.09 p=0.90) (Figure 5 A-B). This finding correlates with changes in rainfall over the years, where increased precipitation in 2022 compared to 2021 was associated to a decline in male body condition (Figure 5 C).



326

327 Figure 4. Variation in body condition of wild individuals of *C. latirostris* in response of the interaction between
328 sex and environmental conditions. Plots shows smoothed lines and confidence intervals (95%) of the
329 relationship between body condition in relation to (A) sex and rainfall season (Dry/Wet season), and in relation
330 to (B) sex and total monthly rainfall for the sum of two months preceding the captures.

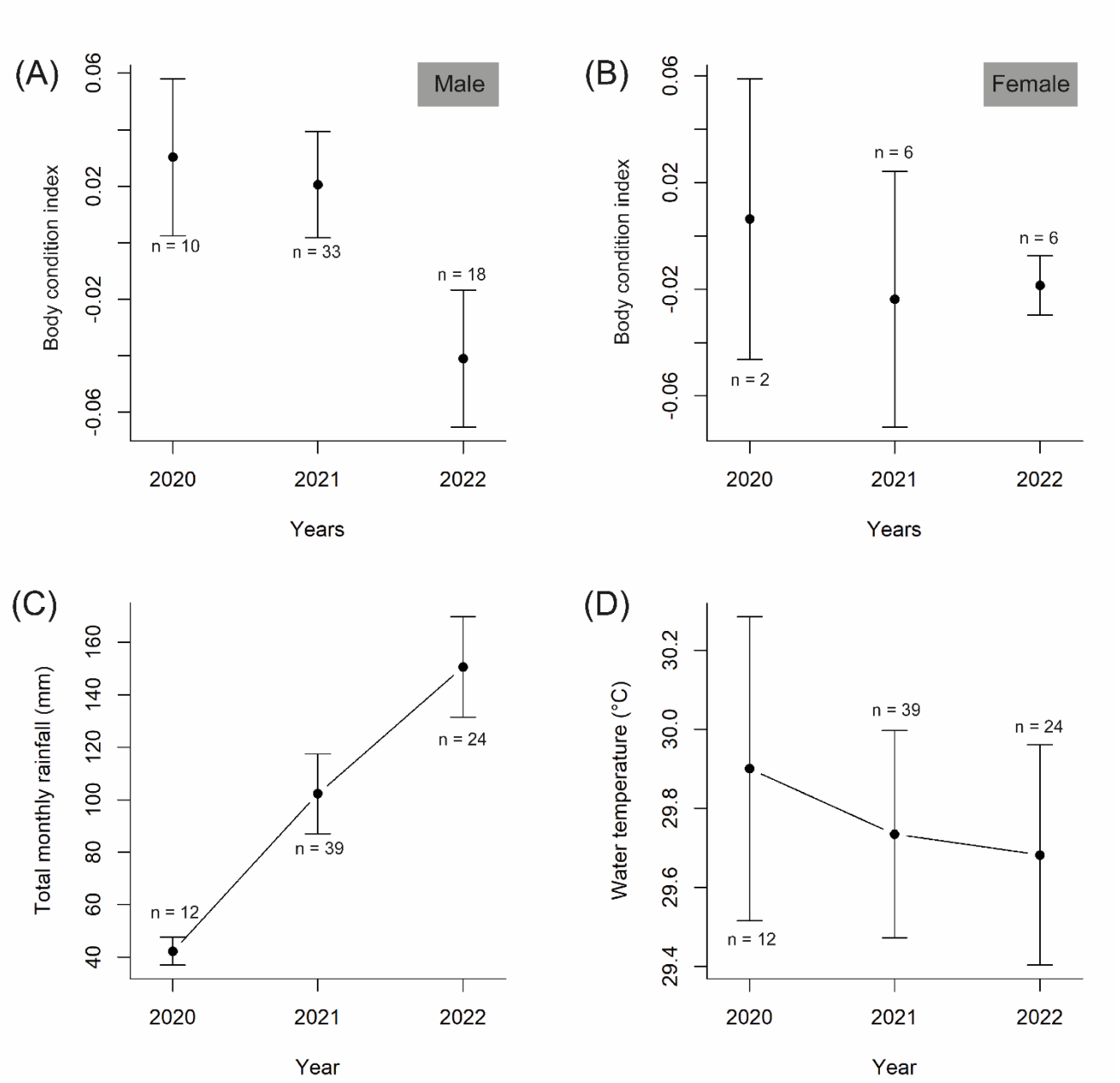
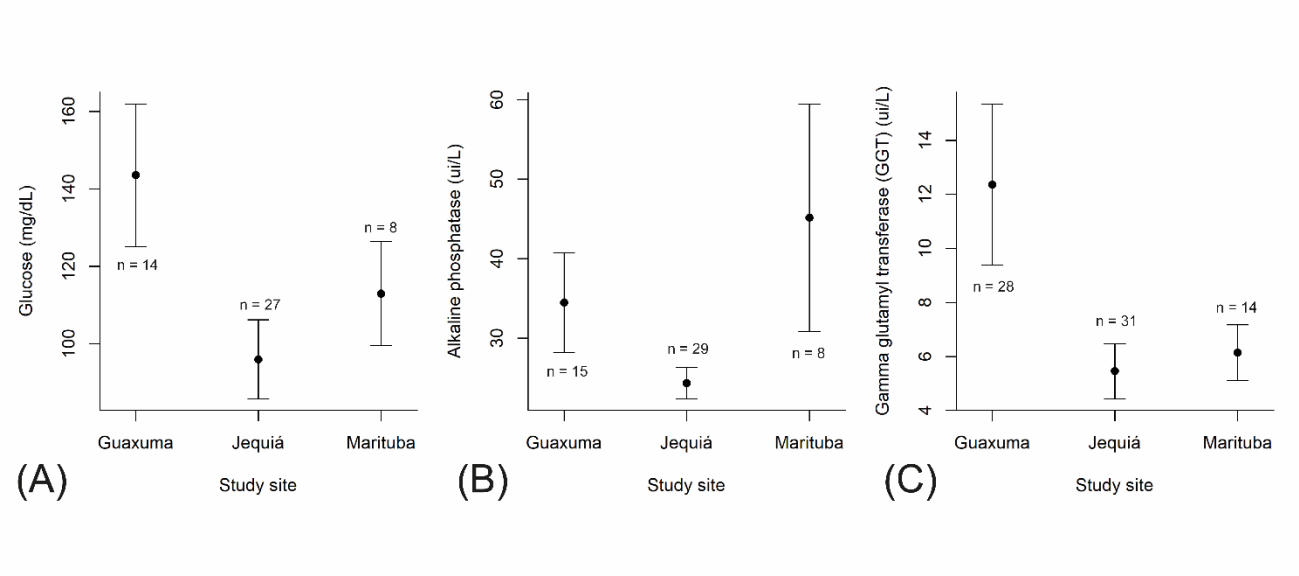


Figure 5. Mean values (points) and standard errors (vertical bars) of inter-annual variation during the sampling period (September 2020 and December 2022) in (A) body condition of males, (B) body condition of females, (C) total monthly rainfall, and (D) water temperature.

338 **Physiological changes in blood components**

339 *Relationships between blood components, environmental and habitat conditions*

340 Levels of glucose ($F_{(2,46)}$=3.321, p=0,04), ALP ($F_{(2,49)}$=3.27, p=0.04) and GGT ($F_{(2,70)}$=3.43, p=0.03)
341 differed between habitats (Figure 6). Guaxuma exhibited higher levels of glucose and GGT, while
342 Jequiá and Marituba presented similar values, but lower when compared to Guaxuma. Levels of ALP
343 were higher in Marituba, followed by Guaxuma and Jequiá (Figure S2, and detailed group-specific
344 values in Table S1).



345

346 Figure 6. Mean values (points) and standard errors (vertical bars) for blood biochemical parameters showing
347 differences among local conditions of study sites (Guaxuma/Jequiá/Marituba) – (A) Glucose (mg/dL); (B)
348 Alkaline phosphatase (ui/L) and (C) Gamma glutamyl transferase (GGT) (ui/L).

349 The effects of rainfall on blood components were captured using two variables, producing similar
350 results, except for creatinine (Figure S3, and detailed group-specific values in Table S2). Rainfall
351 levels (dry/rainy months) were linked to total protein (t=-1.7904, df=50, p=0.03), sodium (t=1.8878,
352 df=73, p=0.03), and potassium (t=-2.2084, df=49, p=0.01), showing higher values in wet season. On
353 the other hand, levels of creatinine (t=1.8878, df=73, p=0.03) and ALT (t=1.778, df=72, p=0.03) were
354 higher in the dry season. Likewise, rainfall season (dry/wet seasons) was correlated with levels of
355 total protein (t=-2.1811, df=50, p=0.01), sodium (t=-2.0276, df=49, p=0.02) and potassium (t = -

2.4746, df = 49, p=0.008), with greater levels in wet season; and ALT (t=2.0919, df=72, p=0.01), with higher values in dry season. An increase in total monthly rainfall was negatively associated to ionized calcium ($F_{(1,48)}$=4.611, r^2= 0.08764, p= 0.03) and ALT ($F_{(1,71)}$= 5.26, r^2= 0.06898, p= 0.02).

Variation in water temperature showed a negative association with total protein ($F_{(1,44)}$=5.88, r^2= 0.11, p= 0.01); and a positive association with creatinine ($F_{(1,65)}$=6.4, r^2= 0.08, p= 0.01), ionized calcium ($F_{(1,43)}$=17.65, r^2= 0.29, p< 0.01) and ALT ($F_{(1,64)}$= 11.13, r^2= 0.14, p< 0.01).

Effects of sex, body condition and ontogeny on blood components

Differing patterns in blood biochemistry were observed in response to sex, body condition index and ontogeny (Figure S4, S5, and detailed group-specific values in Table S3). Urea (t=-1.7818, df=72, p=0.03) was the only blood component that differed between sex, with males [Mean (SD): 4.21 (3.07)] presenting higher levels than females [Mean (SD) 2.71 (1.32)].

Individuals classified as overweight exhibited higher levels for glucose (x2=3.73, df=1, p=0.05), total protein (t = -1.7365, df = 50, p = 0.04), albumin (t = -3.504, df = 50, p < 0.01), triglycerides (t = -2.0957, df = 72, p = 0.01), fructosamine (t = -2.669, df = 50, p < 0.01) and phosphorus (t = -2.3565, df = 72, p = 0.01), whereas under-weight individuals showed higher levels for AST (t = 1.8286, df = 72, p = 0.03) (Table S4). Continuous BC was positively associated with glucose (x^2=6,03, df=1, p=0,01), albumin ($F_{(1,50)}$=17.55, r^2: 0.245, p<0.01), fructosamine ($F_{(1,49)}$=6.134, r^2: 0.09312, p= 0.01), and phosphorus ($F_{(1,72)}$=5.811, r^2= 0.06183, p= 0.01); and negatively correlated with AST ($F_{(1,72)}$= 6.29, r^2= 0.06757, p= 0.01).

Size (SVL) showed a positive correlation with glucose (x2=6,03, df=1, p=0,01), total protein (F(1,50)=17.55, r2: 0.245, p<0.01), albumin (F(1,50)=17.55, r2: 0.245, p<0.01), fructosamine (F(1,49)=6.134, r2: 0.09312, p= 0.01). When individuals were classified into size classes according to their SVL, a difference was found for glucose (x2=8,47, df=1, p=0,01) and albumin (t = -2.8884, df = 6.0242, p=0.01), where Class III/Adults showed higher values than Class II/Juveniles, followed by Class I/Hatchlings. Regardless of similar results among continuous and categorical size variables,

381 the effects of size on total protein and fructosamine levels were only possible to be observed using
382 the continuous variable.
383
384 ## 4. Discussion
385 Our results indicate a substantial variability in body condition and blood biochemical values of free-
386 range *C. latirostris* in Alagoas Atlantic Forest, associated to a range of factors including sex, ontogeny,
387 habitats and seasonal changes in climate condition. Sex-specific responses to intra- and inter-annual
388 seasonal changes in weather conditions were observed in our study area. Seasonal variation in
389 rainfall were also found to affect key blood parameters, with certain levels peaking during wetter
390 periods as we further detail. We also demonstrated that individual size and body condition are
391 associated to shifts in blood biochemical values. In the following sections, we explore how these
392 physiological differences may relate to species ecology, by first discussing patterns in individual body
393 condition, and then on blood biochemistry responses. By demonstrating that physiological responses
394 show great variability in wild conditions, this research adds new information to current knowledge of
395 the species, and about crocodilians, as most studies are derived from captive populations. Our
396 findings also contribute to a more informed approach to the development of site-specific management
397 strategies and health assessments for *C. latirostris*, drawing attention for existent differences across
398 populations, geographic locations, management conditions.

399 **Seasonal patterns in individual body condition**

400 In our study area, the relationship between body condition and rainfall varied according to sex:
401 increased rainfall may contribute to increase female body condition, whereas the same is associated
402 to reduced body condition in males (Figure 7). First, we identified marked intra-annual variation in
403 body condition among sexes. High mean body condition of females during the wet season (July -
404 September) coincide with the onset of breeding activity, and lower body condition during the dry
405 season, correspond to egg laying and nest protection in the northeast Atlantic Forest (Rodrigues et
406 al., 2021). Conversely, males showed lower body condition during the early wet season when

compared to higher body condition during the dry season. Additionally, we also identified marked inter-annual sex-specific variation in body condition. For instance, due to an increase rainfall in 2022, males presented lower mean body condition when compared to males captured in 2021 (Figure 5). These seasonal changes in body condition likely result from a complex combination of factors as observed in other crocodilians, including seasonal movements driven by annual fluctuations in water availability, reproductive behavior, and with feeding habits (Barão-Nóbrega et al., 2018; Campbell et al., 2008; Coutinho, 2000; Fujisaki et al., 2009; Hutton, 1987; Labarre et al., 2020).

Body condition is an attribute that reflects an individual's ability to acquire food resources to meet metabolic needs (Barão-Nóbrega et al., 2018; Jakob et al., 1996; McNamara & Houston, 1996). Seasonal movements and changes in water availability likely influenced body condition dynamics at our study sites. Increased water availability during the wet season may have enhanced females' access to food resources, as evidenced by their higher body condition during this period compared to males. The reduced body condition of males during the wet season may be linked to their seasonal movements to explore new areas for feeding, which requires significant energy (Campbell et al., 2013; Coutinho, 2000). This behavior may reduce local densities, avoiding intraspecific competition and, thereby increasing food availability for females. Inter-annual climate variability also affects *C. yacare* ecophysiological responses such as body condition, hormone levels, nesting activity and population densities (Campbell et al., 2008; Coutinho, 2000). The American crocodile (*C. acutus*) exhibits temporal variation in body condition over a thirteen-year period, initially showing a negative impact up to three years after major hurricanes, followed by a long-term improvement (Labarre et al., 2020). Similarly, inter-annual changes in body condition of American alligators (*A. mississippiensis*) were linked to fluctuating hydrological regimes in the Everglades (Fujisaki et al., 2009).

Reproductive behavior is another crucial factor, with energy allocation differing significantly between males and females. Energy investment in reproduction varies between sexes due to different behavioral characteristics: males expend energy on courtship, mating, and territorial defense, while females focus on mating, nest building, egg laying and nest protection (Labarre et al., 2020; Thorbjarnarson, 1989). Similar seasonal and inter-annual patterns were noted in female American

alligators (*Alligator mississippiensis*), which had high body condition in the early breeding season during the spring dry season (Fujisaki et al., 2009). American crocodiles (*Crocodylus acutus)* exhibited lower body condition at the end of the dry season, aligning with nesting site selection and egg laying (Labarre et al., 2020). In the Brazilian Pantanal, *C. yacare* had lower body condition in the cool-dry season, concurrent with increased gonad activity, which is of high metabolic cost for females (Campbell et al., 2008; Coutinho, 2000). In Central Amazonia, *Caiman crocodilus* females had lower body condition during the dry season when nesting, compared to non-nesting females and males, while no such differences were observed for *Melanosuchus niger*, indicating species-specific nesting strategies influencing body condition (Barão-Nóbrega et al., 2018).

Feeding habits and seasonal dietary shifts further contribute to variations in crocodilian body condition, reflecting changes in resource availability and dietary composition. Changes in environmental conditions throughout the year are also associated with shifts in dietary composition and resource availability (Borteiro et al., 2009; Coutinho, 2000; Magnusson et al., 1987). The reduced body condition of *C. yacare* during dry season (July - September) is linked to low water levels, low temperatures, and low feeding success, with a diet primarily consisting of invertebrates (Coutinho, 2000). Conversely, during the hot-wet season (January - March) in the Pantanal, *C. yacare* exhibits increased body condition due to a diet rich in vertebrates (Coutinho, 2000). Low water levels also favor the body condition of American alligators (*A. mississippiensis*), as prey becomes more concentrated (Fujisaki et al., 2009). In Uruguay, adult *C. latirostris* display a predominant vertebrate diet during the hot season in the austral summer (January - March) (Borteiro et al., 2009). Seasonal dietary shifts from invertebrates to vertebrates significantly influence body condition and are typically related to changes in temperature and water availability (Borteiro et al., 2009; Coutinho, 2000; Hutton, 1987).

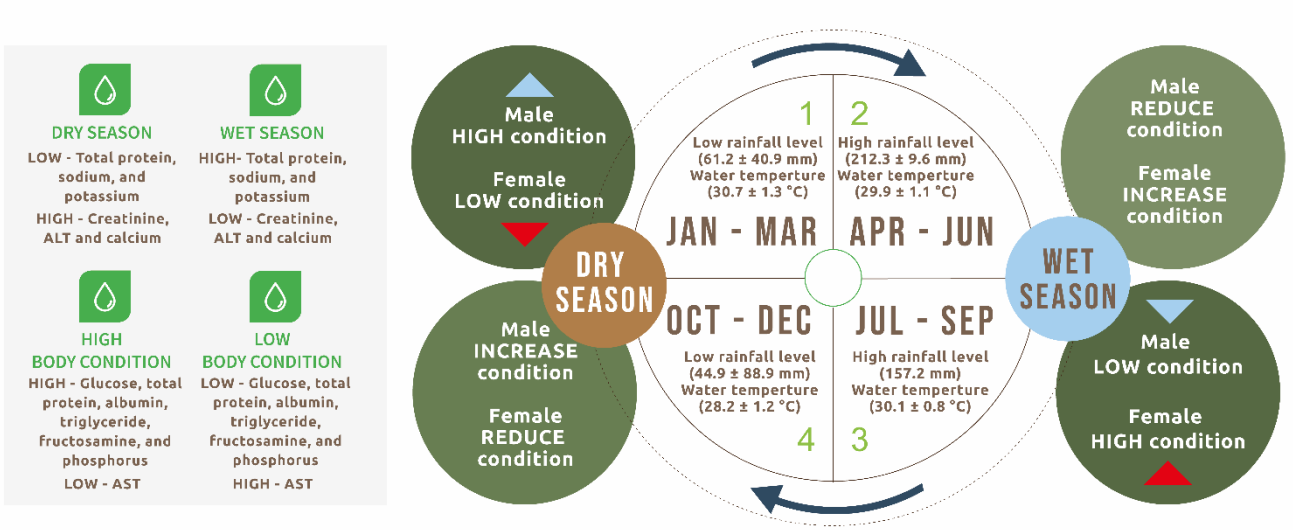


Figure 7. Diagram showing relationships between blood parameters, body condition, and environmental factors in *Caiman latirostris*. The left panel illustrates the relationships between blood biochemical markers, seasonal variations, and body condition, while the right panel highlights sex-specific associations between body condition and seasonal changes. The diagram also shows how body condition in males and females correlates with annual fluctuations in environmental conditions, including rainfall levels and water temperature. In parentheses are the mean values and standard deviations for total monthly rainfall and mean water temperature for each trimester.

Patterns on blood biochemistry responses

Body condition and size were associated with blood parameters related to energetic metabolism, with increased body condition and larger size linked to higher levels of glucose, total protein, albumin, triglycerides, and fructosamine. The positive relationship among body condition and blood parameters related to energetic metabolism, associated to seasonal and sex-specific behavioral changes, have already been observed in other crocodilians. For instance, *C. yacare* exhibits higher mean body condition during the hot-wet season, corresponding with increased glucose and triglyceride levels (Campbell et al., 2008). In *C. crocodilus*, nesting females show lower blood concentrations of glucose and triglycerides compared to non-nesting females and males, reflecting the metabolic costs of nest attendance (Barão-Nóbrega et al., 2018). Similarly, in captive *C. latirostris*, higher body condition is

476 linked to elevated glucose and albumin levels (Moleón et al., 2023). Size also plays a crucial role in
477 influencing blood parameters linked to energetic metabolism. Like in our study area, positive
478 correlations between size and total protein and albumin have also been reported for *C. latirostris*
479 (Moleón et al., 2023), Nile crocodiles *Crocodylus niloticus* (Lovely et al., 2007) and for *C. acutus*
480 (Balaguera-Reina et al., 2022). Among these, glucose and triglyceride are considered the primary
481 short and long-term energy sources for crocodilians (Dessauer, 1970).

482 These results may reflect differences in seasonal, sex-specific, and size-related ecological and
483 behavioral patterns influencing caiman physiology (Dessauer, 1970; Divers & Stahl, 2019; Grigg &
484 Kirshner, 2015; Hill et al., 2016; Kleiber, 1932). These patterns align with the seasonal and sex-
485 specific variations in body condition discussed earlier, highlighting the interplay between ecological
486 factors and physiological responses. The relationship of body condition and body size to blood
487 parameters related to energetic metabolism suggest that larger individuals may have different
488 metabolic demands and greater energy storage, as evidenced by their blood biochemistry. Greater
489 energetic reserves in larger individuals may benefit them with them with a larger metabolic scope —
490 the ability to elevate metabolic rates beyond maintenance levels (Killen et al., 2007). This expanded
491 metabolic scope allows larger individuals to sustain energetically demanding activities, such as
492 reproduction and migration providing them with a physiological advantage to cope with their ecological
493 demands. Additionally, the concept of weight-specific metabolic rate provides further explanation for
494 these differences. Smaller individuals exhibit higher specific metabolic rates, leading to faster
495 depletion of energetic reserves compared to larger animals (Hill et al., 2016; Kleiber, 1932). This could
496 explain the lower levels of glucose and albumin observed in smaller caimans, as their elevated energy
497 expenditure results in reduced reserves.

498 Conversely, higher AST levels, a liver marker of hepatic damage, were observed in individuals with
499 lower body condition. The negative relationship among body condition and AST levels may indicate
500 liver stress or muscle damage, particularly in wild individuals facing environmental challenges, due to
501 food restriction, changes in dietary composition, and long-distance movements. In wild *A.*
502 *mississippiensis* low body condition is also associated with elevated AST levels (Hamilton et al.,

503 2016). Here, AST levels exceeded maximum reference values for captive *C. latirostris* (Zayas et al.,
504 2011), drawing attention for differences among wild and controlled conditions of food, temperature,
505 and water availability.

506 Seasonal environmental shifts between wet and dry season were associated to variation in
507 parameters related to energetic metabolism (total protein), electrolyte balance (sodium, potassium,
508 calcium), renal (creatinine) and hepatic function (ALT). The dry season was linked to higher creatinine,
509 calcium and ALT levels. Elevated creatinine and ALT concentrations in the dry season can be
510 associated with low food intake and pathogen exposure, as these are related to protein catabolism
511 for energy supply, muscle damage, and liver and kidney dysfunction (Divers & Stahl, 2019). In captive
512 *C. latirostris*, creatinine values were higher in spring and summer, which were attributed to muscular
513 mas increment during growth in this period (Coppo et al., 2006). Additionally, the wet season
514 corresponded to higher levels of total protein, sodium, and potassium. Elevated total protein values
515 during the wet season could be correlated with high protein intake (Borteiro et al., 2009; Coutinho,
516 2000; Divers & Stahl, 2019), especially in larger individuals, as we found that protein levels are
517 positively associated to size; and by females, who exhibited higher body condition in this period.

518 Considering sexes, urea values were the only parameter that showed differences in concentrations
519 with males exhibiting higher values than females. Urea values, as well as creatinine, can vary in
520 reptiles according to hydration status, time after feeding (postprandial effects), hepatic and renal
521 disfunction, associated to increased activity and seasonal dehydration (Divers & Stahl, 2019).
522 Differences in urea values in captive *C. latirostris* among seasons (high in summer), and between
523 feeding systems, were attributed to diet (quantity, quality and frequency of the feeding) (Coppo et al.,
524 2006). In turn, in wild *C. latirostris*, there were no differences in urea values in response to diet
525 differences, and also to salinity, and although the effects of sex and seasonality were not measured,
526 it was suggested that changes in were related to water loss. Therefore, variation in urea values among
527 males and females could be physiological responses of sex-specific behaviors, as extensively
528 discussed in this paper, due to seasonal dietary differences, activity, and hydration status. (Coppo et
529 al., 2006; Divers & Stahl, 2019).

530 Differences in glucose, ALP, and GGT levels across study sites indicate that local habitat conditions
531 may influence the physiological health of *C. latirostris*. While glucose and GGT levels were higher in
532 Guaxuma lagoon, caimans in the APA Marituba reserve showed higher ALP values. While glucose
533 reflects nutritional status as it is a primary energy source; ALP is a marker for liver and bone disease;
534 and GGT is associated with liver and kidney diseases. Although our study did not directly measure
535 pollution levels or human disturbance, the observed variation in blood parameters is likely linked to
536 differences in habitat characteristics. For instance, APA Marituba, a sustainable use reserve, faces
537 pollution from sugarcane industries and overexploitation of resources, while RESEX Jequiá is
538 relatively less affected by these impacts. Guaxuma lagoon supports intense fish farming production,
539 which is associated to water eutrophication due to release of effluents from aquaculture (Herath &
540 Satoh, 2015).

541 Similar patterns in response to local habitat conditions have been documented for *C. latirostris* in and
542 for *C. acutus*. In southeast Brazil, *C. latirostris* exhibited elevated GGT levels in eutrophicated lakes
543 (Nóbrega, 2017; Nóbrega et al., 2022). In Florida, *C. acutus* populations highly human-influenced
544 areas showed elevated phosphorus levels (Balaguera-Reina et al., 2022). Although phosphorus
545 levels did not vary across our study sites, individuals with higher body condition displayed elevated
546 phosphorus levels [Mean (SD): 6.76 (3.28)], exceeding previously reported levels for *C. latirostris*
547 (Barboza et al., 2008; Bassetti & Verdade, 2014; Nóbrega, 2017; Nóbrega et al., 2022; Troiano et al.,
548 1997). In this context, our results suggests that the association between high body condition and
549 elevated phosphorus may reflect a complex interaction between diet and environmental
550 contamination rather than a straightforward indicator of better health. Both glucose and phosphorus
551 are absorbed through diet, and while elevated glucose typically indicates increased food intake,
552 elevated phosphorus levels may reflect metabolic responses to environmental eutrophication due to
553 agricultural runoff, sewage, and excess fish feed in aquaculture (Balaguera-Reina et al., 2022; Herath
554 & Satoh, 2015). This excess phosphorus can disrupt calcium-phosphorus balance, leading to health
555 issues such as tissue mineralization, weakened bones, and skeletal deformities (Divers & Stahl,
556 2019).

Such anthropogenic influences, particularly agricultural pollutants, have been extensively studied in *C. latirostris,* reinforcing its role as an indicator of environmental health (Poletta et al., 2008; Rojas-Hucks et al., 2022; Tavalieri et al., 2020). Numerous studies have demonstrated how pesticides and metals impact *C. latirostris* gene expression, reproductive health, growth, and survival across various life stages, highlighting significant consequences for natural populations and their habitats (Odetti et al., 2024; Rojas-Hucks et al., 2022; Santos et al., 2024). The present findings further contribute to this body of knowledge by providing clear evidence of the physiological effects of human impacts on the blood biochemistry—and ultimately the overall health—of free-ranging crocodilians. These results emphasize that presence/absence assessments alone are insufficient indicators of environmental health. Instead, they underscore the critical need for habitat protection measures that extend beyond preserving physical landscapes to include effective pollution control policies in agricultural regions.

Quantitative analysis of blood biochemistry values

Similar to our results, reported ranges and reference intervals for *C. latirostris* exhibit wide variability, both in wild populations (Bassetti, 2016; Grigg et al., 1998; Nóbrega et al., 2022) and in captive individuals (Barboza et al., 2008; Coppo et al., 2006; Moleón et al., 2023; Troiano et al., 1997; Zayas et al., 2011). This variation highlights important physiological and ecological insights about the species, and crocodilians in general (detailed values for *C. latirostris, C. yacare*, *C. crocodilus*, *P. palpebrosus*, and *M. niger*, are provided in Suplemmentary Table S4).

Parameters related to energetic metabolism, such as glucose, total protein, albumin, cholesterol, triglycerides, and fructosamine exhibited higher mean levels compared to previous studies on *C. latirostris* (Bassetti & Verdade, 2014; Moleón et al., 2023; Nóbrega et al., 2022; Zayas et al., 2011). These elevated levels are likely linked to increased energy demands driven by sex-specific seasonal movements, reproductive behavior, and changes in food availability. Liver enzymes such as FAL, ALT, AST also exhibited elevated mean values than those previously reported values for the species (Bassetti & Verdade, 2014; Moleón et al., 2023; Nóbrega et al., 2022; Zayas et al., 2011). This may indicate increased hepatic activity, potentially reflecting dietary shifts, food restriction, and exposure

583 to toxin and environmental stressors. As liver function plays a vital role in detoxification and metabolic
584 regulation, these markers are critical for assessing the health of individuals and populations,
585 particularly under varying habitat conditions. The wide variability of hepatic function markers (ALP,
586 ALT, AST, and GGT) and energetic metabolism indicators (glucose and triglyceride) reinforce the
587 complexity of interpreting blood parameters in *C. latirostris*, as these variations likely reflect the
588 species' adaptive responses to changing environmental and habitat condition, and seasonal dietary
589 changes.

590 Conversely, renal markers such as creatinine and urea displayed lower mean values compared to
591 previously reported ranges for free-range *C. latirostris* (Grigg et al., 1998; Nóbrega, 2017; Nóbrega
592 et al., 2022). Electrolyte balance parameters, including phosphorus, ionized calcium, sodium,
593 potassium, and chloride, exhibited significant variability. High phosphorus levels in individuals with
594 higher body may reflect differences in diet and habitat quality as discussed in the previous section.

595 Understanding these physiological responses in broader ecological contexts and how *C. latirostris*
596 copes with fluctuating with habitat conditions, is crucial for assessing the health and resilience of
597 populations, particularly in the face of human activities and extreme weather events. The present
598 results provide key insights into crocodilians physiology, particularly regarding blood biochemical
599 parameters, which exhibit significant intra- and interspecific variability (Dessauer, 1970). Similar to
600 other ectotherms like turtles and lizards, this variability arises from adaptive regulatory mechanisms
601 (Dessauer, 1970), and factors such as sex, ontogeny, diet composition, reproductive cycles, habitat,
602 and environmental conditions (e.g., temperature, rainfall, salinity) contribute to this variation. Our
603 findings align with existing data, revealing high variability in blood biochemical values and their
604 associations with predictor variables. In wild crocodilians, uncontrolled dietary and environmental
605 conditions make it difficult to establish normal reference values, emphasizing the need for further
606 research under varied conditions to better understand species-specific biological responses. The
607 crocodylia exhibit a remarkable evolutionary convergence, particularly morphologically. Patterns in
608 intra and interspecific shifts in body condition and, similar interspecific blood biochemistry responses
609 to biotic and abiotic factors in different geographical regions suggest a phylogenetic convergence in

610 the way crocodilians body condition and blood parameters behave in response to environmental
611 changes. A deeper understanding of these variations is critical for predicting how long-lived,
612 iteroparous species like crocodilians adapt to changing ecosystems.

613

614 **Conclusion**

615 Our study reveals substantial variability in body condition and blood biochemical values of free-range
616 *C. latirostris* in Alagoas Atlantic Forest, driven by factors such as sex, ontogeny, habitat, and seasonal
617 climatic changes. We identified sex-specific responses to seasonal and inter-annual weather
618 changes, with females showing higher body condition during the wet season, while males peaked
619 during the dry season. There was a positive relationship of body condition and size with energetic
620 metabolism markers, such as glucose, total protein, albumin, triglycerides, and fructosamine.
621 Conversely, there was a negative relationship between body condition and aspartate
622 aminotransferase (AST), indicating liver stress or muscle damage, likely caused by environmental
623 challenges or physiological stress. Seasonal rainfall also affected blood biochemistry: the dry season
624 was linked to higher creatinine, calcium and alanine aminotransferase levels; and the wet season
625 corresponded to higher levels of total protein, sodium, and potassium. Additionally, differences in
626 glucose, ALP, and GGT levels across study sites point to the potential physiological effects linked to
627 of human activities, including agricultural pollution and eutrophication, on free-ranging *C. latirostris*
628 populations. Values of blood components exhibited high variability, and some values observed in this
629 study exceeded reported ranges and intervals for *C. latirostris*, likely reflecting the species'
630 ecophysiological responses to seasonal and environmental fluctuations. These variabilities
631 emphasize the importance of interpreting physiological data within the context of local habitat and
632 environmental conditions. Importantly, our results demonstrate that the presence of *C. latirostris* in
633 anthropized areas does not necessarily indicate a healthy ecosystem, as physiological responses
634 may reveal underlying environmental degradation. In this context, there is an evident need for
635 conservation strategies that extend beyond species presence and habitat preservation to include

pollution control policies, ensuring the long-term health of crocodilian populations and their ecosystems. Overall, our study contributes to a deeper understanding of *C. latirostris* ecophysiology, with important implications for the conservation and management of crocodilian populations in both wild and captive conditions

Acknowledgements

We thank to the staff at RESEX Jequiá and APA Marituba; and to ICMBio; Brazil's São Francisco and Parnaíba valley development company (CODEVASF, Portuguese acronym); Centrovet Veterinary Diagnostic Center; and to the Postgraduate Program in Ecology, Conservation and Management of Wild Fauna at the Federal University of Minas Gerais. We are especially grateful for local community members who actively participated in this project, generously offering their personal fishing boats, piloting expertise, and field guidance – Special thanks to Jeciel, Isael, Rony, and Gugu from RESEX Jequiá; Lailson and Jó, from APA Mariituba; and Rafael, Alex and Nado from Guaxuma. Our research received funding from the Brazilian Biodiversity Fund (FUNBIO, Portuguese acronym) in collaboration with Instituto Humanize (046/2021), the IUCN-SSC Crocodilian Specialist Group, and the Rufford Foundation (31840-1). The research was authorized by the Biodiversity Authorization and Information System (SISBio 74257). Gabriela is supported by a doctoral scholarship granted by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brazil).

References

Alagoas. (2006). *Plano de Manejo da Área de Proteção Ambiental da Marituba do Peixe* (p. 217). Secretaria de Meio Ambiente e dos Recursos Hídricos (SEMARH). https://www2.ima.al.gov.br/app/uploads/2023/03/PLANO-DE-MANEJO_APA-DA-MARITUBA.pdf

Balaguera-Reina, S. A., Jennings, N. D., Godfrey, S. T., Brandt, L. A., Daykin, B., Squires, M. A., &

Mazzotti, F. J. (2022). Hematology and biochemistry reference intervals for American crocodiles (*Crocodylus acutus*) in South Florida. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.919488

Barão-Nóbrega, J. A. L., Marioni, B., Botero-Arias, R., Nogueira, A. J. A., Lima, E. S., Magnusson, W. E., Da Silveira, R., & Marcon, J. L. (2018). The metabolic cost of nesting: body condition and blood parameters of *Caiman crocodilus* and *Melanosuchus niger* in Central Amazonia. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *188*(1), 127–140. https://doi.org/10.1007/s00360-017-1103-8

Barboza, N. N., Mussart, N. B., Coppo, J. A., Fioranelli, S. A., & Koza, G. A. (2008). El medio interno de *Caiman latirostris* en cautiverio. Influencia del sexo, crecimiento y estación del año. *Revista Veterinaria*, *19*(1), 33–41.

Barros, A. H. C., Filho, J. C. de A., Silva, A. B. da, & Santiago, G. A. C. F. (2012). Climatologia do Estado de Alagoas. *Boletim de Pesquisa e Desenvolvimento, EMBRAPA Solos, 211*, 32. http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/950797

Bassetti, L. A. B. (2016). *Estado sanitário do jacaré-de-papo-amarelo (*Caiman latirostris) *em paisagens antropizadas no Estado de São Paulo* [Universidade de São Paulo]. https://www.teses.usp.br/teses/disponiveis/91/91131/tde-05072016-153031/publico/Luis_Antonio_Bochetti_Basset.pdf

Bassetti, L. A. B., & Verdade, L. M. (2014). Crocodylia (Jacarés e Crocodilos). In C. Z.S., S. J.C.R., & C.-D. J.L. (Eds.), *Tratado de animais selvagens* (2 ed., pp. 154–169). Roca.

Borteiro, C., Gutiérrez, F., Tedros, M., & Kolenc, F. (2009). Food habits of the Broad-snouted Caiman (*Caiman latirostris*: Crocodylia, Alligatoridae) in northwestern Uruguay. *Studies on Neotropical Fauna and Environment*, *44*(1), 31–36. https://doi.org/10.1080/01650520802507572

Brommer, J. E. (2000). The evolution of fitness in life-history theory. *Biological Reviews*, *75*(3), 377–

404. https://doi.org/10.1111/j.1469-185X.2000.tb00049.x

Campbell, H. A., Dwyer, R. G., Irwin, T. R., & Franklin, C. E. (2013). Home Range Utilisation and Long-Range Movement of Estuarine Crocodiles during the Breeding and Nesting Season. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0062127

Campbell, H. A., Micheli, M. A., & Abe, A. (2008). A seasonally dependent change in the distribution and physiological condition of *Caiman crocodilus* yacare in the Paraguay River Basin. *Wildlife Research*, *35*(2), 150–157. https://doi.org/10.1071/WR07169

Carvalho, A. L. de, Souza, J. L. de, Lyra, G. B., & Wanderley, H. S. (2017). Aplicação da cadeia de markov para dias secos e chuvosos. *Revista Brasileira de Meteorologia*, *32*(2), 207–214. https://doi.org/10.1590/0102-77863220001

CITES. (2023). *Notification to the Parties No. 2023/005. Amendments to Appendices I and II of the Convention adopted by the Conference of the Parties at its 19th meeting (Panama, 14 – 25 November 2022)*. https://cites.org/sites/default/files/notifications/E-Notif-2023-005.pdf

Coppo, J. A., Mussart, N. B., Barboza, N. N., Fioranelli, S. A., Koza, G. A., & Prado, W. R. (2006). Physiological changes in serum glucidic and nitrogenic analytes from captive Argentine authoctonous caimans. *Revista Veterinaria*, *17*(2), 103–108.

Coulson, R. A., & Hernandez, T. (1953). Glucose studies in crocodilia. *Endocrinology*, *53*, 311–320.

Coulson, R. A., & Hernandez, T. (1983). Alligator metabolism studies on chemical reactions in vivo. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *74*, 1–175.

Coutinho, M. E. (2000). *Population ecology and the conservation and management of* Caiman yacare in the Pantanal, Brazil. University of Queensland.

Coutinho, M. E., Marioni, B., Farias, I. P., Verdade, L. M., Vieira, T. Q., Magnusson, W. E., Campos, Z., Pires, I. P., Verdade, L. M., Bassetti, L., Mendonça, S. H. S. T. de, Vieira, T. Q., Magnusson, W. E., & Campos, Z. (2013). Avaliação do risco de extinção do jacaré-de-papo-

amarelo *Caiman latirostris* (Daudin, 1802) no Brasil. *Biodiversidade Brasileira*, *3*(1), 13–20. http://www.icmbio.gov.br/revistaeletronica/index.php/BioBR/article/view/403

Dessauer, H. C. (1970). Blood chemistry of reptiles: Physiological and evolutionary aspects. In C. Gans & T. C. Parsons (Eds.), *Biology of the Reptilia* (Vol. 3, pp. 1–71). Academic Press.

Divers, S. J., & Stahl, S. J. (2019). Mader's Reptile and Amphibian Medicine and Surgery. In *Mader's Reptile and Amphibian Medicine and Surgery*. https://doi.org/10.1016/C2014-0-03734-3

Filogonio, R., Assis, V. B., Passos, L. F., & Coutinho, M. E. (2010). Distribution of populations of broad-snouted caiman (*Caiman latirostris*, Daudin 1802, Alligatoridae) in the São Francisco River basin , Brazil. *Brazilian Journal of Biology*, *70*(4), 961–968.

Fujisaki, I., Rice, K. G., Pearlstine, L. G., & Mazzotti, F. J. (2009). Relationship between body condition of American alligators and water depth in the Everglades, Florida. *Hydrobiologia*, *635*(1), 329–338. https://doi.org/10.1007/s10750-009-9925-3

Grigg, G. C., Beard, L. A., Moulton, T., Melo, M. T. Q., & Taplin, L. E. (1998). Osmoregulation by the broad-snouted caiman, *Caiman latirostris*, in estuarine habitat in southern Brazil. *J Comp Physiol B*, *168*, 445–452.

Grigg, G. C., & Kirshner, D. (2015). *Biology and evolution of crocodylians*. CSIRO Publishing. https://doi.org/10.1146/annurev.ento.52.110405.091303

Gutberlet, J., Seixas, C. S., Thé, A. P. G., & Carolsfeld, J. (2007). Resource conflicts: Challenges to fisheries management at the São Francisco River, Brazil. *Human Ecology*, *35*(5), 623–638. https://doi.org/10.1007/s10745-007-9132-7

Hamilton, M. T., Kupar, C. A., Kelley, M. D., Finger, J. W., & Tuberville, T. D. (2016). Blood and plasma biochemistry reference intervals for wild juvenile American alligators (*Alligator mississippiensis*). *Journal of Wildlife Diseases*, *52*(3), 631–635. https://doi.org/10.7589/2015-

734 10-275

735 Herath, S. S., & Satoh, S. (2015). Environmental impact of phosphorus and nitrogen from
736 aquaculture. In *Feed and feeding practices in aquaculture* (pp. 369–386). Woodhead
737 Publishing.

738 Hill, R. W., Wyse, G. A., & Anderson, M. (2016). *Animal Physiology* (S. Associates (ed.); 4th ed.).
739 Oxford University Press.

740 Hutton, J. . M. . (1987). Growth and feeding ecology of the nile crocodile *Crocodylus niloticus* at
741 Ngezi, Zimbabwe. *Journal of Animal Ecology*, *56*(1), 25–38.

742 Jakob, E. M., Marshall, S. D., & Uetz, G. W. (1996). Estimating Fitness: A Comparison of Body
743 Condition Indices. *Oikos*, *77*(1), 61–67. https://doi.org/10.2307/3545585

744 Killen, S. S., Costa, I., Brown, J. A., & Gamperl, A. K. (2007). Little left in the tank: Metabolic scaling
745 in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B:*
746 *Biological Sciences*, *274*(1608), 431–438. https://doi.org/10.1098/rspb.2006.3741

747 Kleiber, M. (1932). Body Size and Metabolism. *Hilgardia Journal of Agricultural Science*, *6*(11), 315–
748 353.

749 Labarre, D., Charruau, P., Parsons, W. F. J., Larocque-Desroches, S., & Gallardo-Cruz, J. A.
750 (2020). Major hurricanes affect body condition of American crocodile *Crocodylus acutus*
751 inhabiting Mexican Caribbean islands. *Marine Ecology Progress Series*, *651*, 145–162.
752 https://doi.org/10.3354/meps13425

753 Labocha, M. K., Schutz, H., & Hayes, J. P. (2014). Which body condition index is best? *Oikos*,
754 *123*(1), 111–119. https://doi.org/10.1111/j.1600-0706.2013.00755.x

755 Lovely, C. J., Pittman, J. M., & Leslie, A. J. (2007). Normal haematology and blood biochemistry of
756 wild Nile crocodiles (*Crocodylus niloticus*) in the Okavango Delta, Botswana. *Journal of the*
757 *South African Veterinary Association*, *78*(3), 137–144. https://doi.org/10.4102/jsava.v78i3.305

758 Magnusson, W. E., Vieira Da Silva, E., & Lima, A. P. (1987). Diets of Amazonian Crocodilians.
759 *Journal of Herpetology*, *21*(2), 85–95.

760 Marques, T. S., Bassetti, L. A. B., Lara, N. R. F., Millan, C. H., Piña, C. I., & Verdade, L. M. (2016).
761 Population structure of the broad-snouted caiman (*Caiman latirostris*) in natural and man-made
762 water bodies associated with a silvicultural landscape. *Salamandra*, *52*(1), 1–10.

763 Mazzotti, F. J., Best, G. R., Brandt, L. A., Cherkiss, M. S., Jeffery, B. M., & Rice, K. G. (2009).
764 Alligators and crocodiles as indicators for restoration of Everglades ecosystems. *Ecological*
765 *Indicators*, *9*(6 SUPPL.), 137–149. https://doi.org/10.1016/j.ecolind.2008.06.008

766 McNamara, J. M., & Houston, A. I. (1996). State-dependent life histories. *Nature*, *380*, 215–221.
767 https://doi.org/10.2173/bow.yebtit4.01

768 Moleón, M. S., Santiago, G., Hilevski, S., & Siroski, P. A. (2023). Blood biochemistry parameters of
769 Broad snouted caiman, *Caiman latirostris* subjected to stress conditions, appliying micro-
770 volume techniques. *Journal of Experimental Zoology Part A: Ecological and Integrative*
771 *Physiology*, *April*, 1–6. https://doi.org/10.1002/jez.2705

772 Murray, C. M., Easter, M., Merchant, M., Cooper, A., & Crother, B. I. (2013). Can reproductive
773 allometry assess population marginality in crocodilians? A comparative analysis of gulf coast
774 american alligator (*Alligator mississippiensis*) populations. *Copeia*, *2*, 268–276.
775 https://doi.org/10.1643/CH-11-136

776 Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity
777 hotspots for conservation priorities. *Nature*, *403*(6772), 853–858.
778 http://www.ncbi.nlm.nih.gov/pubmed/10706275

779 Nóbrega, Y. C. (2017). *Avaliação da saúde de jacarés-de-papo-amarelo (*Caiman latirostris*) em*
780 *condições in situ e ex situ no Espírito Santo, sudeste do Brasil.* Universidade Vila Velha – ES.

781 Nóbrega, Y. C., Curbani, F., Paulino Alvarenga, F., Quadros de Menezes, P., Targino da Silva, T.,

Abreu da Fonseca, L., De Deus Santos, M. R., & Srbek-Araujo, A. C. (2022). Baseline data on hematological and biochemical parameters for the use of wild juvenile broad-snouted caiman (*Caiman latirostris*) as bioindicators in Atlantic forest, southeastern Brazil. *Science and Animal Health*, *9*(3), 230–245. https://doi.org/10.15210/sah.v9i3.22522

Odetti, L. M., Paravani, E. V., Simoniello, M. F., & Poletta, G. L. (2024). Micronucleus test in reptiles: Current and future perspectives. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *897*(May), 503772. https://doi.org/10.1016/j.mrgentox.2024.503772

Passos, L. F., Coutinho, M. E., & Young, R. J. (2014). Demographic and growth analysis of broad snouted caiman (*Caiman latirostris*) in a disturbed environment in southeastern Brazil. *Herpetological Journal*, *24*, 223–228.

Poletta, G. L., Larriera, A., Kleinsorge, E., & Mudry, M. D. (2008). *Caiman latirostris* (broad-snouted caiman) as a sentinel organism for genotoxic monitoring: Basal values determination of micronucleus and comet assay. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *650*(2), 202–209. https://doi.org/10.1016/j.mrgentox.2007.12.001

R Core Team. (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. http://www.r-project.org

Rodrigues, C. F. S. N., Barboza, R. S. L., Maranhão, E. S., & Correia, J. M. S. (2021). Communal nesting of broad-snouted caiman, *Caiman latirostris* (Daudin, 1802), in a protected area of Atlantic Forest in northeastern Brazil. *Herpetology Notes*, *14*(April), 677–680.

Rojas-Hucks, S., Rodriguez-Jorquera, I. A., Nimpstch, J., Bahamonde, P., Benavides, J. A., Chiang, G., Pulgar, J., & Galbán-Malagón, C. J. (2022). South American National Contributions to Knowledge of the Effects of Endocrine Disrupting Chemicals in Wild Animals: Current and Future Directions. *Toxics*, *10*(12). https://doi.org/10.3390/toxics10120735

Santos, R. L. dos, Mascarenhas-Júnior, P. B., Dos Santos, E. M., de Moura, G. J. B., Diniz, G. T.

807 N., & Correia, J. M. de S. (2024). Nondestructive Evaluation of Metal Bioaccumulation and
808 Biochemical Biomarkers in Blood of Broad-Snouted Caiman (*Caiman latirostris*) from
809 Northeastern Brasil. *Environmental Toxicology and Chemistry*, *00*(00), 1–18.
810 https://doi.org/10.1002/etc.5823

811 Siroski, P., Bassetti, L. A. B., Piña, C. ., & Larriera, A. (2020). *Caiman latirostris. The IUCN Red List*
812 *of Threatened Species 2020*. https://doi.org/https://dx.doi.org/10.2305/IUCN.UK.2020-
813 3.RLTS.T46585A3009813.en

814 Somaweera, R., Nifong, J., Rosenblatt, A., Brien, M. L., Combrink, X., Elsey, R. M., Grigg, G. C.,
815 Magnusson, W. E., Mazzotti, F. J., Pearcy, A., Platt, S. G., Shirley, M. H., Tellez, M., van der
816 Ploeg, J., Webb, G., Whitaker, R., & Webber, B. L. (2020). The ecological importance of
817 crocodylians: towards evidence-based justification for their conservation. *Biological Reviews*,
818 *March*. https://doi.org/10.1111/brv.12594

819 Tavalieri, Y. E., Galoppo, G. H., Canesini, G., Luque, E. H., & Muñoz-de-Toro, M. M. (2020). Effects
820 of agricultural pesticides on the reproductive system of aquatic wildlife species, with
821 crocodilians as sentinel species. *Molecular and Cellular Endocrinology*, *518*(January).
822 https://doi.org/10.1016/j.mce.2020.110918

823 Thorbjarnarson, J. B. (1989). Ecology of the American crocodile (Crocodylus acutus). In P. M. Hall
824 (Ed.), *Crocodiles: their ecology, management, and conservation* (pp. 228–259). IUCN
825 Publication New Series.

826 Troiano, J. C., Althaus, R. L., & Malinskas, G. (1997). Perfil bioquímico sanguíneo de de las
827 especies argentinas del género Caiman en condiciones de cautividad. *Revista Española de*
828 *Herpetología*, *11*, 31–34.

829 Verdade, L. M. (2001). The São Francisco River 'codfish': the northernmost populations of the
830 broad-snouted caiman (*Caiman latirostris*). *Crocodile Specialist Group Newsletter*, *20*, 80–82.

831 Zayas, M. A., Rodríguez, H. A., Galoppo, G. H., Stoker, C., Durando, M., Luque, E. H., & Muñoz-de-

Toro, M. (2011). Hematology and blood biochemistry of young healthy broad-snouted caimans (*Caiman latirostris*). *Journal of Herpetology*, *45*(4), 516–524. https://doi.org/10.1670/10-158.1

List of figures

Figure 7. Diagram showing relationships between blood parameters, body condition, and environmental factors in *Caiman latirostris*. The left panel illustrates the relationships between blood biochemical markers, seasonal variations, and body condition, while the right panel highlights sex-specific associations between body condition and seasonal changes. The diagram also shows how body condition in males and females correlates with annual fluctuations in environmental conditions, including rainfall levels and water temperature. In parentheses are the mean values and standard deviations for total monthly rainfall and mean water temperature for each trimester.