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Author(s) :M. A. Zayas, H. A. Rodríguez, G. H. Galoppo, C. Stoker, M. Durando, E. H. Luque, and M. Muñoz-de-Toro

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Hematology and Blood Biochemistry of Young Healthy Broad-Snouted Caimans (*Caiman latirostris*)

M. A. ZAYAS,¹ H. A. RODRÍGUEZ,¹ G. H. GALOPPO, C. STOKER, M. DURANDO, E. H. LUQUE, AND M. MUÑOZ-DE-TORO²

Laboratorio de Endocrinología y Tumores Hormonodependientes, School of Biochemistry and Biological Sciences, Universidad Nacional del Litoral,
Casilla de Correo 242, (3000) Santa Fe, Argentina

ABSTRACT.—*Caiman latirostris* (Broad-Snouted Caiman) is widely distributed in wetlands and rivers of South America. Hematological and blood chemistry reference values are necessary for detecting the effects of environmental, infectious, parasitic, or toxicological stress on *C. latirostris* health. Peripheral blood samples were obtained from 24 healthy 6- to 18-month-old caimans. Blood cell dimensions and cytochemistry profiles were described; and reference intervals for hematological parameters, enzyme activities, and clinical analytes were established. Based on the caiman mass frequency distribution, two classes were distinguished: 125–900 g and 901–3,100 g. Although an overlap in age ranges was observed, total length and snout–vent length range values from both mass classes differed. This finding is particularly useful because, in the wild, caiman age is unknown, whereas growth parameters can be easily recorded. Caiman blood cells exhibited morphological features similar to those of other reptiles, with lymphocytes being the most numerous type of leukocytes. Significant positive correlations between mass and hemoglobin concentration, hematocrit, red blood cell, and white blood cell counts were observed. Neither sex nor age was associated with differences in these parameters. Analysis of blood chemistry values found in this study was done by comparing with values reported for related species. Both similarities and discrepancies with values from other crocodiles are discussed. This study provides baseline information from healthy juvenile caimans to which subsequent measurements can be compared. These data will aid in the medical management of caiman farms, zoo conservation programs, and field studies.

Caiman latirostris (Broad-Snouted Caiman) is widely distributed in wetlands and rivers of South America. In Argentina, because of protective legislation and rearing programs, the Broad-Snouted Caiman, which is one of two crocodilian species inhabiting Argentinean wetlands, has recovered recently from near extinction. As a result of its sustainable use, caiman is now assuming increasing importance as an economic resource and experimental animal model (CITES, 2009). In fact, many ranching programs, which represent a commercial option for rearing caimans, have been established (Larriera and Imhof, 2006). Besides that, evidence that *C. latirostris* possesses suitable characteristics for use as a sentinel of environmental pollution has been presented (Stoker et al., 2003; Rey et al., 2006, 2009; Beldomenico et al., 2007; Poletta et al., 2008; Stoker et al., 2008). Conservation programs, appropriate management, and the effective use of *C. latirostris* in research require a consistent base of knowledge regarding the physiological parameters of this species. Hematological and blood chemistry reference values are necessary for detecting the effects of environmental, infectious, parasitic, or toxicological stress on these animals (Campbell, 1996). Although reference ranges have been reported for many reptile species (Alleman et al., 1999; Dickinson et al., 2002; Casal and Oros, 2007), including several crocodilians (Glassman et al., 1981; Mateo et al., 1984; Millan et al., 1997; Stacy and Whitaker, 2000; Schoeb et al., 2002), little is known about the baseline values for *C. latirostris* (ISIS, 1999). Therefore, to better understand *C. latirostris* physiology and to use the information provided by hematological and biochemical markers to its full extent in controlled laboratory research, ranching programs, or to assess the health status of wild-caught animals, reliable baseline values must be available. The aims of this study were to describe the morphological and cytochemical staining features of peripheral blood cells and to establish baseline values for hematology and blood chemistry parameters in healthy juvenile *C. latirostris* raised in a pollutant-free environment.

MATERIALS AND METHODS

Animals and Housing.—Animals used in this study were hatched from eggs collected immediately after oviposition in “El Cachape,” Chaco province (Argentina). “El Cachape” is a farm that has been incorporated into the Refuge Program of Argentinean Wildlife Foundation and is characterized by low anthropogenic intervention. Eggs from eight clutches were transported and incubated following procedures described previously (Stoker et al., 2003). Viable eggs were distributed randomly into two incubators stabilized at either male ($33 \pm 0.5^\circ\text{C}$) or female ($30 \pm 0.5^\circ\text{C}$) producing temperatures (Stoker et al., 2003). Upon hatching, neonates were identified using tags (style 1005-1; National Band and Tag Co.) and then held in controlled conditions. Housing facilities have been designed to provide consistent thermal ranges ($28.0 \pm 2.0^\circ\text{C}$) and the opportunity to choose diverse microenvironments from a dry area to a water feature large enough to facilitate ad libitum full-body soaking, swimming, and social interaction. Water temperature was stabilized by a radiant underfloor heating system at $26.0 \pm 2.0^\circ\text{C}$. Other environmental factors were controlled, including dark-light cycles (lights on from 0600 to 2000 h), humidity ($70\% \pm 5\%$), and air renewal (air automatically renewed every 15 min).

Caimans were fed three times a week with an amount of food that represented 15% of their mass. The wet food portion consisted of 70% premium low fat ground beef and 30% chicken carcasses; this was supplemented with 1% of a vitamin and mineral complex (Vionates-S, Novartis Laboratory, Argentina). Ad libitum access to wet food and satisfactory growth rates were ensured by this feeding schedule. Additionally, animals were exposed daily to a 30-min cycle of ultraviolet B light (Reptistar, Sylvania, Germany) for normal calcium and vitamin D metabolism. Water quality and the caimans' sanitary status were checked daily. Twenty-four caimans (at least one male and one female from each clutch) were assigned to this study; remaining animals were used in other experiments. Blood samples were collected at 6, 12, and 18 months of age. At the time of blood sample collection, growth parameters such as mass, total length (TL), and snout–vent length (SVL) were recorded. At the studied ages, caimans can be restrained manually; thus, anesthetics were not used.

¹These authors contributed equally to this work.

²Corresponding Author. E-mail: monicamt@fbc.unl.edu.ar

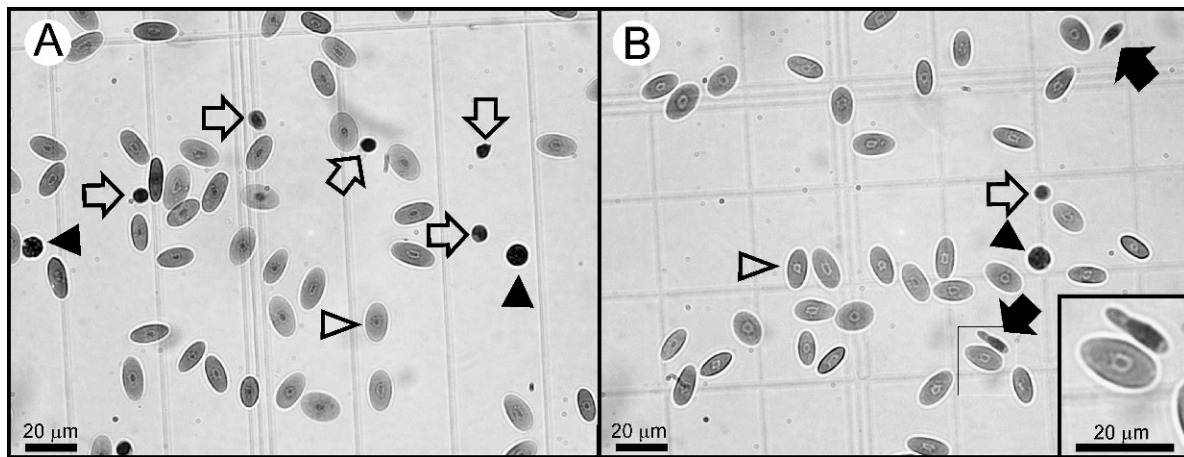


FIG. 1. Differential cell counts in the Neubauer chamber. Open triangle: erythrocytes; filled triangle: granulocytes; open arrow: lymphocytes; filled arrow: thrombocytes. (A) Peripheral caiman blood diluted 1 : 100 in Natt-Herrick's stain solution. Erythrocytes and granulocytes were clearly identified. Differentiation between lymphocytes and thrombocytes was difficult, mainly because of a variation in thrombocyte shape from ovoid to spherical, causing thrombocytes to be mistakenly identified as lymphocytes. (B) Modified Natt-Herrick's solution preserved the differences between lymphocytes and thrombocytes, allowing reliable cell counting. Inset: high magnification image of caiman erythrocyte and thrombocyte. Thrombocyte, elliptical cell with oval nucleus and tail-like cytoplasm at one pole.

Blood Samples.—Peripheral blood samples were obtained from 24 healthy 6- to 18-month-old caimans (14 males and 10 females) by venipuncture of the supravertebral occipital venous sinuses with a 25 G \times 5/8" (0.5 \times 16 mm) needle. To minimize handling time and stress, anesthetics were not used for blood sampling. Specimens were collected after 24 h of fasting. Blood smears for differential leukocyte counts, morphological evaluation, and cytochemical characterization were made from a drop of blood that contains no anticoagulant and was taken from the needle immediately after collection and then was air-dried. Hematocrit, hemoglobin concentration, and total cell counts were performed with anticoagulated blood samples. Ethylenediaminetetraacetic acid (EDTA, 0.342 mol/L; pH 7.2; Wiener Lab, Argentina) was the anticoagulant of choice because cellular constituents remained free in the plasma and preserved their morphology (Glassman et al., 1981; Millan et al., 1997; Stacy and Whitaker, 2000; Strik et al., 2007). At 12 months of age, 1 mL of blood was collected from nine caimans and allowed to clot for serum harvest. Serum samples were stored at -20°C for determination of blood chemistry parameters.

Hematology.—Total cell counts: red blood cell (RBC), white blood cell (WBC), and thrombocyte counts were performed using a 1 : 100 dilution of EDTA-anticoagulated blood in modified Natt-Herrick's solution (see below). Duplicate counting was performed manually in a Neubauer hemacytometer chamber following standard procedure (McPherson and Pincus, 2007; Weiss and Wardrop, 2010). The formulas that were applied considered the average number of cells counted, the dilution factor and the chamber volume (counting area \times depth).

Differentiation between lymphocytes and thrombocytes was difficult using the original formulation of Natt-Herrick's stain solution, mainly because of a variation in thrombocyte shape from ovoid to spherical. Differential cell counts using Natt-Herrick's stain solution were, therefore, substantially altered because of the mistaken identification of thrombocytes as lymphocytes. To improve the assessment of the relative numbers of these two blood cell types, a modified Natt-Herrick's stain solution was used. One milliliter of sodium bicarbonate (0.1% w/v) was mixed with 3 mL of Natt-Herrick's stain solution (sodium sulfate 2.50 g; sodium chloride 3.88 g; sodium phosphate monobasic 2.91 g; potassium phosphate dibasic 0.25 g; formalin 37% 7.50 mL; crystal violet 2B 0.10 g). All reagents were prepared with analytical

grade chemicals (Merck, Argentina). The use of modified Natt-Herrick's solution as a diluting fluid preserved the differences between lymphocytes and thrombocytes allowing reliable cell counting. Representative photomicrographs are shown in Figure 1A, B.

Hematocrit: hematocrit (Hct) was obtained by measuring packed cell percentage through the use of microhematocrit capillary tubes. Capillary tubes were filled with whole blood by capillary action to within 1 cm of the end of the tube. The unfilled end was sealed, and the tubes were spun for 5 min at 10,000 rpm in a centrifuge (Cavours VT-1224, Cavours, Argentina). After centrifugation, the capillary tubes were promptly placed in a reading device, and packed cell volume was determined. Hct was determined in duplicate.

Hemoglobin: hemoglobin (Hb) concentration was determined by a modified cyanmethemoglobin method (Campbell, 2004) using a commercial kit (Hemoglobin test, Wiener Lab, Argentina). In the modified method, free RBC nuclei were removed by spinning the samples for 5 min at 2,500 rpm, before reading the absorbance in a spectrophotometer at a wavelength of 540 nm. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using established formulas (McPherson and Pincus, 2007).

Differential leukocyte counts: for differential leukocyte counts, blood smears were stained with May-Grünwald Giemsa (Biopur, Argentina) following the manufacturer's recommendations. Leukocytes were classified as heterophils, eosinophils, basophils, lymphocytes, and monocytes, with thrombocytes and erythrocytes considered as distinct cell types (Glassman et al., 1981). Two slides per animal and 250 leukocytes per slide were counted to determine the percentage of each individual cell type.

Cytochemical Staining.—Cytochemical staining features of blood cells were evaluated using air-dried blood smears. Peroxidase (Px) was detected by Washburn's method; carbohydrates (polysaccharides, mucopolysaccharides, glycoproteins, and glycolipids) were stained with periodic acid Schiff (PAS) using a commercial kit from Biopur (Tinción PAS, Biopur, Argentina); and the presence of lipids was demonstrated by Sudan Black B (SBB) stain. All reagents were prepared with analytical grade chemicals (Merck, Argentina). The presence and pattern of staining in individual blood cells were recorded (Campbell, 2004). Human blood smears were used as positive controls.

TABLE 1. Hematological and biochemical data for juvenile *Caiman latirostris* raised under controlled conditions. Numbers of observations (N) vary because not all parameters were evaluated for every caiman. Asterisk indicates significant differences between mass classes. Wilcoxon matched pairs test, $P < 0.05$. MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

| Parameter | Mass | | | | | |
|---|-----------|--------------------|---------------|-------------|------------------------|--------------------|
| | 125–900 g | | | 901–3,100 g | | |
| | N | Mean \pm SE | Range | N | Mean \pm SE | Range |
| <i>General</i> | | | | | | |
| Mass (g) | 15 | 486.90 \pm 62.66 | 126.00–878.00 | 11 | 1,986.00 \pm 184.90* | 1,103.00–3,056.00 |
| Snout–vent length (cm) | 15 | 23.47 \pm 0.99 | 16.00–29.50 | 11 | 39.41 \pm 1.50* | 31.50–48.00 |
| Total length (cm) | 15 | 48.72 \pm 2.05 | 32.00–62.50 | 11 | 80.93 \pm 2.74* | 65.75–95.00 |
| Age (months) | 15 | 7.53 \pm 0.65 | 6.00–18.00 | 11 | 13.73 \pm 0.69* | 12.00–18.00 |
| Sex ratio (Female : Male) | 15 | 7:8 | | 11 | 4:7 | |
| <i>Hematology</i> | | | | | | |
| Red blood cells ($\times 10^5$ cells/mm ³) | 15 | 5.58 \pm 0.17 | 4.40–6.70 | 9 | 7.27 \pm 0.53* | 5.18–9.15 |
| White blood cells ($\times 10^3$ cells/mm ³) | 15 | 16.57 \pm 2.12 | 5.52–39.33 | 9 | 25.61 \pm 1.66* | 19.75–34.75 |
| Hematocrit (%) | 15 | 22.74 \pm 0.61 | 18.00–28.00 | 11 | 28.59 \pm 1.17* | 23.50–35.00 |
| Hemoglobin (g/dL) | 8 | 7.07 \pm 0.21 | 6.14–8.15 | 9 | 8.51 \pm 0.42* | 7.21–10.84 |
| Lymphocytes (%) | 15 | 58.35 \pm 2.04 | 45.13–74.26 | 11 | 62.73 \pm 2.50 | 50.00–81.68 |
| Heterophils (%) | 15 | 30.54 \pm 2.03 | 17.07–47.06 | 11 | 24.48 \pm 2.27 | 9.42–34.38 |
| Eosinophils (%) | 15 | 2.24 \pm 0.32 | 0.83–5.69 | 11 | 3.52 \pm 0.50* | 1.31–6.25 |
| Basophils (%) | 15 | 4.85 \pm 0.66 | 0.99–12.20 | 11 | 4.25 \pm 0.84 | 0.91–10.00 |
| Monocytes (%) | 15 | 4.01 \pm 0.58 | 0.80–8.00 | 11 | 5.02 \pm 0.76 | 1.20–9.33 |
| Lymphocyte : Heterophil ratio | 15 | 2.09 \pm 0.20 | 1.00–3.57 | 11 | 3.05 \pm 0.60 | 1.45–8.67 |
| Thrombocytes ($\times 10^3$ cells/mm ³) | 2 | 26.81 \pm 4.12 | 22.69–30.94 | 9 | 28.00 \pm 1.72 | 20.44–38.25 |
| Polychromatic erythrocytes (%) | 15 | 2.38 \pm 0.26 | 0.53–4.15 | 11 | 1.36 \pm 0.19* | 0.51–2.69 |
| MCV (μm^3) | 8 | 388.90 \pm 9.76 | 346.20–445.20 | 9 | 404.10 \pm 15.67 | 335.30–478.90 |
| MCH (pg) | 8 | 124.60 \pm 3.94 | 111.20–144.40 | 9 | 119.70 \pm 6.24 | 96.51–153.10 |
| MCHC (g/dL) | 8 | 32.17 \pm 1.15 | 28.01–37.04 | 9 | 29.53 \pm 0.58 | 27.33–31.96 |
| <i>Clinical analytes</i> | | | | | | |
| Glucose (g/L) | — | — | — | 9 | 1.24 \pm 0.07 | 0.78–1.51 |
| Total proteins (g/dL) | — | — | — | 9 | 4.76 \pm 0.12 | 4.24–5.26 |
| Albumin (g/dL) | — | — | — | 9 | 2.38 \pm 0.05 | 2.07–2.62 |
| Globulin (g/dL) | — | — | — | 9 | 2.38 \pm 0.08 | 2.10–2.75 |
| Albumin : Globulin ratio | — | — | — | 9 | 1.01 \pm 0.03 | 0.91–1.17 |
| Uric acid (mg/dL) | — | — | — | 9 | 3.63 \pm 0.49 | 2.37–6.59 |
| Creatinine (mg/dL) | — | — | — | 9 | 0.41 \pm 0.05 | 0.20–0.62 |
| Total bilirubin (mg/dL) | — | — | — | 9 | 0.11 \pm 0.01 | 0.10–0.16 |
| Cholesterol (mg/dL) | — | — | — | 9 | 159.40 \pm 11.50 | 118.00–217.00 |
| Triglycerides (mg/dL) | — | — | — | 8 | 15.25 \pm 1.36 | 8.00–19.00 |
| Urea (g/L) | — | — | — | 9 | 0.02 \pm 0.002 | 0.01–0.03 |
| <i>Clinical enzymology</i> | | | | | | |
| Alkaline phosphatase (mU/mL) | — | — | — | 9 | 25.44 \pm 1.94 | 17.40–35.00 |
| Alanine aminotransferase (IU/L) | — | — | — | 9 | 69.44 \pm 6.28 | 45.00–97.00 |
| Aspartate aminotransferase (IU/L) | — | — | — | 9 | 116.20 \pm 9.51 | 73.00–168.00 |
| Lactate dehydrogenase (IU/L) | — | — | — | 9 | 1,085.00 \pm 156.00 | 569.00–1,881.00 |
| Creatine kinase (IU/L) | — | — | — | 9 | 4,213.00 \pm 970.30 | 1,487.00–10,290.00 |

Blood Cell Morphometry.—Peripheral blood cell morphometric analysis was performed in May-Grünwald Giemsa-stained blood smears. Cellular area, perimeter, and maximum diameter and nuclear maximum diameter were measured on digitized images recorded using a Dplan \times 100 objective (numerical aperture = 1.25) on an Olympus BH2 microscope (Olympus Optical, Tokyo, Japan) with a Spot Insight version 3.5 color video camera (Diagnostic Instruments). Image analysis was performed using the Image Pro-Plus 4.1.0.1 system (Media Cybernetics) as previously described (Ramos et al., 2002; Rodriguez et al., 2003).

Blood Chemistry.—Serum levels of glucose, urea, cholesterol, uric acid, triglycerides, creatinine, total bilirubin, total proteins, albumin, and globulins and serum activity of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK) were measured by using an automated biochemical analyzer (Vitalab Selectra E Mod. 6002-160, Vital Scientific, EU).

Data Analysis.—Frequency distribution histograms for each growth parameter were plotted. Mass exhibited a bimodal frequency distribution (each bin covering a range of 200 g).

Hematological and biochemical data separated by sex or by mass class were normally distributed (Kolmogorov-Smirnov test). The lower and upper 95% confidence intervals (CI) of the mean values were used to suggest reference intervals (Oliveira-Junior et al., 2009). In spite of all data that exhibited normal distributions, the nonparametric Wilcoxon matched pairs test was used to assess differences between classes. Samples from the same animal evaluated in both mass classes were paired. The significance level was set at $P < 0.05$. The Mann-Whitney test was used to assess differences between male and female caimans. Correlations were performed by using Pearson analysis (Siegel, 1956).

RESULTS

Body size parameters and the hematological and biochemical data that we collected from juvenile *C. latirostris* are summarized in Table 1. Because no differences were found between male and female values, data from both sexes were combined. Positive correlations between hematological cell counts and caiman mass were found, although no correlations were found with caiman ages. Based on the caiman mass frequency

TABLE 2. Reference intervals for juvenile *Caiman latirostris* raised under controlled conditions. Numbers of observations (N) vary because not all parameters were evaluated for every caiman.

| Parameter | Mass | | | |
|---|----------|--|------------|--|
| | 125–900g | | 901–3,100g | |
| | N | Suggested reference interval (95% CI of the mean) | N | Suggested reference interval (95% CI of the mean) |
| <i>General</i> | | | | |
| Snout–vent length (cm) | 15 | 21.3–25.6 | 11 | 36.1–42.8 |
| Total length (cm) | 15 | 44.3–53.1 | 11 | 74.8–87.0 |
| <i>Hematology</i> | | | | |
| Red blood cells ($\times 10^5$ cells/mm ³) | 15 | 5.2–5.9 | 9 | 6.1–8.5 |
| White blood cells ($\times 10^3$ cells/mm ³) | 15 | 12.0–21.0 | 9 | 22.0–30.0 |
| Hematocrit (%) | 15 | 21.4–24.1 | 11 | 26.0–31.2 |
| Hemoglobin (g/dL) | 8 | 6.6–7.5 | 9 | 7.5–9.5 |
| Thrombocytes ($\times 10^3$ cells/mm ³) | — | — | 9 | 24.0–32.0 |
| Polychromatic erythrocytes (%) | 15 | 1.8–2.9 | 11 | 0.9–1.8 |
| <i>Clinical analytes</i> | | | | |
| Glucose (g/dL) | — | — | 9 | 1.1–1.4 |
| Total proteins (g/dL) | — | — | 9 | 4.5–5.0 |
| Albumin (g/dL) | — | — | 9 | 2.3–2.5 |
| Globulin (g/dL) | — | — | 9 | 2.2–2.6 |
| Albumin : Globulin ratio | — | — | 9 | 0.9–1.1 |
| Uric acid (mg/dL) | — | — | 9 | 2.5–4.8 |
| Creatinine (mg/dL) | — | — | 9 | 0.3–0.5 |
| Total bilirubin (mg/dL) | — | — | 9 | 0.09–1.1 |
| Cholesterol (mg/dL) | — | — | 9 | 132.9–186.0 |
| Triglycerides (mg/dL) | — | — | 8 | 12.0–18.5 |
| Urea (g/L) | — | — | 9 | 0.02–0.03 |
| <i>Clinical enzymology</i> | | | | |
| Alkaline phosphatase (mU/mL) | — | — | 9 | 21.0–29.9 |
| Alanine aminotransferase (IU/L) | — | — | 9 | 55.0–83.9 |
| Aspartate aminotransferase (IU/L) | — | — | 9 | 94.3–138.2 |
| Lactate dehydrogenase (IU/L) | — | — | 9 | 736.3–1,434.0 |
| Creatine kinase (IU/L) | — | — | 9 | 1,975.0–6,450.0 |

distribution, two mass classes were distinguished: 125–900 g ($N = 15$) and 901–3,100 g ($N = 11$). Although these two caiman groups differed in the mean animal age, an overlap was observed in the age ranges. Therefore, we established hematological and biochemical reference values for these two mass classes, independent of animal age (Table 2).

Erythrocytes: As shown in Table 1 and Figure 2, not only was an increase in the total erythrocyte counts observed in the heaviest caimans, but a significant positive association was established between Hct or Hb concentration and juvenile caiman mass (Pearson r : 0.675, $P < 0.001$, and r : 0.583, $P = 0.0032$, respectively). Erythrocytes (Fig. 3) appeared as ellipsoidal cells with centrally positioned, oval to round nuclei with condensed and dark-stained chromatin. Few erythrocytes showed an eccentrically located nucleus. The cytoplasm stained uniformly red-orange to brick-red. Immature erythrocytes (round to irregular cells with large, round nuclei and polychromatophilic or basophilic cytoplasm) were occasionally present and represented 2.38% of the erythrocyte population in the 125–900 g and 1.36% in the 901–3,100 g groups (Fig. 4A; Table 1). Erythrocytes were negative for PAS and SBB and positive for Px (Fig. 3; Table 3).

Leukocytes were classified as heterophils, lymphocytes, eosinophils, basophils and monocytes (Table 1). A significant positive association was found between total leukocyte count and juvenile caiman mass, Pearson r : 0.6125, $P = 0.0002$.

Heterophils (Fig. 3) were generally round or oval cells with abundant cytoplasmic spindle-shaped granules. The nucleus was lenticular, oval, or rarely, bilobed and eccentrically positioned with densely clumped chromatin. Heterophils stained with PAS, SBB, and Px (Fig. 3; Table 3).

Lymphocytes: As shown in Table 1, lymphocytes were the most numerous cells in the leukocyte differential count, followed by heterophils, basophils, monocytes, and eosinophils. Lymphocytes were the smallest cells in *C. latirostris* blood smears (Table 4) and showed a well-defined round nucleus (Fig. 3). Typically, lymphocytes displayed a large nucleus : cytoplasm ratio. Their chromatin was heavily clumped. The scant cytoplasm was typically basophilic. Lymphocytes did not take up the cytochemical stains from any of the assays we performed (Fig. 3; Table 3).

Eosinophils (Fig. 3) were scarce in juvenile caiman blood smears. Eosinophils were oval or round cells, with abundant spherical, eosinophilic granules. The nucleus stained purple and was usually located at one pole of the cell, causing a slight outward bulge of the cell outline. Eosinophils had a positive reaction with Px and a moderately positive reaction with PAS and SBB (Fig. 3; Table 3).

Basophils (Fig. 3) were round and contained a dense, violet-blue, generally eccentric nucleus that presented a clumped chromatin pattern. The cytoplasm showed numerous large basophilic granules that often obscured the nucleus. When visible, the nucleus was slightly eccentric in position and nonlobed. Basophils were negative for all of the cytochemical assays we performed (Fig. 3; Table 3).

Monocytes appeared as the largest leukocytes in *C. latirostris* (Table 4), varying in shape from round to ameboid (Fig. 3). The eccentric nucleus was variable in shape, ranging from round to oval or being lobed. The nuclear chromatin was less condensed compared with the nuclei of lymphocytes. The abundant basophilic cytoplasm may contain shiny vacuoles, and it exhibited numerous delicate cytoplasmic projections. Monocytes were negative for PAS, SBB, and Px (Fig. 3; Table 3).

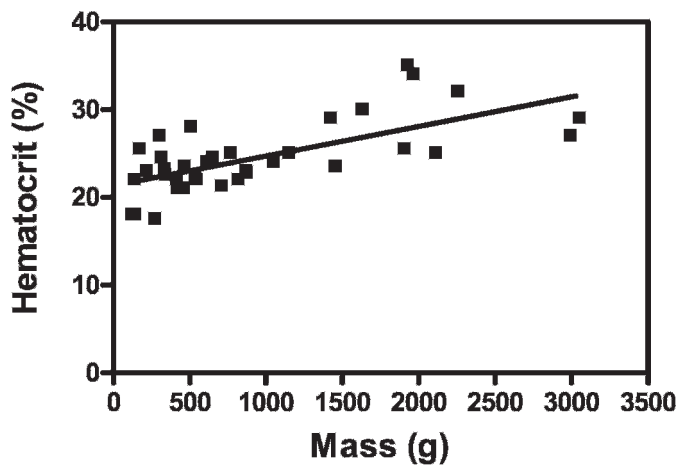


FIG. 2. Relationship between hematocrit and mass in healthy juvenile caimans. Hematocrit exhibited a significant positive correlation with mass (Pearson r : 0.675, P < 0.001).

Thrombocytes (Fig. 3) appeared as oval to elliptical nucleated cells. The centrally positioned nucleus was generally oval, strongly violet-blue stained and with condensed chromatin. The scant cytoplasm accumulated at the two poles of the cell when the thrombocyte presented an oval morphology. When the cell acquired a round shape, a slim cytoplasmic halo around the nucleus was seen, making the distinction between thrombocytes and lymphocytes difficult. Although thrombocytes were mainly observed as being isolated, small multicellular thrombocyte clusters were found in the peripheral blood in May-Grünwald Giemsa-stained films (Fig. 4C). As shown in Figure 3 and Table 3, cytochemical stains were helpful tools to establish differences between thrombocytes that were PAS positive and lymphocytes, which failed to stain with any of the cytochemical stains. No association was found between thrombocyte counts and caiman mass.

DISCUSSION

The establishment of reference ranges is required to help in assessing the causes of disease and to evaluate populations that are potentially at risk (Casal and Oros, 2007; Oliveira-Junior et al., 2009). A better understanding of caiman hematological variables is important as we attempt to provide medical care for these animals in the wild and in captivity. Even though hematological and blood chemistry parameters can present valuable, noninvasive diagnostic and prognostic tools to examine caiman health status, these parameters can be highly variable, because of the influence of various intrinsic and extrinsic factors such as the quality of the aquatic environment, geographical location, genetic variations, maturity, sex, breeding status, and diet. To define the reference ranges for health indicators, caimans were bred in controlled environmental conditions to meet physiological requirements, and stress was reduced during handling to avoid any interference. We proposed reference intervals for two caiman mass classes: 125–900 g and 901–3,100 g. Although mass classes exhibited an overlapping in age ranges, no overlap was observed for SVL and TL values. This finding from our data is of particular use because, in the wild animal, age is unknown, and mass can be easily recorded. We observed that none of the studied variables were influenced by sex in 6- to 18-month-old caimans.

Caiman blood cells exhibited morphological features similar to those of other reptilian species. Lymphocytes were the most numerous leukocytes in the peripheral blood of juvenile *C. latirostris*. This is consistent with the data reported by ISIS (1999), but it differs from the results obtained in most

chelonians and crocodilians, in which heterophils appear to be the most commonly observed blood leukocytes (Mateo et al., 1984; Stacy and Whitaker, 2000; Schoeb et al., 2002; Casal and Oros, 2007). Mussart et al. (2006) reported that lymphocytes represented approximately 75% of the total leukocytes in the peripheral blood of a mixed sample of subadult *C. latirostris* and *Caiman yacare*. Even though, reptile thrombocyte morphological features and ultrastructural characteristics were described (Glassman et al., 1981; Mateo et al., 1984; Leal de Moura et al., 1997; Alleman et al., 1999; Casal and Oros, 2007), reports of reptile thrombocyte quantitation are infrequent and often contradictory. The lack of a direct and adequate method for thrombocyte quantitation (Mateo et al., 1984), forced investigators to deduce total thrombocyte counts from blood smears (Oliveira-Junior et al., 2009). No thrombocyte data were previously available for *C. latirostris*. However, in the present study, the use of modified Natt-Herrick's solution as the diluting fluid in the Neubauer chamber preserved the differences between lymphocytes and thrombocytes and allowed us to count these two blood cell types properly. The resulting mean thrombocyte levels are comparable with values reported for American Alligators (Glassman et al., 1981; Mateo et al., 1984).

Polychromatophilic erythrocytes were present in the peripheral blood of healthy caimans. Polychromatophilic erythrocytes were more prevalent in caimans from the first mass class (125–900 g) than in the heaviest one (901–3,100 g), and the established reference intervals were 1.8–2.9% and 0.9–1.8% of the total erythrocyte count, respectively. The degree of polychromasia is a good indicator of the erythrocytic regenerative response (Campbell, 2004). Preliminary results indicated that higher proportions of immature erythrocytes may be used as indicators of increased erythropoietic activity as compensatory responses in the caiman anemic processes (M. Zayas, unpubl. data).

We observed significant positive correlations between caiman mass and hematological parameters such as Hb concentration, Hct, and RBC count. The correlations we observed in the present study are supported by results that have been reported for other reptiles. A positive association between turtle weight and Hct was previously detected (Stamper et al., 2005). Furthermore, adult *Crocodylus palustris* had a greater RBC count than juveniles (Stacy and Whitaker, 2000). In other words, the larger the animal, the higher its oxygen-carrying capacity, which is probably because larger caimans, crocodiles, and turtles might need a higher oxygen supply to dive for longer periods of time.

We found both similarities and differences when comparing the enzyme activities and levels of carbohydrates, proteins, and lipids of juvenile *C. latirostris* with values from other healthy crocodiles (Stacy and Whitaker, 2000; Schoeb et al., 2002; Barboza et al., 2008). Levels of glucose are highly influenced by the fasting period. Glucose levels reported here, after 24 h fasting, were higher than those of Stacy and Whitaker (2000) in which all animals were fasted at least 48 h prior to blood collection. When fasting period was unknown, like in free-living animals (Schoeb et al., 2002) or not available (Barboza et al., 2008), no comparisons of glucose levels may be done.

The low levels of cholesterol and triglycerides reported here could reflect low-fat intake, resulting from the low-fat diet used in the present study. On the other hand, the amount of creatinine produced is related to the muscle mass, and the diet has little influence. In accordance with this, creatinine levels reported in this study were similar to those reported for animals in the same mass range (Stacy and Whitaker, 2000) and lower than those observed in heavier caimans (Barboza et al., 2008). The values found for enzymes related to hepatocellular damage fell within the ranges reported for other healthy crocodiles (Stacy and Whitaker, 2000). To define the reference

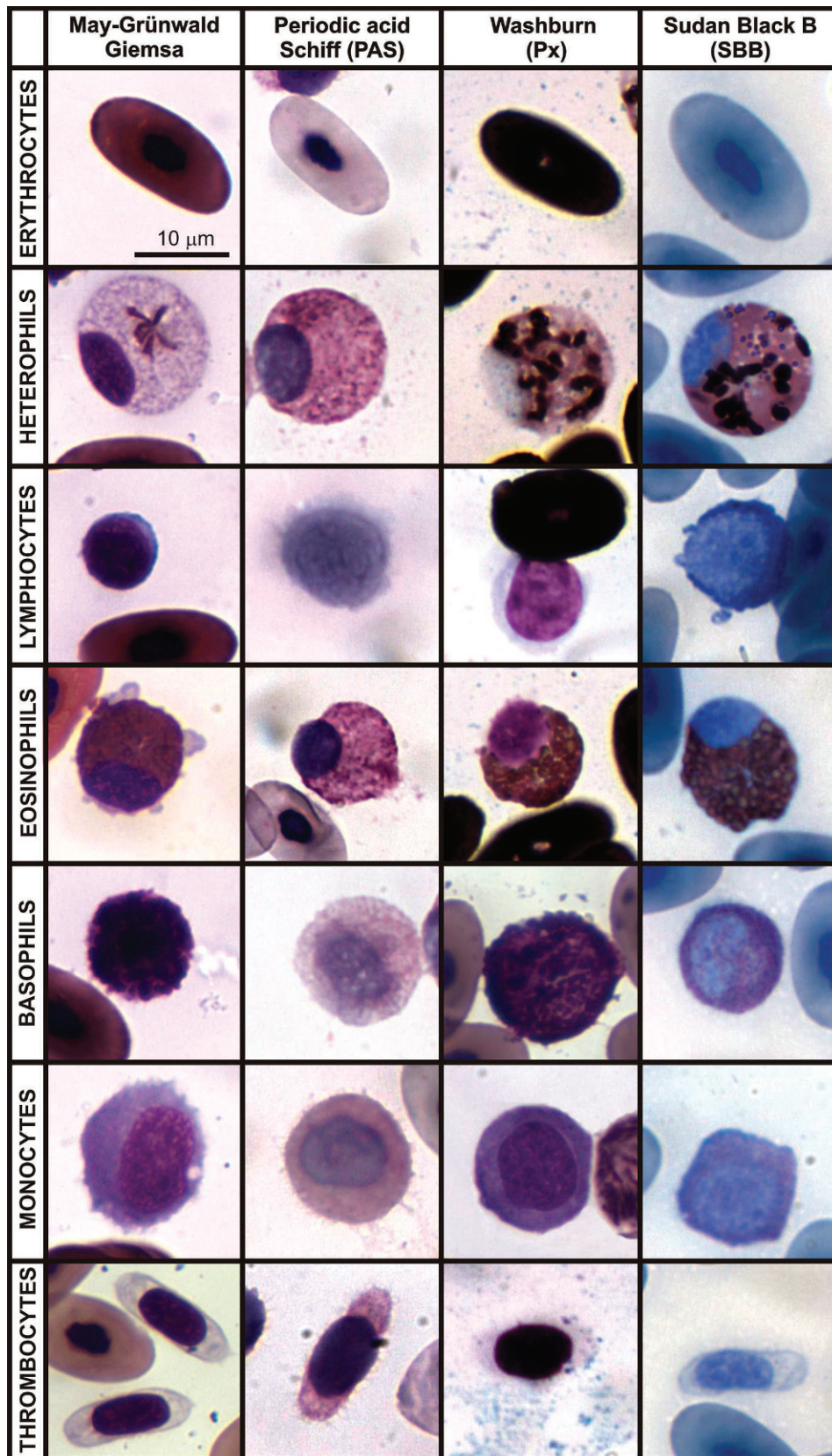


FIG. 3. Staining pattern within individual caiman blood cells. General features were described by using May-Grünwald Giemsa stain. Carbohydrates were stained with periodic acid Schiff (PAS); peroxidase (Px) was demonstrated by Washburn's method; and the presence of lipids was demonstrated by Sudan Black B (SBB) stain. To define a stain as positive or negative, a comparison with staining patterns established for human blood cells was made. Color reproduction supported by the Thomas Beauvais Fund.

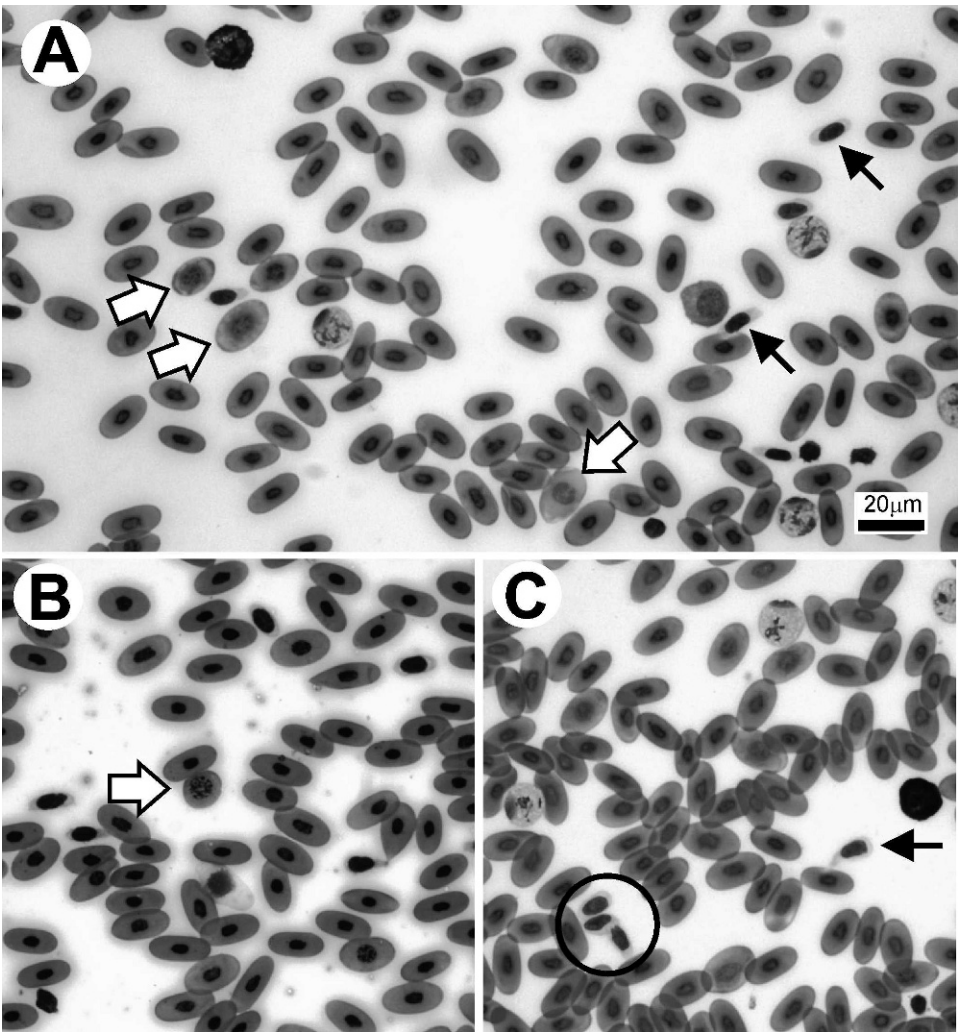


FIG. 4. Peripheral blood smears of young healthy caiman. (A) Panoramic photomicrograph of May-Grünwald Giemsa-stained smear showing mature erythrocytes and immature polychromatophilic erythrocytes (open arrow), various leukocytes, and isolated thrombocytes (arrow). (B) Immature erythrocytes (open arrow) often appear smaller than mature erythrocytes, and they are round to slightly oval and exhibit cytoplasmic polychromasia or basophilia and less pyknotic nuclei. (C) Thrombocytes are small oval cells with colorless cytoplasm and densely clumped chromatin. In addition to isolated thrombocytes (arrow), clusters of thrombocytes (circle) can be observed in peripheral blood smears.

ranges for health indicators, not only a control population free from contaminant exposure need to be assessed but also environmental parameters such as temperature, diet, and stocking densities need to be considered, because all of these variables could have an impact on hematological and biochemical parameters.

In this study, hematological and blood chemistry baseline values, as well as blood cell dimensions and cytochemical profiles for healthy young *C. latirostris*, were established. Hematological and blood chemistry parameters can provide predictive information about animal health and, therefore, can be used as a quick tool for diagnosis. Evaluation of the clinical status of reptiles is made difficult by the limited diagnostic techniques that can be performed in these species. In addition, because reptiles are a heterogeneous group of vertebrates, it is difficult to draw inferences from one species to another.

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involving caimans was performed following procedures revised and approved by the Institutional Committee of Animal Use and Care of Universidad Nacional del Litoral, Santa Fe, Argentina (permit 6-45). This study was supported by grants from ANPCyT (PICT 16-25575), CONICET (PIP 112-200801-00218), and Universidad Nacional del Litoral (CAID

TABLE 3. Cytochemical staining characterization of *Caiman latirostris* peripheral blood cells. Carbohydrates were stained with periodic acid Schiff (PAS); the presence of lipids was demonstrated by Sudan Black B (SBB) stain and Peroxidase (Px) was detected by Washburn’s method. Staining intensity was considered: intense positive (+); slight positive (±); or negative (–).

| Cells | Stain | | |
|-------------|-------|-----|----|
| | PAS | SBB | Px |
| Erythrocyte | – | – | + |
| Heterophil | + | + | + |
| Lymphocyte | – | – | – |
| Eosinophil | ± | ± | + |
| Basophil | – | – | – |
| Monocyte | – | – | – |
| Thrombocyte | + | – | – |

TABLE 4. *Caiman latirostris* peripheral blood cell dimensions. Cellular area, perimeter, and maximum diameter and nuclear maximum diameter were measured on digitized images from May-Grünwald Giemsa-stained blood smears. One-hundred cells from each blood cell subtype were evaluated. Basophils exhibited large granules that masked their nucleus; thus, basophil nucleus diameter was not determined.

| Cell | Area (μm^2) Mean \pm SE | Perimeter (μm) Mean \pm SE | Cell diameter (μm) Mean \pm SE | Nucleus diameter (μm) Mean \pm SE |
|-------------|---|--|--|---|
| Erythrocyte | 133.65 \pm 2.00 | 44.46 \pm 0.37 | 17.24 \pm 0.13 | 5.15 \pm 0.07 |
| Heterophil | 157.21 \pm 1.80 | 45.79 \pm 0.27 | 14.83 \pm 0.13 | 7.69 \pm 0.11 |
| Lymphocyte | 52.61 \pm 1.43 | 26.44 \pm 0.39 | 8.11 \pm 0.10 | 6.58 \pm 0.12 |
| Eosinophil | 120.31 \pm 1.66 | 38.95 \pm 0.32 | 13.15 \pm 0.14 | 7.24 \pm 0.14 |
| Basophil | 130.29 \pm 2.03 | 41.58 \pm 0.37 | 12.93 \pm 0.11 | — |
| Monocyte | 166.38 \pm 2.74 | 48.00 \pm 0.48 | 14.06 \pm 0.14 | 8.87 \pm 0.11 |
| Thrombocyte | 60.41 \pm 0.94 | 33.17 \pm 0.31 | 12.55 \pm 0.15 | 8.33 \pm 0.09 |

program). MAZ is a Fellow and HAR, CS, MD, and EHL are Career Investigators of the CONICET, and GG is Fellow of Universidad Nacional del Litoral.

LITERATURE CITED

- ALLEMAN, A. R., E. R. JACOBSON, AND R. E. RASKIN. 1999. Morphologic and cytochemical characteristics, and ultrastructural characteristics of blood cells from Eastern Diamondback rattlesnakes (*Crotalus adamanteus*). *American Journal of Veterinary Research* 60:507–513.
- BARBOZA, N. N., N. B. MUSSART, J. A. COPPO, S. A. FIORANELLI, AND G. A. KOZA. 2008. El medio interno de *Caiman latirostris* en cautiverio. Influencia del sexo, crecimiento y estación del año. *Revista Veterinaria* 19:33–41.
- BELDOMÉNICO, P. M., F. REY, W. S. PRADO, J. C. VILLARREAL, M. MUÑOZ-DE-TORO, AND E. H. LUQUE. 2007. In ovum exposure to pesticides increases the egg weight loss and decreases hatchlings weight of *Caiman latirostris* (Crocodylia: Alligatoridae). *Ecotoxicology and Environmental Safety* 68:246–251.
- CAMPBELL, T. W. 1996. Clinical Pathology. In D. R. Mader (ed.), *Reptile Medicine and Surgery*, pp. 248–257. W. B. Saunders Company, Philadelphia, PA.
- . 2004. Hematology of reptiles. In M. A. Thrall (ed.), *Veterinary Hematology and Clinical Chemistry*, pp. 259–276. Lippincott Williams and Wilkins, Philadelphia, PA.
- CASAL, A. B., AND J. ORÓS. 2007. Morphologic and cytochemical characteristics of blood cells of juvenile Loggerhead Sea Turtles (*Caretta caretta*). *Research in Veterinary Science* 82:158–165.
- CITES. 2009. Convention on International Trade in Endangered Species, Appendix II [Internet]. Geneva, Switzerland; 2009 [cited 16 Sep 2009]. Available from: <http://www.cites.org/eng/app/appendices.shtml>.
- DICKINSON, V. M., J. L. JARCHOW, AND M. H. TRUEBLOOD. 2002. Hematology and plasma biochemistry reference range values for free-ranging desert tortoises in Arizona. *Journal of Wildlife Diseases* 38:143–153.
- GLASSMAN, B. A., C. A. BENNETT, AND T. C. HAZEN. 1981. Peripheral blood components in *Alligator mississippiensis*. *Transactions of the American Microscopical Society* 100:210–215.
- ISIS (INTERNATIONAL SPECIES INFORMATION SYSTEM). 1999. Physiological Data Reference Values [Internet]. Kiel, Germany; 1994 [cited 24 Sep 2009]. Available from: www.southcampus.colostate.edu/zoo/book1/INT_BOOK.htm.
- LARRIERA, A., AND A. IMHOF. 2006. Ranching in Argentina (*Caiman latirostris*, *Caiman yacare*) [Internet]. Santa Fe, Argentina; 2006 [cited 30 Jun 2009]. Available from: <http://www.cites.org/common/resources/reports/ranch/AR0607.pdf>.
- LEAL DE MOURA, W., L. W. OLIVEIRA, AND M. I. EGAMI. 1997. Aspectos ultraestructurales de trombocitos eosinófilos y heterófilos de *Caiman crocodilus yacare* (Daudin, 1802) (Reptilia, Crocodylia). *Revista Chilena de Anatomía* 15:201–208.
- MATEO, M. R., E. D. ROBERTS, AND F. M. ENRIGHT. 1984. Morphologic, cytochemical, and functional studies of peripheral blood cells of young healthy American Alligator (*Alligator mississippiensis*). *American Journal of Veterinary Research* 45:1046–1053.
- MCPHERSON, R. A., AND M. R. PINCUS. 2007. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. Saunders Elsevier, Philadelphia, PA.
- MILLAN, J. M., A. JANMAAT, K. C. RICHARDSON, L. K. CHAMBERS, AND K. R. FORMIATTI. 1997. Reference ranges for biochemical and haematological values in farmed saltwater crocodile (*Crocodylus porosus*) yearlings. *Australian Veterinary Journal* 75:814–817.
- MUSSART, N. B., N. N. BARBOZA, S. A. FIORANELLI, G. A. KOZA, W. S. PRADO, AND J. A. COPPO. 2006. Age, sex, year season, and handling system modify the leukocytal parameters from captive *Caiman latirostris* and *Caiman yacare* (Crocodylia: Alligatoridae). *Revista Veterinaria* 17:3–10.
- OLIVEIRA-JÚNIOR, A. A., M. TAVARES-DIAS, AND J. L. MARCON. 2009. Biochemical and hematological reference ranges for Amazon freshwater turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of blood cells. *Research in Veterinary Science* 86:146–151.
- POLETTA, G. L., A. LARRIERA, E. KLEINSORGE, AND M. D. MUDRY. 2008. *Caiman latirostris* (broad-snouted caiman) as a sentinel organism for genotoxic monitoring: basal values determination of micronucleus and comet assay. *Mutation Research* 650:202–209.
- RAMOS, J. G., J. VARAYOUD, V. L. BOSQUIAZZO, E. H. LUQUE, AND M. MUÑOZ-DE-TORO. 2002. Cellular turnover in the rat uterine cervix and its relationship to estrogen and progesterone receptor dynamics. *Biology of Reproduction* 67:735–742.
- REY, F., J. G. RAMOS, C. STOKER, L. E. BUSSMANN, E. H. LUQUE, AND M. MUÑOZ-DE-TORO. 2006. Vitellogenin detection in *Caiman latirostris* (Crocodylia: Alligatoridae): a tool to assess environmental estrogen exposure in wildlife. *Journal of Comparative Physiology B* 176:243–251.
- REY, F., M. GONZÁLEZ, M. A. ZAYAS, C. STOKER, M. DURANDO, E. H. LUQUE, AND M. MUÑOZ-DE-TORO. 2009. Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male *Caiman latirostris*. *General and Comparative Endocrinology* 162:286–292.
- RODRÍGUEZ, H. A., H. H. ORTEGA, J. G. RAMOS, M. MUÑOZ-DE-TORO, AND E. H. LUQUE. 2003. Guinea-pig interpubic joint (symphysis pubic) relaxation at parturition: underlying cellular processes that resemble an inflammatory response. *Reproductive Biology and Endocrinology* 1:113–122.
- SCHOEB, T. R., T. G. HEATON-JONES, R. M. CLEMMONS, D. A. CARBONNEAU, A. R. WOODWARD, D. SHELTON, AND R. H. POPPENG. 2002. Clinical and necropsy findings associated with increased mortality among American Alligators of Lake Griffin, Florida. *Journal of Wildlife Diseases* 38:320–337.
- SIEGEL, S. 1956. *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill Book Company, New York.
- STACY, B. A., AND N. WHITAKER. 2000. Hematology and biochemistry of captive mugger crocodiles (*Crocodylus palustris*). *Journal of Zoo and Wildlife Medicine* 31:339–347.
- STAMPER, M. A., H. CRAIG, P. SHERYAN, J. BRAUN-McNEILL, L. AVENS, AND M. K. STOSKOPF. 2005. Relationship between barnacle epibiotic load and hematologic parameters in Loggerhead Sea Turtles (*Caretta caretta*), a comparison between migratory and residential animals in Pamlico Sound, North Carolina. *Journal of Zoo and Wildlife Medicine* 36:635–641.
- STOKER, C., F. REY, H. RODRÍGUEZ, J. G. RAMOS, P. SIROSKY, A. LARRIERA, E. H. LUQUE, AND M. MUÑOZ-DE-TORO. 2003. Sex reversal effects on *Caiman latirostris* exposed to environmentally relevant doses of the xenoestrogen bisphenol A. *General and Comparative Endocrinology* 133:287–296.
- STOKER, C., P. M. BELDOMÉNICO, V. L. BOSQUIAZZO, M. A. ZAYAS, F. REY, H. RODRÍGUEZ, M. MUÑOZ-DE-TORO, AND E. H. LUQUE. 2008. Developmental exposure to endocrine disruptor chemicals alters follicular

- dynamics and steroid levels in *Caiman latirostris*. *General and Comparative Endocrinology* 156:603–612.
- STRIK, N. I., A. R. ALLEMAN, AND K. E. HARR. 2007. Chapter 3. Circulating inflammatory cells. *In* E. R. Jacobson (ed.), *Infectious Diseases and Pathology of Reptiles*, pp. 167–218. Taylor and Francis Group, Boca Raton, FL.
- WEISS, D. J., AND K. J. WARDROP. 2010. *Schalm's Veterinary Hematology*. Wiley-Blackwell Inc., Ames, IA.

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