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Hematology and serum biochemistry of free-ranging mantled howler monkeys (*Alouatta palliata*) at La Pacifica, Costa Rica

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Abstract

Background: Hematologic and blood biochemical values are key tools for assessing primate health. A long-term behavioral study of howler monkeys at a single site (La Pacífica, Guanacaste, Costa Rica), afforded the opportunity to develop baseline values for a large group of animals, evaluating differences between adult males and females and comparing to a report in the same population two decades later.

Methods: In 1998, 64 free-ranging mantled howler monkeys were anesthetized and sampled for hematologic and biochemical analysis.

Results: Blood analysis is reported for 29 adult females, 9 juvenile females, 19 adult males and 3 juvenile males. Four adults were excluded due to external injury or disease. There were few significant differences between adult females, juvenile females, and adult males.

Conclusions: Baseline blood parameters are useful for determining normal values for howler monkey populations. The values for total protein, blood urea nitrogen, glucose, liver enzymes and potassium differed from a later study in 2019 may indicate changes that are influencing howler monkey health.

KEYWORDS
blood analysis, ecosystem health, portable chemistry analyzer, primate health

1 | INTRODUCTION

Baseline hematologic and blood biochemical values are key tools for assessing the health of free-ranging primates and can be used to characterize changes in health as animals respond to their environments.[1,2] Baseline values can also provide valuable information for comparison with animals in managed care.[3] However, values may vary not only by age, sex, and species, but by geographic location, hydration, body condition, and nutrition.[4–6] The IUCN recognizes 14 species of howler monkeys and this group has a large geographic distribution, occupying a wider range of habitat type than any other group of Central or South American primate.[5,7,8] As such, they are an excellent candidate species for monitoring health status in different environments. Hematologic and serum biochemistry values have been reported for free-ranging *A. macconnelli* and *A. seniculus* in French Guiana, *A. palliata* and *A. pigra* in Mexico, *A. caraya* in Brazil and Argentina, and *A. palliata* in Costa Rica.[1,5,9–13]

Although historically one of the most widely distributed nonhuman primates in Central America, the mantled howler monkey (*A. palliata*) is in sharp decline in recent years, resulting in a change from a species of Least Concern to Vulnerable[5,8]; with the *A. p. palliata* subspecies classified as endangered.[7] This decline has largely been attributed to habitat loss, the pet trade and hunting, but it is not clear that these are the responsible factors in all situations. For

example, a recent report describes a decline in mantled howler monkey populations in La Pacífica, Costa Rica.[10] Although this area has fragmented habitat and agricultural activity, these conditions are reported as stable over the past five decades and the monkeys have largely been protected from hunting and the pet trade[10,14–17] so the cause of the decline of these monkeys is unclear. Hematologic and serum biochemistry analysis was performed on this population in 2019 to evaluate whether this analysis could shed light on causes of population declines.[10] The present study obtained comparative hematologic and biochemistry analyses for the same population of free-ranging mantled howler monkeys in 1998, 21 years prior. Although there are some differences in analytical techniques between the two investigations, these datasets provide a rare opportunity to compare hematologic and biochemistry information for the same population two decades apart.

2 | MATERIALS AND METHODS

2.1 | Humane care guidelines

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines, were adhered to, and that the appropriate ethical review committee approval was received from Duke University. Darting, capture, marking, and release procedures followed established protocols that minimized risk to the study subjects and have been previously detailed.[18,19] This research adhered to legal requirements of MINAE and CONAGEBIO for the Republic of Costa Rica under appropriate permissions.

2.2 | Study subjects and immobilization

In July 1998, 64 free-ranging *A. palliata* were anesthetized for long-term ecological and behavioral studies at the Centro Ecológico La Pacífica near Cañas Guanacaste Province, Costa Rica (10°28′N and 85°07′W).[20] Since this was part of a long-term behavioral study, most animals were of known age within a few months. Animals ≥3 years of age were considered mature adults, while those 1–3 years of age were considered juveniles. Animals were anesthetized with tiletamine/zolazepam (Telazol^{R}, Fort Dodge) by remote IM delivery (Pneudart, Inc) as previously described.[18] Once anesthetized, each animal was weighed using a commercial hanging scale, then transported to a base of operations and assessed for physical condition and general health. A veterinarian conducted a physical examination, documenting any injuries or signs of illness. Each animal was marked with a modified nylon dog collar or ankle bracelet, and all measurements and blood samples were obtained. Each monkey was monitored until capable of spontaneous movement and then placed in a shaded area in a tied burlap bag until sufficiently recovered to climb without assistance. Once fully recovered, each animal was returned to the proximity of their social group and monitored for successful reintroduction.

2.3 | Blood collection and analyses

Blood samples were collected from the femoral vein 20–60 min after anesthetic induction. Whole blood was collected in plain tubes (BD Vacutainer™) without additives (2 mL) as well as in tubes coated with sodium ethylenediamine-tetra-acetate (EDTA BD Vacutainer™) (1 mL) and gently mixed. Immediately thereafter, capillary tubes were used to make blood smears on glass slides; after air drying, these slides were stained (Diff Quik) for evaluation. The remainder of the EDTA-whole blood samples were stored at 4°C until transported to the reference laboratory (Clinical Analysis Laboratory of the School of Veterinary Medicine at the Universidad Nacional, Heredia, Costa Rica), where packed-cell volume (PCV) was determined using a microhematocrit centrifuge (MB Diamond/IEC Division) and hemoglobin (cyanomethahemoglobin) was measured using a spectrophotometer (Coleman Junior II, Model 6/20). The total white blood cell counts (WBC) were performed within 96 h of collection using a Neubauer chamber or reticulum. Differential WBC counts (based on a 100-cell count) and cell morphologic evaluations were measured using thin blood smears made within 3 h of blood collection and were fixed and stained with a commercial Romanowsky stain (Diff-Quick™).

Plain tubes with blood were kept at 4°C until centrifugation. Within 4 h of collection these tubes were centrifuged at 1500 rpm for 10 min and the serum was placed into two aliquots in cryovials; one aliquot of this serum was processed within 4 h of collection using a portable analyzer (*i*-Stat^{R}) while the other was frozen (−20°C) until analyzed at a reference laboratory (Universidad Nacional, Heredia, Costa Rica). At the laboratory, biochemistry parameters, including total protein, albumin, globulin, alkaline phosphatase, calcium, phosphorus glucose, and blood urea nitrogen (BUN), were measured colorimetrically and kinetically using a spectrophotometer (Model 6/20 Junior II, Coleman). In the field, 65 μL of serum was placed into a cartridge (i-STAT EC8+, *i*-Stat^{R}) and inserted into the portable analyzer. Each cartridge assayed samples for glucose, blood urea nitrogen (BUN), sodium, potassium, chloride, pH, and pCO2. Sodium (mmol/L), potassium (mmol/L) and chloride (mmol/L) were measured by direct ion-selective electrode potentiometry. Glucose (mg/dL) was measured by oxidation with glucose oxidase and amperometric measurement of hydrogen peroxide. Blood urea nitrogen (mg/dL) was hydrolyzed to ammonium in a reaction catalyzed by urease and the resultant ammonium ions were measured amperometrically. Hct (%) was determined by conductivity. (i-Stat System Manual. Waukesha, Wis: Sensor Devices, 1995).

2.4 | Data analysis

Data were grouped into samples from adult females, adult males, juvenile females, and juvenile males. Normality of blood analyte data sets was assessed using Kolmogorov–Smirnov tests. Differences between multiple groups were assessed using Kruskal–Wallis tests with a statistical significance assessed at a level of $p < .05$. In addition, lactating females were separately compared to non-lactating

TABLE 1 Hematologic values for free-ranging mantled howler monkeys (Alouatta palliata).

Parameters	Adult female			Juvenile female			Adult male			Juvenile male		
	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*
Hematocrit (%)	37	(34–39)	29	37	(34–42)	9	37	(36–40)	19	39	(35–39)	3
Hemoglobin (g/dL)	10.5	(9.6–11.4)	29	11.2	(9.7–12)	9	11.2	(10.4–11.6)	19	11.6	(9.8–12.2)	3
White blood cells (cells/uL)	8470	(6435–10 505)	13	12 320	(5940–12 870)	3	9020	(7165–10 175)	13	10 670		1
Neutrophils (% of WBC)	62	(48–69)	29	62	(55–75)	9	66	(61–76)	19	47	(35–66)	3
Lymphocytes (% of WBC)	28	(22–42)	29	33	(20–40)	9	24	(17–30)	19	41	(29–51)	3
Monocytes (% of WBC)	4	(3–6)	29	3	(2–5)	9	4	(2–6)	19	8	(3–14)	3
Eosinophils (% of WBC)	3	(2–4)	29	1	(0–4)	9	2	(1–4)	19	0	(0–3)	3
Basophils (% of WBC)	0	(0–1)	29	0	(0–1)	9	0	(0–1)	19	1	(0–2)	3

Abbreviation: IQR, Interquartile Range.

females. Differences between these groups were assessed using a Mann–Whitney *U* Test. Results are expressed as median and interquartile ranges (IQR). For samples where data was obtained for the same analyte from both the benchtop analyzer and the portable analyzer, Bland–Altman blots were generated to determine the average (±SD) bias. Statistics were performed using GraphPad^R Prism 9.5.1 (Insight Partners, GraphPad Holdings LLC).

3 | RESULTS

Monkeys were in good physical condition, although several animals had old, healed injuries such as missing fingertips or broken tail tips, and minor scrapes and cuts were incurred during capture in three animals. Four adult animals had significant abnormalities on physical examination and blood values from these individuals were excluded from statistical analysis, because the animals were not considered clinically healthy. Two of these were adult females that had full-thickness skin lesions on the ventral aspect of the neck caused by irritation from a previously placed collar. The collar was removed from these animals and replaced with an anklet to allow the lesion to heal. These individuals were anesthetized again 3 days later and the wounds had nearly completely healed by that time. The other two animals for which bloodwork was excluded from analysis were an adult female and an adult male, each exhibiting mucoid nasal discharge, increased respiratory sounds, and pharyngeal inflammation. While these two animals were not recaptured, they were observed via binoculars 3 days after capture and no clinical signs were visually detected.

Results from the remaining 60 animals are reported. Twenty-nine of these were adult females, 9 were juvenile females, 19 were adult males, and 3 were juvenile males. No significant difference in age was detected between adult females (9 year; 5–14 year) and adult males (12 year; 7–14 year). Due to the low number of juvenile males, data from this group are reported but not analyzed for statistically significant differences with other groups.

3.1 | Hematology

Due to sampling and logistical limitations, not all blood analyses were performed on all animals and the number of samples analyzed for each blood parameter is noted in the tables. For reference laboratory hematologic evaluation (Table 1), hematocrit (Hct), hemoglobin (Hgb), and differential white blood cell (WBC) counts were performed on all animals, but total WBC counts were only performed on a subset for each group. No statistically significant differences between groups were detected for any of these parameters. For the animals excluded due to signs of inflammation, WBC values ranged from 9240 to 16 610 cells/uL, with 51%–62% neutrophils, 28%–39% lymphocytes, 4%–8% monocytes, 1%–5% eosinophils, and 1% basophils. Total protein ranged from 5.6 to 6.6 g/dL and globulin values ranged from 1.6 to 3.0 g/dL.

TABLE 2 Serum chemistry values for free-ranging mantled howler monkeys (Alouatta palliata).

Parameters	Adult female			Juvenile female			Adult male			Juvenile male		
	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*
Total protein (g/L)	6.2	(6.0–6.6)	27	6.3	(5.7–6.7)	8	6.6	(6.3–7.0)	19	5.9	(5.7–6.1)	2
Albumin (g/L)	4.3	(3.9–4.6)	29	4.2	(3.9–4.6)	8	4.3	(4.2–4.5)	19	4.2	(3.9–4.5)	2
Globulin (g/L)[a]	2.1	(1.8–2.5)	27	2.0	(1.8–2.5)	8	2.3	(1.8–2.7)	19	1.7	(1.6–1.8)	2
Glucose (mg/dL)	90	(83–104)	29	80	(76–118)	8	98	(87–147)	19	100	(94–106)	2
Blood urea nitrogen (mg/dL)	11	(8–16)	29	14	(7–23)	7	11	(8–16)	19	18	(8–27)	2
Alkaline phosphatase (U/L)	61	(40–100)	29	51	(6–288)	8	62	(33–109)	19	92	(75–108)	2
Alanine aminotransferase (U/L)	7	(5–9)	27	8	(5–9)	8	9	(7–12)	18	8	(8, 9)	2
Phosphorous (mg/dL)[b]	5.7	(4.7–6.9)	27	8.2	(7.5–9.8)	6	6.1	(4.7–7.4)	20	8.4	(8.1–8.8)	2
Calcium (g/dL)	9.3	(9.0–10.2)	22	8.9	(6.1–9.8)	5	9.8	(9.0–10.2)	18	11	(8.8–13.0)	2

Abbreviation: IQR, interquartile range.

[a]Calculated from Total Protein and Albumin results.

[b]calculated value?

For serum biochemistry analysis at the reference laboratory, sufficient quantities of serum were available for complete evaluation of only a subset of animals and the number of animals for which each analyte was measured is noted (Table 2). The only biochemistry analyte that was statistically significantly different between sex-age classes was phosphorus, which was significantly higher in juvenile females than in adult females or in adult males ($p = .02$).

Fifteen of 29 adult females were observed to be lactating. Age was not statistically significant between lactating (12 year; 7–13 year) and non-lactating (12 year; 5–20 year) females and hematologic parameters were not significantly different between these groups (Table 4). ALT was significantly higher in the lactating group ($p = 0.04$) than in the non-lactating group (Table 5). When comparing results from the portable analyzer, lactating adult females had significantly higher anion gap values, but no other statistically significant differences were detected (Table 6).

Blood samples were processed at both the reference laboratory and the portable analyzer for 42 monkeys (Table 7). There was good agreement between the two methods of analysis for glucose values and moderate agreement for Hct and BUN values.

4 | DISCUSSION

4.1 | Hematology

Most hematologic parameters remained similar to those reported from this population in 2019.[10] Unlike the 2019 study, we detected no significant differences between adult males and adult females for Hgb when samples were analyzed at the reference laboratory; however, values obtained using the portable analyzer showed a similar trend to the 2019 study, as females in the present study had significantly lower Hgb, as well as Hct. It is notable that reference laboratory Hct values in our study were consistently higher (median = 5%) than portable analyzer values. The reasons for these differences are unclear. It is possible that the delay in analysis with transport to the reference laboratory may have allowed red blood cells to swell and caused an artificial increase in Hct. Stability of hematologic samples at refrigeration temperatures for up to 24 h has been demonstrated using human blood, but if samples are kept at 4°C longer than 24 h, Hct and Hgb may increase.[21,22] The discrepancy may also have been due to differences in method of analysis. The i-STAT portable analyzer uses conductivity to measure Hct, with measured conductivity inversely proportional to the number of cells in the sample. The formula for this correlation may be different in howler monkeys than in humans or domestic animals for which the i-STAT has been validated. Differences in Hgb between the reference laboratory and the portable analyzer are difficult to interpret. The reference laboratory measured Hgb by spectrophotometry while the portable analyzer calculated Hgb using a standard equation, using an assumption of mean corpuscular volume. In addition, the portable analyzer only reported values within 1 g/dL, so subtle differences in values would have been difficult to discern.

TABLE 3 Portable analyzer chemistry values for free-ranging mantled howling monkeys.

	Adult female			Juvenile female			Adult male			Juvenile male	
Parameters	**Median**	**IQR**	***n***	**Median**	**IQR**	***n***	**Median**	**IQR**	***n***	**Value**	***n***
Hematocrit (%)[b]	29	(28–34)	21	34	(33–35)	7	36	(31–38)	12	31	1
Hemoglobin[a,b]	10	(10–12)	21	12	(12)	7	12	(11–13)	12	11	1
Glucose (mg/dL)	89	(83–96)	21	90	(76–119)	7	88	(79–125)	12	91	1
Blood urea nitrogen (mg/dL)	6	(4–8)	21	10	(4–14)	7	8	(6–10)	12	18	1
Sodium (mmol/L)	136	(135–137)	21	136	(135–137)	7	136	(134–138)	12	138	1
Potassium (mmol/L)	3.8	(3.6–4.2)	21	3.6	(3.4–4.2)	7	4.1	(3.8–4.6)	12	3.6	1
Chloride (mmol/L)	104	(102–108)	21	103	(103–104)	7	104	(102–107)	12	107	1
pH	7.48	(7.42–7.52)	20	7.5	(7.45–7.54)	7	7.45	(7.42–7.58)	12	7.4	1
pCO2	42	(34–48)	20	40	(37–43)	7	38	(35–42)	10	47	1
TCO2[a]	29	(26–36)	20	31	(29–34)	7	30	(28–33)	11	31	1
Anion Gap[a]	6	(3–10)	20	6	(4–7)	7	8	(1–9)	11	6	1
Base Excess[a]	22	(2–10)	20	6	(5–10)	7	4	(1–9)	11	4	1

Abbreviation: IQR, Interquartile Range.

[a]Calculated value.

[b]Statistically significant difference between adult females and other groups.

TABLE 4 Hematologic values for lactating and non-lactating free-ranging mantled howler monkeys.

	Lactating adult female			Non-lactating adult female		
Parameters	**Median**	**IQR**	***n***	**Median**	**IQR**	***n***
Hematocrit (%)	37	(30–42)	15	36	(34–39)	14
Hemoglobin (g/dL)	10.4	(9.0–12.6)	15	10.5	(9.8–11.2)	14
White blood cells (cell/ul)	8580	(5858–11 245)	8	7750	(7040–10 230)	5
Neutrophils (% of WBC)	64	(51–68)	15	64	(47–72)	14
Lymphocytes (% of WBC)	28	(23–42)	15	32	(20–42)	14
Monocytes (% of WBC)	5	(3–8)	15	3	(3–5)	14
Eosinophils (% of WBC)	2	(1–4)	15	4	(2–4)	14
Basophils (% of WBC)	0	(0–1)	15	0	(0–1)	14

Abbreviation: IQR, Interquartile Range.

In general, both our study and the subsequent study in 2019 suggest that female mantled howler monkeys may have slightly lower Hct and/or Hgb than males. Lower Hgb and Hct in adult females compared to males have been previously noted in captive howler monkey populations,[23,24] as well as other species.[25] The reasons for these differences remain speculative and have not been documented in all howler populations studied,[13] but it has been suggested that differences in Hgb and Hct could be due to inhibitory effects of estrogens on erythropoiesis.

White blood cell counts were comparable between our study, the 2019 investigation, and other reports in *Alouatta* spp.[9,10,13,23] They are notably lower than values reported for captive *A. caraya*.[26] However it should be noted that our investigation excluded from statistical evaluation four individuals with clinical evidence of inflammation; these animals had relatively high white blood cell counts which, if included in the data set, would have skewed median values somewhat higher. The cause for leukocytosis in these animals appeared to be skin wounds or pharyngeal inflammation; however, the etiology of the pharyngeal inflammation and accompanying mucus nasal discharge is unknown. The animals with these respiratory signs appeared to be clinically normal several days later, so a transient infection or environmental irritant seems likely; however, evaluation was done remotely so it is possible that signs of disease were not appreciated, rather than not present.

TABLE 5 Serum chemistry values for lactating and non-lactating free-ranging mantled howler monkeys.

	Lactating adult female			Non-lactating adult female		
Parameters	**Median**	**IQR**	***n***	**Median**	**IQR**	***n***
Total Protein (g/L)	6.2	(6.0–6.6)	14	6.4	(6.1–6.9)	13
Albumin (g/L)	4.2	(3.9–4.7)	15	4.3	(3.9–4.5)	14
Globulin (g/L)[a]	2.0	(1.6–2.3)	14	2.3	(2.0–2.7)	13
Glucose (mg/dL)	90	(84–100)	15	89	(79–111)	14
Blood urea nitrogen (mg/dL)	11	(7–17)	15	10	(9–13)	14
Alkaline phosphatase (U/L)	57	(39–79)	15	75	(41–115)	14
Alanine aminotransferase (U/L)[b]	8	(6–11)	14	6	(3–8)	13
Phosphorous (mg/dL)	5.4	(4.8–6.0)	13	5.8	(4.6–7.4)	14
Calcium	0.9	(0.8–1.0)	10	1.0	(0.9–1.0)	12
Sodium (mmol/L)	202	(191–210)	15	192	(188–202)	14
Potassium (mmol/L)	6.7	(6.2–8.0)	15	6.9	(6.0–7.5)	14
Chloride (mmol/L)	101	(85–113)	15	104	(92–116)	14

Abbreviation: IQR, Interquartile Range.

[a]Calculated from Total Protein and Albumin results.

[b]Statistically significant difference between lactating and non-lactating females.

TABLE 6 Portable analyzer chemistry values for lactating and non-lactating adult female mantled howler monkeys.

	Lactating adult female			Non-lactating adult female		
Parameters	**Median**	**IQR**	***n***	**Median**	**IQR**	***n***
Hematocrit (%)	29	(28–34)	10	31	(27–34)	11
Hemoglobin[a]	10	(10, 11)	10	11	(9–12)	11
Glucose (mg/dL)	88	(85–97)	10	90	(82–98)	11
Blood urea nitrogen (mg/dL)	6	(4–11)	10	6	(4–8)	11
Sodium (mmol/L)	136	(135–138)	10	136	(135–137)	11
Potassium (mmol/L)	3.7	(2.7–4.2)	10	4.0	(3.6–4.6)	11
Chloride (mmol/L)	102	(102–108)	10	105	(104–110)	11
pH	7.47	(7.41–7.50)	10	7.48	(7.42–7.53)	10
pCO2	39	(32–47)	10	42	(32–47)	10
TCO2[a]	28	(25–36)	10	30	(28–36)	10
Anion Gap[a,b]	8.5	(5.5–10.5)	10	3.0	(0.0–7.2)	10
Base excess[a]	3.0	(−0.2–10.0)	10	6.5	(2.8–11.0)	10

Abbreviation: IQR, Interquartile Range.

[a]Calculated Value.

[b]Statistically significant difference betweenlactating and non-lactating females

Differential white blood cell counts (percentages) were also similar to other reports, except that the percentage of monocytes was higher in the present study than in the 2019 investigation at La Pacifica, which had reported median monocyte percentages of 0% with a min–max of 0%–5%.[10] Our investigation reports median values of 3%–8% with interquartile ranges from 3% to 14%, depending on age class and sex. Our results are more comparable to reports in other captive and free-ranging howler populations.[9,11,27] The cause for the small, but statistically significant difference with the 2019 study is unclear, but may be due to differences in injuries or in exposure to infectious disease agents between the two points in time We documented several chronic, healing injuries such as broken tail tips or missing finger tips. Although the etiology of these injuries is unknown, the associated inflammation with these chronic injuries could explain a higher monocyte percentage in 1998.

4.2 | Biochemistry

Biochemistry parameters were similar to other reports, but there were some notable differences from the 2019 study in La Pacifica. Total protein in our investigation was lower (Table 2) than reported

TABLE 7 Comparison of blood values for free-ranging mantled howling monkeys using a reference laboratory and a portable analyzer (i-STAT).

Parameters	*n*	Median difference between methods (IQR)	Method	Median	IQR
Hematocrit (%)	42	5 (3 to 8)	Ref lab	38	(35–40)
			i-stat	32	(29–35)
Hemoglobin (g/dL)	42	−0.2 (−0.8 to 0.6)	Ref lab	11	(10–12)
			i-stat	11	(10–12)
Glucose (mg/dL)	42	3 (−3 to 7)	Ref lab	89	(79–114)
			i-stat	89	(81–108)
Blood urea nitrogen (mg/dL)	41	3 (3 to 4)	Ref lab	8	(2–11)
			i-stat	4	(3–8)

Abbreviation: IQR, Interquartile range.

from the 2019 study (median = 7.0–7.2 g/dL). Our results are more consistent with values reported from *A. palliata mexicana* and *A. pigra* in Mexico.[12,28] Increases in total protein may be caused by infection and inflammation, relative dehydration, some neoplastic processes, or better nutritional status. Albumin levels in our study were similar to the 2019 report, while globulin levels (Table 2) were lower than in 2019. This suggests there may have been a change in inflammatory stimulation in the population between the two time points, although it is also possible that the differences were due to differing analytical techniques or the exclusion of animals with clinical signs of inflammation in our study. Inflammatory stimulation could be caused by a wide variety of bacterial, viral, fungal, or parasitic infections, and further investigation would be needed to determine a cause.

Blood urea nitrogen (BUN) was lower in our investigation (Table 2) than in 2019 (19 ± 9 and 21 ± 9 for males and females, respectively), but it seems unlikely that these are statistically or clinically significant differences. The reference laboratory and the portable analyzer provided similar values (Tables 2, 3, and 7). Glucose values were lower (Table 2) compared to the 2019 study (109 ± 28 mg/dL and 100 ± 27 for males and females, respectively), but the magnitude of these differences also is unlikely to be clinically significant.

In contrast, alkaline phosphatase (ALP) was substantially lower in 1998 (Table 2) compared with 310 ± 232 and 276 ± 174 U/L for males and females, respectively, in 2019. ALP can be elevated due to active bone formation or due to liver disease. Given that these differences were seen in adults, the elevation seems most likely due to a hepatic source, rather than from bone growth. Increases seen from 1998 to 2019 could certainly have been due to differences in analytical techniques but may also reflect hepatic insult. In humans, this enzyme is elevated with hepatic cirrhosis, hepatitis, and bile duct blockage; of these hepatic inflammation from an infectious agent seems most likely, but exposure to pathogens that cause hepatic injury was not investigated. The ALP values in 2019 were similar to values reported from *A. palliata* and *A. pigra* sampled in Mexico[1,12,28] and captive *A. guariba* in Brazil,[23] while samples from 1998 were more similar to *A. seniculus* in French Guiana.[2,9] The cause for differences in these different investigations remains unknown, ALP may be a useful parameter to continue to monitor, particularly if it can be paired with reports of hepatic pathology in live animals or at necropsy.

Alkaline aminotransferase (ALT) was similarly lower during the 1998 La Pacífica investigation (Table 2) as compared to the 2019 investigation (26 ± 10 and 21 ± 6 U/L, for males and females, respectively) and to other reports.[1,2,9,23,28] Results from our study are similar to *A. caraya* sampled in Brazil.[24,26] As an indicator of active liver damage, ALT may be a parameter worth monitoring as a clinical sign of environmental hepatic insult in howler monkeys. It should be noted that ALT was the only analyte that was statistically significantly different between lactating and non-lactating females (Table 5); however, this difference was small and unlikely to be of any clinical significance. As such, we suggest that the difference between lactating and non-lactating females may be a Type I statistical error, rather than a true difference, while the more substantial difference between 1998 and 2019 may reflect real changes in environment or diet. Evaluation of the environmental changes that have occurred in the past 20 years could help elucidate possible contributing factors.

Phosphorus is typically elevated in growing animals with active bone metabolism, and as expected, phosphorus values were higher in juvenile females (Table 2) than in adult females or males. Interestingly, the phosphorus values from adult animals in this study were higher (Table 2) than what has been reported in other studies of multiple howler species including the 2019 study of this population.[1,2,9–12] Given the consistently low values in other populations, it seems likely the higher values in our investigation may be due to a difference in sample handling or analytical technique. One of the more common causes of artificially elevated serum phosphorus is hemolysis, as ruptured red blood cells release phosphorus into the serum; however, samples were refrigerated immediately after acquisition, processed promptly, and gross hemolysis was not noted, so hemolysis seems unlikely to be the reason for higher phosphorous values in this study.

Total serum calcium values were only slightly lower in our investigation (Table 2) compared to the 2019 study (10.5 ± 0.5 and 10.0 ± 0.4 mg/dL). Total serum calcium comprises both protein-bound calcium and ionized (free) calcium, so will be affected by the protein content of the blood, particularly the albumin content.[29] Total protein values were lower in 1998 than in 2019, but albumin values were similar between the two study years, so serum protein differences do not explain the lower calcium values in 1998. Differences

in dietary calcium intake or differences in analytic technique may better explain the differences in serum calcium, but would require detailed description of dietary habits in order to substantiate this as the source for the difference.

Sodium values (Table 3) were similar to those reported in 2019[10] as well as those reported in *A. palliata* rescued after electrocution[11] and in *A. pigra* in Mexico.[12] These latter values were substantially lower than those reported in *A. seniculus*[2,9] which were 150 ± 3 mmol/L and 156 ± 7 mmol/L; wild *A. pigra* have been reported to have sodium values of 157 ± 3 mmol/L and 142 ± 4 mmol/L for males and females, respectively.[12] These differences may be due to dietary differences in sodium intake or differences in hydration, with more hydrated animals having lower sodium levels. In future studies of howler monkeys, it may be useful to assess Hct, TP, and sodium, as well as serum creatinine, potassium, osmolarity and osmolality. Urinalysis may also be helpful in assessing hydration status, with urine specific gravity being particularly helpful. Potassium values were lower (Table 3) than values measured in this population in 2019, which may also support a difference in hydration between years.[10] Chloride values in this investigation were consistent with other reports in howler monkey species,[2,9,11,12] but chloride levels were not reported in the 2019 investigation of the La Pacifica population.

5 | CONCLUSIONS

This investigation provides baseline hematological and biochemical data for a population of mantled howling monkeys that can be compared to values from more recent reports. Although there were few significant differences between sex or age groups in 1998, there were notable differences with a subsequent study in liver enzymes (ALT, ALP) and values related to protein metabolism (BUN, total serum protein and albumin). This report also highlights potential opportunities and possible frustrations with using hematology and biochemistry values to assess health under field conditions in free-ranging populations. Not only does careful attention to species differences and differences in environmental conditions need to be made, but also differences in methods of analysis and sample processing. Ideally, samples would be handled similarly across studies and analysis would be performed using the same portable analyzers and/or reference laboratory techniques. However, this is often difficult, if not impossible due to differences in logistical considerations, geographic availability of analyzers and analytical techniques, and technological advances that inevitably occur as equipment is updated and replaced. In addition, some reports present data on relatively small numbers of animals, making it even more difficult to make definitive conclusions.

We also suggest that animals be critically evaluated for signs of clinical disease and excluded from "normal" values. While results from potentially sick individuals are valuable, care should be taken not to incorporate this information with healthy individuals, particularly if baseline or normal values are being established.

Significant differences were not found between sexes or age groups for most values, so grouping age-sex classes may be appropriate and could provide more robust normal ranges. An exception is the lower Hct and Hgb in adult females compared to males. This trend has been documented in multiple studies of non-human primates.[5,10,30–32] The tendency for juveniles to have higher phosphorus values than adults should also be noted.

After comparing between ages and sexes, over time, and with other studies, use of bloodwork to more comprehensively evaluate hydration may be useful. Populations that exhibit blood values suggestive of dehydration, may be under pressure from diminished water resources through food, lack of access to drinking water, or due to higher environmental temperatures resulting in higher water loss.[6] These may be important indicators of habitat degradation and climate change.

Other biochemistry values worth noting are ALP and ALT. Animals with elevations in both enzymes suggest liver damage; if present, an investigation of infectious or toxic agents in the environment is warranted. However, it should be noted that the differences in ALT values were not particularly large. Furthermore, ALT is not considered a useful parameter in domestic herbivores, so ALT values in herbivorous primates should be interpreted in that context.

In general, some of the differences observed between time points may indicate changes that are having an effect on mantled howler monkey health. Increases in total protein, blood urea nitrogen, liver enzymes, and glucose may suggest changes in hydration, diet, exposure to disease and toxins or stress. Further investigation of infectious disease, hydration, contaminants, or other sources of stress may be warranted. However, it is also recognized that these are only two points in time and that normal seasonal or other cyclic variation could also cause changes in blood parameters. Further investigation, including evaluation at different times of year, may help clarify the significance of any blood biochemistry changes.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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