Hematological values of wild *Caiman latirostris* (Daudin, 1802) in the Atlantic Rainforest in Pernambuco, Brazil

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Abstract. Hematological studies in crocodilians are important tools in the evolutionary diagnosis and control of sicknesses, such as anaemia, malnutrition, dehydration, inflammation, and parasitism, among others. We aimed to obtain reference intervals for the hemogram of *Caiman latirostris* in wild populations that inhabit Recife's Metropolitan Region, Pernambuco. We obtained blood samples from 42 caimans, from different sexes (22 males and 20 females) and ages classes (eight hatchlings, 24 subadults and 10 adults) in two areas of Atlantic Rainforest domain. We found that hematological parameters were included within the reference intervals for other crocodilian species. It was possible to observe differences between the areas for the mean corpuscular volume values, suggesting a possible difference between adult and juvenile individuals in the two study areas. When comparing sexes, there was no significant difference between the study parameters, but it was possible to observe differences in the mean corpuscular volume, mean corpuscular hemoglobin and hemoglobin in the Estação Ecológica de Tapacurá region. Although small differences have been observed between the two populations, we can infer that the hematological parameters are similar. We can use this information to evaluate animal's health in nature and for comparations with captive individuals, allowing the establishment of ideal maintenance conditions and assisting in the identification of possible pathologies.

Keywords. Broad-snouted Caiman, crocodilians, hematimetric indices, hematology.

INTRODUCTION

The broad-snouted caiman (*Caiman latirostris*) occurs in rivers, mangroves and flooded areas in Argentina, Bolivia, Brazil, Paraguay, and Uruguay. In Brazil, this species is found in the Cerrado, Caatinga, Atlantic Forest and Pampas biomes, from the coastal region of Rio Grande do Norte, distributed through the basins of the rivers São Francisco and Paraná/Paraguay to Rio Grande do Sul (Coutinho et al., 2013). In the wild, these caimans can live in large aggregations or in small groups and are always present in the mangrove areas of rivers, lagoons, wetlands, and lakes (Filogonio et al., 2010). This species, classified as Least Concern (LC), presents a large geographical area of distribution and an apparent ability to colonise anthropic environments (Júnior et al., 2018). However, anthropic pressure caused by the constant growth of human populations which, to a certain extent, triggers the destruction of their habitat, mainly through the drainage of water bodies, deforestation, pollution,

intensive pesticide use, as well as illegal hunting in certain regions. These impacts can affect connectivity and consequently gene flow between populations on a micro and macrogeographic scale, with such processes resulting in the decline of populations (Coutinho et al, 2013; Bassetti and Verdade, 2014).

Reference hematological and blood biochemistry values are necessary for detecting the effects of environmental, infectious, parasitic, or toxicological stress in these animals, providing information on their health and therefore, can be used as a rapid diagnostic tool (Heatley and Russel, 2019). Hematological studies in wild or captive broad-snouted caimans have a scientific, educative and production improvement outcomes, and can be applied to conservation, reproduction, and reintroduction projects (Barboza et al., 2006; Adelakun et al., 2017). Blood analysis is a relatively non-invasive method which can provide important clinical data as well as information about the physiological conditions and health of animals (Padilha et al., 2011; Adelakun et al., 2017). The hemogram is comprised of the determination of the total erythrocyte count, hematocrit, hemoglobin concentration and hematological indices such as the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (Saggese, 2009; Heatley and Russel, 2019). The erythrogram data serve to aid in the evolutionary diagnosis, characterisation, and control of sicknesses, such as anaemia, malnutrition, dehydration, inflammation, and parasitism among others (Barboza et al., 2007; Saggese, 2009; Heatley and Russel, 2019).

The clinical interpretation of the leukogram is challenging for many reasons, primarily because of the strict reference intervals, including the total leukocyte count and smear cell percentages which are not available for all species of healthy reptiles (Campbell, 2006; Saggese, 2009; Zayas et al., 2011). Normal hematological values for reptiles, including crocodilians, when determined by different laboratories, demonstrate ample inter and intraspecific variation due to the differences in blood sample collection, handling of the specimen, analysis techniques, physiological state of the reptiles, age, sex, nutrition, population dynamics, environmental conditions and use of anaesthetics (Stacy and Whitaker, 2000; Campbell, 2006; Saggese, 2009; Zayas et al., 2011). Reference intervals can be found for several reptile species, including crocodilians (Stacy and Whitaker, 2000; Padilha et al., 2011; Zayas et al., 2011). Since the mean hematological parameter values can vary between species, the reference values obtained for healthy animals can serve as a guide for dealing with sick animals (Stacy and Whitaker, 2000). Lastly, the effects on the leukocyte

response to bacterial, fungal, viral, and parasitic infections or stress agents have been minimally investigated for this species (Heatley and Russel, 2019). Thus, when referring to a set of reference values, it is important to determine the conditions in which the blood samples were obtained. Understanding and recognising possible variations in hematological results for reptiles contributes to a critical interpretation of the published reference values and their clinical significance. Thus, the aim of this study was to describe the morphological characteristics of the peripheral blood of *C. latirostris* and establish erythrogram reference indices for this species in wild protected areas in Recife's Metropolitan Region.

MATERIAL AND METHODS

Study area

Between 2014 and 2015, we collected samples in water bodies located in the municipalities of Recife and São Lourenço da Mata, both belonging to the Recife's Metropolitan Region (RMR), the main socio-economic centre of the state of Pernambuco, Brazil. The region is dominated by Atlantic Forest and the climate is tropical and humid, with autumn-winter rains (Alvares et al., 2013). Furthermore, the area has a large water network (Carvalho, 2004) with the Capibaribe river, and great influence on RMR, occupying an area of 7,545.88 km^2, which represents 7.6% of Pernambuco (APAC, 2020).

In the municipality of São Lourenço da Mata, we collected samples in the Estação Ecológica de Tapacurá (EET) reservoir (8°2'S, 35°11'E), a lentic environment in a rural area, formed by the damming of the Tapacurá river. The water body is approximately 7.5 km^2 and is surrounded by sugar cane matrices (Almeida and Oliveira, 2009), open areas, agricultural areas, livestock, and riverside communities (Mascarenhas-Junior et al., 2020). It also has 5.35 km^2 of forest fragments, of which 1.72 km^2 belong to Conservation Units of Integral Protection (Mata do Camucim, Mata do Toró, Mata do Outeiro de Pedro) (CPRH, 2020) (Fig. 1). In the reservoir areas, there are also constant fishing activities, with recurring recordings of bycatch of local fauna (Mascarenhas-Junior et al., 2018; Santos et al., 2020).

Approximately 25 km from Tapacurá, we also collected samples from caimans located in the Parque Estadual de Dois Irmãos (PEDI) (8°0'S, 34º56'E), in the municipality of Recife, capital of Pernambuco. The area comprises approximately 11.6 km^2 of protected forests (Mata de Dois Irmãos, 3.8 km^2; Fazenda Brejo dos Macacos, 7.8 km^2) and in these fragments there is approximately 1.4 km^2 of water bodies forming the Prata microbasin, the Açude do Meio (~0.024 km^2), Açude do Prata (~0.025 km^2), Açude de Dentro (~0.015 km^2) and Açude de Dois Irmãos (~0.135 km^2). Despite this area is in an urban environment with anthropic pressures, the presence of a mature marginal forest aids in the maintenance of the water quality (Lima, 2004). In this study, we only accessed the Açude de Dentro and Açude de Dois Irmãos.
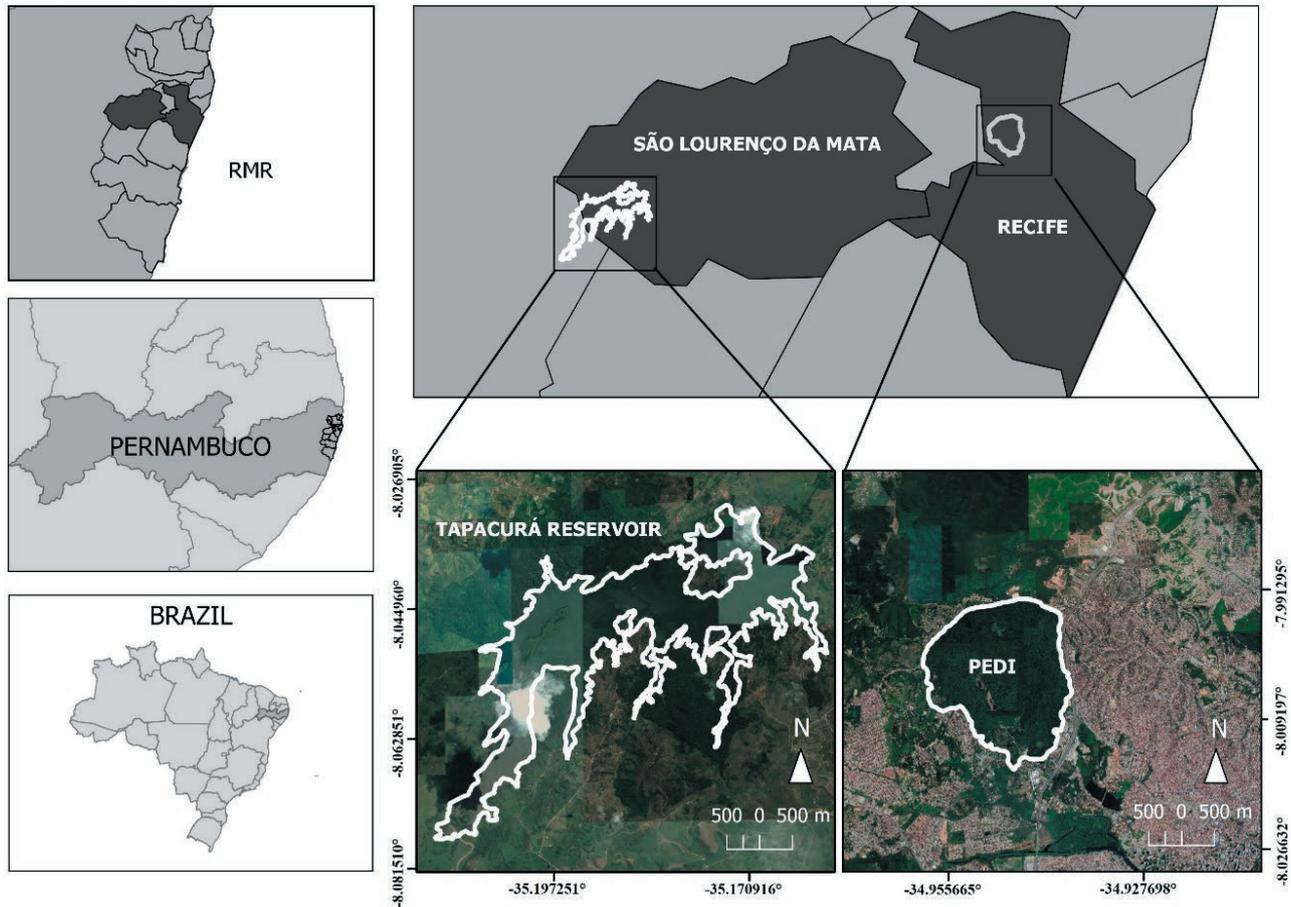
Fig. 1. Recife's Metropolitan Region (RMR) with Tapacurá reservoir and Parque Estadual de Dois Irmãos highlighted in white lines.

Data collection

Captures were performed bimonthly between 2014 and 2015, with active captures during the nocturnal period and with the aid of aluminium boats. The individuals were identified in the environment through the reflection of eyeballs (*tapetum lucidum*) from the interception of a beam of concentrated light using a spotlight (Magnusson, 1995). The captures were performed manually or with the use of cables snares connected to a telescopic pole (up to 5 m). Additionally, aquatic funnel traps with baits to attract the caimans were used. All physical post-capture restraint was performed using adhesive tapes.

The caimans were evaluated in terms of trauma, body scars and the absence of clinical signs and symptoms of diseases. Following this evaluation, blood samples were collected through supravertebral occipital venous sinus punctures using 20-gauge needles without anticoagulants and disposable needles. Immediately following the collection of samples, blood smears were prepared, air dried and stained with modified May-Grünwald-Giemsa colouration (Rosenfeld, 1947). A small volume of blood (0.5 mL) was collected in a microtube containing heparin for the determination of hematological parameters. The total hemocyte count (THC), total leukocyte count (TLC) and total thrombocyte count (TTC) were performed manually in a Neubauer chamber after 10 mL of blood being diluted in 2.0 mL of Natt and Herrick solution. The hematocrit (HT) was determined using the microhematocrit method, through the centrifugation of microcapillaries at 10000 rpm (Coles, 1986) and the hemoglobin concentration (HB) was determined using the cyanmethemoglobin method, mixing 20 mL of blood with 2.5 mL of Drabkin solution (Labtest Diagnostica®). Through conventional calculations, hematometric indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were calculated. To count the differential leukocytes, a total of 100 cells were counted using a microscope under immersion (1000X).

After the collection of the blood material, the individuals were measured, weighed, sexed, and marked. The size class was determined through the snout-vent length (SVL), following the proposal by Leiva et al. (2019): Class I or hatchlings (< 25cm); Class II or subadults (25 cm to 67.9 cm); Class III (68 cm to 99.9 cm) and Class IV (over 99 cm) or adults. The weight was determined using scales with a limit of 40 kg. Furthermore, sex was determined for larger individuals using a cloacal palpation technique (Yanosky, 1990) and, in smaller caimans, surgical tweezers were inserted into the cloaca to separate the edges for

the observation of the genitalia (Webb et al., 1984). Lastly, all the individuals were marked with cuts in the scales of the single and double crests and with the subcutaneous implantation of microchips. At the end of data collection, caimans were released back into the water bodies from where they were captured.

Data analysis

To test the normality and homogeneity of the variables included in the study, we used Shapiro Wilk and Bartlett tests, respectively. For the analyses of the quantitative variables, we used a Bartlett test for homogeneity and the Shapiro Wilk test for the normality of the variables included in the study. The mean differences for the independent variables were evaluated using an ANOVA followed by Tukey's post-hoc test when observing the presumption of homogeneity. Otherwise, Kruskal-Wallis tests were used followed by the post-hoc test using Fisher's criteria of the lowest significant difference (LSD). A student t test was also used to observe when there was a presumption of normality, and when there was not, a Mann-Whitney test was used to evaluate the medians of the variables included in the study. We used categorical analyses for the comparative analysis between the qualitative variables, such as Pearson's χ^2 test andm when necessary, Fisher's exact test. All the conclusions were taken at a significance level of 5%. The R software (R Core Team, 2021) was used for the evaluation of the study results.

RESULTS

Cells morphological aspects

The erythrocytes presented an elliptical shape, with abundant cytoplasm, acidophilic, pinkish in colour, occupying approximately 80% of the cell. The nucleus presented condensed chromatin, basophilia, predominantly elliptical and occupies a central position in the cell. Larger erythrocytes with spherical nuclei and more rounded forms were also occasionally found (Fig. 2a). No intracellular inclusions or hemoparasites were observed.

The thrombocytes were predominantly elliptical with abundant cytoplasm, hyaline, also presenting a less elongated shape with little cytoplasm. Eventually, azurophilic granules were observed in the cytoplasm. The predominantly elliptical nucleus can present morphological variations, with chamfers, indentation or grooves, violet in colour with a central location (Fig. 2a, 2f and 2h).

The lymphocytes presented a large variation in terms of size and shape. The presence of irregularly shaped or spherical cells was common. The cytoplasm is scarce, basophilic, with azurophilic granules, commonly exhibiting cytoplasmic projections (Fig. 2a, 2b and 2g).
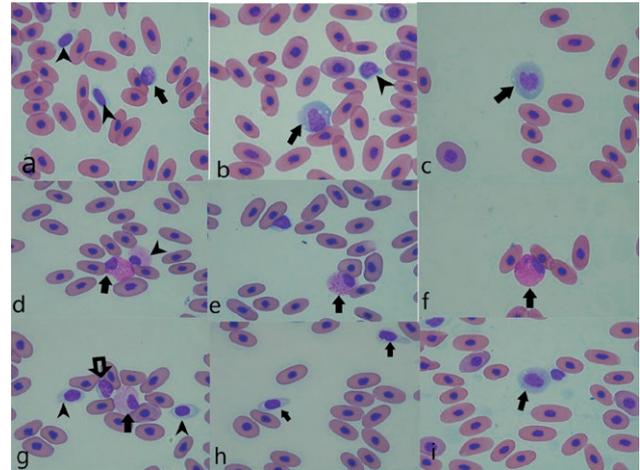

Fig. 2. Photomicrographs of *Caiman latirostris* blood cells coloured with modified May-Grunwald-Giemsa (Rosenfield, 1947) (magnification 1000X). (a) Mature erythrocytes in high quantity, lymphocytes (arrow), thrombocytes (head of arrow); (b) monocytes (arrow), lymphocyte (head of arrow); (c) monocyte; (d) eosinophil (arrow), heterophil (head of arrow); (e) heterophil; (f) eosinophil; (g) heterophile (arrow), thrombocytes (head of arrow), lymphocyte (outlined arrow); (h) thrombocytes; (i) monocytes with vacuoles.

The monocytes presented a spherical to oval shape, often presenting an irregular outline (Fig. 2b, 2c and 2i). The pale to moderately basophilic cytoplasm may contain cytoplasmic vacuoles of varying sizes. The nucleus may be spherical, oval or even U-shaped, generally with irregular outlines, occupying an off-centre position.

The eosinophils generally have a spherical shape. The cytoplasm is abundant and homogenously filled by acidophilic granules which are compact, spherical, oval or slightly elongated, pinkish in colour with a relatively homogenous colour. The nucleus is violet in colour and is generally spherical or lenticular and is in an off-centre position and may often be bilobulated (Fig. 2d and 2f).

The heterophils are large and are spherical, oval or irregular. The cytoplasm is generally abundant, full of compact granules whose acidophilia varies in its intensity, depending on the granule aspect and is of a dark pink or salmon colour. In terms of their morphology, the granules have varying aspects, both in terms of their colour intensity and their shape, where they can be spherical, fusiform with intense colouration or even stick, drumstick or oval shaped. There is a clear predominance of fusiforms compared to the others. The spherical nucleus is located off-centre or peripherally (Fig. 2d, 2e and 2g).

The basophils have a spherical shape and are smaller when compared to the other granulated leukocytes. The cytoplasm presents strongly basophilic spherical gran-

Table 1. Hematological reference values for wild *Caiman latirostris* and differences between the two study areas (Pernambuco, Brazil). SD (Standard deviation), P (p value), SVL (Snout-Vent Length), TL (Total Length), TEC (Total Erythrocyte Count), TLC (Total Leukocyte Count), HB (Hemoglobin), Ht (Hematocrit), MVC (Mean corpuscular volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), TTC (Total Thrombocyte Count).

Parameter	PEDI (n = 22)			EET (n = 20)			P
	Mean	SD	Range	Mean	SD	Range	
SVL (cm)	47.2	36.14	17.3-100	58.88	25.5	17.8-100.04	0.09
TL (cm)	81.77	41.62	36-209	106.31	60.64	28-211	0.30
Weight (g)	5287	11934	125-51380	8140	12030	114.2-38300	0.42
TEC (10^3 cells/mm^3)	178.86	73.58	30-355	219.75	63.58	120-345	0.06
TLC (10^3 cells/mm^3)	5.23	2.87	0.75-11.75	3.84	2.01	1.25-8.25	0.07
HB (g/dl)	6.85	0.82	5.2-8.0	7.22	0.6	5.8-8	0.11
Ht (%)	20.18	6.13	3-29	21.5	3.61	15-29	0.40
MCV (fl)	1.53	1.18	0.76-5.45	1.02	0.18	0.75-1.42	**0.04**
MCH (pg)	0.5	0.4	0.22-2	0.35	0.09	0.22-0.59	0.15
MCHC (%)	42.66	40.91	25-223.3	33.9	4.33	22.3-41.76	0.67
TTC (10^3 cells/mm^3)	2.98	1.94	0.5-8.37	4.5	1.59	1.5-7	**0.01**
Lymphocytes (%)	54.32	12.37	33-81	51.5	12.95	31-77	0.48
Monocytes (%)	5.18	2.72	1.0-9.0	3	2.18	0-9	**0.01**
Basophils (%)	2.73	1.75	0-5	1.4	1.79	0-8	**0.01**
Heterophils (%)	34.82	12.94	6.0-60.0	41.8	13.83	9.0-63.0	0.10
Eosinophils (%)	2.95	1.73	0-6	1.8	1.4	0-5	**0.02**

ules of varying sizes and when located on top of the nucleus it can be impossible to distinguish between their outlines.

General parameters of caimans

Blood samples from 42 caimans were collected, with 22 from PEDI and 20 from EET. The sex proportions of the captured individuals were 22 males (PEDI = 12, EET = 10, 52.38%), 18 females (PEDI = 10, EET = 8, 42.86%) and 2 undetermined individuals (4.76%).

From the total number of animals, 10 were adults (PEDI = 3, EET = 7, 23.9%), eight hatchlings (PEDI = 5, EET = 3, 19.0%) and 24 subadults (PEDI = 14, EET = 10, 57.1%). Between the two locations it was possible to observe differences in the capture of different age classes, with a greater number of adults in the EET and more subadults in PEDI.

The biometric and hematological parameters collected for *Caiman latirostris* in the two locations are presented in Table 1. The SVL varied from 17.30 cm to 100.04 cm and the weight varied from 114.2 g to 51,380 g. It was possible to observe differences in the hematological parameters for the different areas for the values of MCV (P = 0.04), thrombocytes (P ≤ 0.01), monocytes (P = 0.01), basophils (P = 0.01) and eosinophils (P = 0.02; Table 1).

The hematological data and their reference intervals for the different age groups are established in Table 2 and Table 3. The MCV values did not indicate difference between adult and subadult individuals (P = 0.06). In the comparisons between the areas, differences in the values for hatchling thrombocytes (P = 0.04) and adult eosinophils (P = 0.03) were identified (Table 2 and Table 3).

For the comparison between the sexes in the samples, there was no significant difference between the hematological parameters, with the only significant difference being for TL (P = 0.04). However, when performing this evaluation between individuals of the same area, it was possible to observe differences in the red blood cells (P = 0.02), MCV (P < 0.01), MCH (P = 0.01) and monocytes (P = 0.01) in Tapacurá and for the hemoglobin values in the PEDI (P = 0.02) with higher values for red blood cells in females. The other parameters, MCV, MCH, monocytes and hemoglobin, were higher for males (Table 4 and Table 5).

DISCUSSION

In general, the hematological parameters found for *Caiman latirostris* in wild environments in the State of Pernambuco fall within the reference intervals for other species of crocodilians (Stacy and Whitaker, 2000; Padil-

Table 2. Hematological reference values for different age groups of wild *Caiman latirostris* (Parque Estadual de Dois Irmãos, Pernambuco, Brazil). SD (Standard deviation), P (p value), SVL (Snout-Vent Length), TEC (Total Erythrocyte Count), TLC (Total Leukocyte Count), HB (Hemoglobin), Ht (Hematocrit), MVC (Mean corpuscular volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), TTC (Total Thrombocyte Count).

Parameter	Adults (n = 3)			Hatchlings (n = 5)			Subadults (n = 14)			P
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
SVL (cm)	99.13	62.39	70-109	22.17	6.48	17.3-21.0	44.25	4.83	32.4-50.0	**<0.01**
Weight (g)	23553	21138	3834-51380	330	370.63	125-1140	1799	965.94	166-3210	**<0.01**
TEC (10^3 cells/mm³)	171.25	101.77	30-255	175.71	26.21	150-210	183.64	88.29	55-355	0.28
TLC (10^3 cells/mm³)	5.87	3.05	1.5-8.5	4.86	1.04	3.5-6.5	5.23	3.69	0.7-11.75	0.34
HB (g/dl)	6.45	0.7	6-7.5	7.11	0.73	5.8-8	6.84	0.91	5.2-8	0.44
Ht (%)	18.5	3.42	14-22	21.57	4.72	12-26	19.91	7.71	47178	0.80
MCV (fl)	1.88	1.87	0.76-4.67	1.24	0.28	0.80-1.73	1.58	1.31	0.82-5.45	0.22
MCH (pg)	0.73	0.85	0.26-2	0.41	0.03	0.37-0.46	0.48	0.3	0.22-1.21	0.16
MCHC (%)	35.45	5.74	30.5-42.86	34.21	6.8	26.54-48.34	50.66	57.78	25-223	0.86
TTC (10^3 cells/mm³)	2.75	2.01	0.75-5.5	2.54	0.82	1-3.5	3.35	2.45	0.5-8.37	**0.04**
Lymphocytes (%)	43.25	8.88	35-55	59.29	8.12	48-68	55.18	13.82	33-81	0.22
Monocytes (%)	5.25	3.5	1-9	4.57	2.94	2-9	5.55	2.5	2-9	0.07
Basophils (%)	3.25	2.22	0-5	2.29	1.5	0-5	2.82	1.83	0-5	0.11
Heterophils (%)	44	7.12	38-52	30.43	9.25	18-45	34.27	15.41	6-60	0.28
Eosinophils (%)	4.25	0.96	3-5	3.43	1.62	2-6	2.18	1.72	0-4	**0.03**

Table 3. Hematological reference values for different age groups of wild Caiman latirostris (Estação Ecológica de Tapacurá, Pernambuco, Brazil). SD (Standard deviation), P (p value), SVL (Snout-Vent Length), TEC (Total Erythrocyte Count), TLC (Total Leukocyte Count), HB (Hemoglobin), Ht (Hematocrit), MVC (Mean corpuscular volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), TTC (Total Thrombocyte Count).

Parameter	Adults (n = 7)			Hatchlings (n = 3)			Subadults (n = 10)			P
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
SVL (cm)	74.71	18.39	70-100.4	41.1	35.12	17.8-24.0	44.24	14.97	59.0-67.5	**<0.01**
Weight (g)	15495	13641	1015-38300	709.73	827.8	114-216	829	512.35	540-1740	**<0.01**
TEC (10^3 cells/mm³)	223.64	47.17	130-290	246.25	70.28	185-345	190	89.93	120-345	0.28
TLC (10^3 cells/mm³)	4.34	1.92	1.75-7.75	3.19	0.24	3-3.5	3.25	2.9	1.25-8.25	0.34
HB (g/dl)	7.19	0.65	5.8-8	7.4	0.54	6.9-8	7.12	0.61	6.2-7.8	0.44
Ht (%)	21.45	3.24	15-26	23.25	4.35	19-29	20.2	4.02	16-26	0.80
MCV (fl)	0.98	0.15	0.75-1.26	0.96	0.08	0.84-1.02	1.15	0.25	0.75-1.42	0.22
MCH (pg)	0.33	0.05	0.27-0.45	0.31	0.06	0.22-0.37	9.42	0.13	0.22-0.59	0.16
MCHC (%)	33.91	3.37	28.33-33.33	31.33	6.16	22.33-36.31	35.92	4.51	30-41.76	0.86
TTC (10^3 cells/mm³)	4.02	1.5	1.5-6	6.12	0.77	5.25-7	4.25	1.61	1.75-5.75	**0.04**
Lymphocytes (%)	54.09	13.68	37-77	53.5	14.84	32-66	44.2	8.64	31-55	0.22
Monocytes (%)	2.18	1.54	0-5	4.25	1.5	3-6	3.8	3.27	1-9	0.07
Basophils (%)	1	1.1	0-3	1	0	1	2.6	3.13	0-8	0.11
Heterophils (%)	40.36	15.59	9-59	39	16.06	30-63	47.2	7.6	35-54	0.28
Eosinophils (%)	1.45	0.82	0-3	2.25	2.22	0-5	2.2	1.79	0-4	**0.03**

ha et al., 2011; Zayas et al., 2011; Bassetti and Verdade, 2014). Some factors that may have affected the results obtained in previous studies may be related to the methodology used, the environmental conditions and the diet of the population sampled.

Among the hematological parameters, it was possible to observe differences between the areas in the MCV values. These values suggest a possible difference between adult individuals and subadults between the two study areas. The causes of physiological changes in

Table 4. Hematological reference values for male and female wild *Caiman latirostris* (Parque Estadual de Dois Irmãos, Pernambuco, Brazil). SD (Standard deviation), P (p value), SVL (Snout-Vent Lenght), TL (Total Length), TEC (Total Erythrocyte Count), TLC (Total Leukocyte Count), HB (Hemoglobin), Ht (Hematocrit), MVC (Mean corpuscular volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), TTC (Total Thrombocyte Count).

Parameter	Males (n=12)			Females (n=10)			P
	Mean	SD	Range	Mean	SD	Range	
SVL (cm)	37.77	25.43	17.3-100	49.51	13.3	42-86.50	0.07
TL (cm)	74.21	51.07	36-209	90.85	26.18	75.5-163	0.11
Weight (g)	5944	14631	125-51380	4498	8332	166-28000	0.22
TEC (10^3 cells/mm^3)	203.33	39.9	150-255	149.5	94.5	30-355	0.12
TLC (10^3 cells/mm^3)	5.77	2.3	2.75-11	4.57	3.45	0.75-11.7	0.36
HB (g/dl)	7.21	0.69	5.8-8	6.43	0.78	5.2-8	**0.02**
Ht (%)	22.25	4.22	12-28	17.7	7.3	3-29	0.10
MCV (fl)	1.12	0.27	0.76-1.73	2.01	1.64	0.82-5.45	0.12
MCH (pg)	0.36	0.07	0.26-0.46	0.67	0.56	0.22-2	0.21
MCHC (%)	33.3	5.6	25.71-48.34	53.9	60.09	25-223	0.62
TTC (10^3 cells/mm^3)	2.77	1.13	1-5.75	3.23	2.67	0.5-8.37	0.69
Lymphocytes (%)	56.17	12.38	38-81	52.1	12.64	33-73	0.46
Monocytes (%)	5.08	3	1-9	5.3	2.5	2-9	0.85
Basophils (%)	2.67	1.56	0-5	2.8	2.04	0-5	0.87
Heterophils (%)	32.92	13.06	6-52	37.1	13.09	17-60	0.46
Eosinophils (%)	3.16	1.99	0-6	2.7	1.42	0-4	0.53

Table 5. Hematological reference values for male and female wild *Caiman latirostris* (Estação Ecológica de Tapacurá, Pernambuco, Brazil). SD (Standard deviation), P (p value), SVL (Snout-Vent Lenght), TL (Total Length), TEC (Total Erythrocyte Count), TLC (Total Leukocyte Count), HB (Hemoglobin), Ht (Hematocrit), MVC (Mean corpuscular volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), TTC (Total Thrombocyte Count).

Parameter	Males (n=10)			Females (n=8)			P
	Mean	SD	Interval	Mean	SD	Interval	
SVL (cm)	67.73	27.31	28.5-100.04	59.75	14.14	41-73.5	0.49
TL (cm)	104.33	78.14	28-211	124.3	25.24	82-147	0.50
Weight (g)	12032	15975	540-38300	5584	4447	1015-11260	0.57
TEC (10^3 cells/mm^3)	192.5	67.17	120-345	259.37	46.17	195-345	**0.02**
TLC (10^3 cells/mm^3)	3.72	2.34	1.25-8.25	4.15	1.9	1.75-7.75	0.67
HB (g/dl)	7.04	0.69	5.8-8	7.5	0.44	7-8	0.11
Ht (%)	20.8	4.29	15-29	22.75	2.87	18-26	0.27
MCV (fl)	1.12	0.17	0.84-1.42	0.89	0.11	0.75-1.02	**0.00**
MCH (pg)	0.39	0.1	0.22-0.59	0.29	0.04	0.22-0.35	**0.01**
MCHC (%)	34.21	5.54	22.33-41.76	33.28	3.16	30-40	0.66
TTC (10^3 cells/mm^3)	4.62	1.66	1.75-7	4.09	1.67	1.5-6	0.51
Lymphocytes (%)	47.1	10.88	31-66	58.75	12.52	44-77	0.06
Monocytes (%)	3.9	2.28	1-9	1.5	1.07	0-3	**0.01**
Basophils (%)	1.8	2.3	0-8	1.25	1.2	0-3	0.49
Heterophils (%)	45	10.87	30-59	36.25	15.56	9-54	0.20
Eosinophils (%)	2.2	1.69	0-5	2.25	0.89	0-3	0.15

erythrogram parameters for reptiles are numerous. With increasing age, the total erythrocyte count, the MCV, MCH, MCHC, hematocrit and hemoglobin, tend to increase (Heatley and Russel, 2019).

A relative increase in the total erythrocyte count, hemoglobin and/or hematocrit occurs in males of some

reptile species. However, many species may not correspond to this expectation (Heatley and Russel, 2019). In this study, most parameters did not present significant differences between males and females, but we observed differences in the MCV, MCH and hemoglobin values, which were higher in males, whereas the total erythrocyte count was higher in females in the EET region.

The main aims of the blood smear readings include the differentiation of cell types, the evaluation of cell morphology, the observation of anomalies in cell morphology and the observation of cell inclusions or extracellular anomalies, such as hemoparasites (Heatley and Russel, 2019). In this study, no intracellular inclusions nor hemoparasites were found.

This study corroborates the findings of Basset (2016) for *C. latirostris* and Moura et al. (1999) for *Caiman yacare*, in relation to the types of cells found in peripheral blood. The leukocytes were classified as lymphocytes, azurophilic monocytes, eosinophils, heterophils and basophils. For many authors, azurophils are a variation of monocytes, as can be observed (Zayas et al., 2011), for the same species, whereas other researchers recommend that these cells could be counted separately in snakes but should be grouped for other reptile species (Moura et al., 1999). In this study all the cells were grouped as as monocytes, and we observed we observed differences in the number of monocytes between areas, with the PEDI presenting higher values compared to the EET. Likewise, when comparing sexes within the same area, males in the EET presented higher values than females.

In relation to the morphological characteristics of the other leukocytes, this study corroborates the findings described for other species of crocodilians (Moura et al., 1999; Stacy and Whitaker, 2000; Padilha et al., 2011; Zayas et al., 2011). The reptile heterophile has larger cytoplasmic granules, despite being fewer in number, compared to lizards and snakes (Clever and Quaglia, 2009; Sacchi et al., 2011).

The lymphocytes are the most common leukocytes, accounting for up to 80% of the differential count in healthy reptiles (Heatley and Russel, 2019). In this study the lymphocytes were the most numerous leukocytes, followed by heterophils, monocytes, eosinophils, and basophils, which differs from the results found by Zayas et al. (2011), for the same species. They observed a higher number of lymphocytes followed by heterophils. However, they found more basophils compared to monocytes and eosinophils. Stress factors may be responsible for the increase in total leukocyte count and differential count; however, the extent of these changes has not been comprehensively investigated in reptiles. With a few exceptions, we lack fundamental knowledge on the timing of inflammation and associated cellular responses in reptiles (Heatley and Russel, 2019).

Although we attempted, in this study, to relate the differences found between ages, sex and sample areas, additional factors should be considered, as the influence of environmental parameters such as climate, seasonal period, nutritional state and population dynamics (Moura et al., 1999; Stacy and Whitaker, 2000; Heatley and Russel, 2019).

This study corroborates the idea presented by Basset (2016) where the results obtained from experiments involving blood hematology and biochemistry parameters should always be considered as a diagnostic tool for an animal's or even of a population's state of health.

As affirmed by Stacy and Whitaker (2000) in their study on *Crocodylus palustris*, the differences found may not be a true reflection of the differences between species, therefore making it difficult to come to any firm conclusion about the biological significance of these differences. However, it is still worth considering this data when interpreting the blood parameters of one species, since these are the best data available currently.

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