HEMATOLOGICAL AND BLOOD BIOCHEMISTRY PARAMETERS OF CAPTIVE BIG-HEADED AMAZON RIVER TURTLES, 
*Peltocephalus dumerilianus* (TESTUDINES: PODOCNEMIDIDAE)

Parámetros hematológicos y bioquímica sanguínea de tortugas amazónicas cautivas de cabeza grande, *Peltocephalus dumerilianus* (Testudinas: Podocnemidae)

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**ABSTRACT**

The determination of hematological values is used to obtain knowledge about the health conditions of animal species. The big-headed Amazon River turtles, (*Peltocephalus dumerilianus*) are considered one of the least known testudine species concerning their biology and health status. Herein, we determined the hematological and plasma biochemical parameters of 17 (eight males and nine females) adult *P. dumerilianus* to provide reference interval values for clinically healthy individuals. We collected the blood samples by puncturing the femoral vein using long heparinized hypodermic syringes. Sexual dimorphism for individuals was determined by external observation of the shape of the plastron. The average values obtained for the ten hematological and biochemical parameters analyzed were red blood cell count = 0.32 million µL⁻¹; hematocrit = 20.6 %; hemoglobin = 8.5 g dL⁻¹; mean corpuscular volume = 681.6 fl; mean corpuscular hemoglobin = 267.8 pg; mean corpuscular hemoglobin concentration = 41.9 g dL⁻¹; glucose = 80.6 mg dL⁻¹, total protein = 4.1 g dL⁻¹, triglycerides = 388.9 mg dL⁻¹, and total cholesterol = 79.3 mg dL⁻¹. Despite the sexual dimorphism evidenced for the species, there was no significant statistical difference between males and females for both hematological and biochemical parameters analyzed herein. Based on these results, the population is considered healthy, with parameter values coinciding with previously reported reference ranges for testudines species in the region. The results obtained in this study can be used for assessing the health status of other Amazonian turtle populations, especially in actions aimed at cultivation strategies, management, and species conservation.

**Keywords:** Amazon, blood, hematology, physiology, reptiles.

**RESUMEN**

La determinación de valores hematológicos se ha utilizado para conocer las condiciones sanitarias de algunas especies animales. La tortuga cabezona del río Amazonas, *Peltocephalus dumerilianus*, se considera una de las especies de testudines menos conocidas en relación a su biología y estado de salud. Aquí, determinamos los parámetros bioquímicos hematológicos y plasmáticos de 17 adultos (ocho machos y nueve hembras) de *P. dumerilianus* con el fin de proporcionar valores de intervalo de referencia sobre los
peltocephalus -1, 2006; 2014; (roschel, 1848) and population from the amazon are commonly exploited for their meat, eggs, paws, and vertebrate: reptilia) have great economic value, since they the preservation of animal health and environmental health. ecological conditions of vertebrate species and can be used in the determination of both hematology and biochemistry reference values is used for obtaining knowledge regarding the health status of wild populations (aride et al., 2015; oliveira et al., 2016; nascimento et al., 2020). according to stevenson et al. (2005), this practice has led to the strengthening of a research field known as conservation physiology. this is an important concept since it aims to understand in detail the mechanisms that cause conservation problems based on the hematological, metabolic, endocrine, and immunological parameters of animals (stevenson et al., 2005).

these evaluations are important to characterize distinctive physiological parameters and determine specific values for different types of disease (aguirre and balazs, 2000; kakizoe et al., 2007; oliveira et al., 2017). therefore, this type of clinical study has often been applied to research involving endangered species, such as frequently is the case for the species of sea turtle (prieto-torres et al., 2012; 2013; montilla et al., 2014), or even in species that have potential for commercial use (stevenson et al., 2005; lópez-martínez et al., 2020). maceda-veiga et al. (2015) affirm that research on blood components contributes to the monitoring of ecological conditions of vertebrate species and can be used in the preservation of animal health and environmental health.

in the amazon region, the testudines (animalia: vertebrate: reptilia) have great economic value, since they are commonly exploited for their meat, eggs, paws, and the carapace, as well as for use as adornments (oliveira et al., 2011; tavares-dias et al., 2012). due to this scenario, and because they are very easy to capture in the natural environment, several species have virtually disappeared in some regions. nevertheless, the brazilian government has created laws aimed at protecting these species, as well as encouraging their rearing in a captive environment (andrade, 2008). however, currently, few studies assess the physiological state and clinical health of individuals from both natural and captive environments.

the big-headed amazon river turtle (peltoccephalus dumerilianus schweigger, 1812) is considered to be one of the least known testudine species regarding its biology and health status. recent information suggests that big-headed amazon river turtles are being widely used as a food source in locations where populations of larger species, such as podocnemis unifilis (roschel, 1848) and podocnemis expansa (schweigger, 1812), have become reduced. because of its economic and social relevance, the conservation status of p. dumerilianus has become ever more critical, and it is now classified by the iucn (2015) as a species that is vulnerable to extinction. moreover, due to a variety of reasons, baseline health data are not available for many populations throughout the amazon region. therefore, it is very important to perform local studies to establish reference values for the conditions of animals and their environment (aguirre and balazs, 2000; montilla et al., 2006; 2014; stevenson et al., 2005; kakizoe et al., 2007; prieto-torres et al., 2012; 2013; lara resendiz, 2020).

the aims of this study were as follows: 1) obtain the hematological and blood biochemistry reference values for the p. dumerilianus population from the amazon region; 2) compare the blood chemistry values with data previously reported for testudine populations; 3) determine hematological and blood biochemistry differences within the population, according to sex and size of the animals.

materials and methods

sample size, blood collection, and physical examination

all procedures developed in this study were performed according to the license (number 41350) provided by the instituto chico mendes de conservação da biodiversidade (icmbio) and approved by the ceua (animal ethics committee) at the universidade federal do amazonas (ufam) under protocol number. 005/2016. from january to march 2018, we collected blood samples from the seventeen adult p. dumerilianus (eight male and nine female) from rearing systems located in the municipality of manaus, amazonas, brazil. the animals were kept in a
Hematological parameters and biochemical analysis

Erythrocyte counts (RBC) were conducted in a Neubauer chamber, after dilution of the samples in a formalin-citrate solution (Oliveira-Júnior et al., 2009); hematocrit (Ht) was determined using the microhematocrit method; and the hemoglobin (Hb) concentration was obtained using the cyanmethemoglobin method (Oliveira-Júnior et al., 2009). Via these data, the following red cell indexes were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Wintrobe, 1934).

Blood smears were prepared and stained following the recommendations of Oliveira et al. (2011). Subsequently, these were used for morphological identification of leukocyte and total thrombocyte counts (Oliveira-Júnior et al., 2011), and for leukocyte differential counts, which were based on the counts of 200 leukocyte types of interest.

After blood centrifugation, plasma was obtained to determine glucose (mg dL\(^{-1}\)), total protein (g dL\(^{-1}\)), triglycerides (mg dL\(^{-1}\)), and total cholesterol (mg dL\(^{-1}\)) concentrations with the aid of specific commercial kits (Labtest Diagnóstica®, Brazil).

Statistical analyses

Data were expressed as mean, standard deviation (SD), and reference intervals for each hematological and biochemical parameter. To evaluate data normality, we used the Shapiro-Wilk test. Because our sample size was low, reference interval values were only calculated for the whole population. We calculated these values in two ways (Prieto-Torres et al., 2012;2013): 1) all values between the mean and two SDs were included for normally distributed variables, and 2) data found between the 2.5\(^{th}\) and 97.5\(^{th}\) percentiles were selected for variables that were not normally distributed. All analyses were performed using a significance level of 95 % (\(p < 0.05\)) using R software, v. 3.5.3.

RESULTS

All the big-headed Amazon River turtle individuals were alert and active during capture and were considered clinically healthy upon physical examination (Thomson et al., 2009; Ferrando Gaibisso, 2010; Harris et al., 2011). Captured animals were classified as adults based on their morphological measurements. We observed statistically significant differences (\(p < 0.05\)) among mostly their morphological measurements among individuals according to their sex; the males were those with the higher values for SCL, SCW, SLP, and weight (Table 1).

Regarding the leukocyte and thrombocyte morphology in \(P.\) dumerilianus, the staining technique used did not

| Parameters                        | Male (n= 8)     | Female (n= 9)  | \(P\)     |
|----------------------------------|----------------|---------------|----------|
| Straight carapace length (cm)    | 43.0 ± 8.5 (27.0 – 52.0) | 33.0 ± 6.3 (23.0 – 42.0) | 0.025*   |
| Straight carapace width (cm)     | 38.7 ± 8.9 (22.0 – 48.0) | 30.2 ± 4.9 (22.0 – 37.0) | 0.029*   |
| Straight plastron length (cm)    | 31.0 ± 6.7 (18.0 – 37.0) | 23.9 ± 4.4 (17.0 – 30.0) | 0.023*   |
| Straight plastron width (cm)     | 28.9 ± 5.8 (18.0 – 35.0) | 24.5 ± 4.2 (18.0 – 31.0) | 0.096    |
| Weight (kg)                      | 18.2 ± 8.2 (3.2 – 26.2)  | 9.2 ± 5.5 (2.4 – 17.8)   | 0.020*   |

* denotes a significant difference between males and females: Student’s t-test (\(p < 0.05\)).
present satisfactory results, making the morphological and quantitative analysis of leukocytes and thrombogram impossible. This difficulty may have been caused by a specific biological agent in the blood of *P. dumerilianus*, demonstrating that the blood extension staining technique used in other species of Amazonian turtles (Oliveira-Júnior et al., 2009; Oliveira et al., 2011; Tavares-Dias et al., 2012) is not effective for all species of testudinids in the Amazon region.

Despite the sexual dimorphism evidenced for the species, there was no significant statistical difference in the hematomal parameters analyzed between males and females (Table 2). No statistically significant differences in the results of the erythrogram were found between adult *P. dumerilianus* males and females (Table 3).

**DISCUSSION**

Sexual dimorphism has been previously observed in studies conducted with *P. dumerilianus* individuals in natural environments. Males are generally larger than females (De La Ossa-V et al., 2011). The study by Pritchard and Trebbau (1984) recorded male specimens with SCL ranging from 37.4 to 42.2 cm, while females showed values between 27.1 and 27.6 cm, both of which are lower than the average values obtained in this study. Similar results to those found in our study were also recorded in studies by Pezzutti (2003) and Iverson and Vogt (2002). On the other hand, we observed a maximum bodyweight of 26.2 kg for males and 17.8 kg for females, which is higher than the weight recorded for wild individuals (15 kg) by Iverson and Vogt (2002) and Pezzutti (2003).

Differences observed for the morphological variables among individuals from different populations (including captive populations) may be explained by several factors, such as aquatic environment quality, geographical location, genetic variability, gender, age, and nutritional status (Oliveira-Júnior et al., 2009). For the present study, we considered that nutritional status was the most probable factor for such differences regarding previous studies.

### Table 2. Hematological and biochemical parameters of the *Podocnemis* species. Animals from a captive breeding system located in the city of Manaus (Brazil).

| Parameters        | Species                  | Adult male (n= 8) | Adult female (n= 9) | p* | Adult male (n= 68) | Adult female (n= 35) | Adult male and female (n= 28) | Adult male and female (n= 28) | Adult male and female (n= 28) |
|-------------------|--------------------------|-------------------|---------------------|----|-------------------|----------------------|-----------------------------|-----------------------------|-----------------------------|
| **Hematological** |                          |                   |                     |    |                   |                      |                             |                             |                             |
| Ht (%)            | *P. dumerilianus*         | 20.6 ± 3.7        | 20.5 ± 3.6          | 0.945 | 21.5 ± 0.4        | 20.8 ± 0.4           | 25.1 ± 6.9                 | 21.8 ± 6.6                  | 23.2 ± 4.7                  |
|                   | *P. erythrocephala*       | 7.9 ± 2.4         | 9.0 ± 2.7           | 0.384 | 6.3 ± 1.3         | 5.7 ± 1.1            | 6.5 ± 1.21,3               | 5.1 ± 2.3                   | 7.0 ± 1.6                   |
|                   | *P. expansa*              | 0.34 ± 0.08       | 0.29 ± 0.05         | 0.171 | 0.44 ± 0.08       | 0.41 ± 0.08          | 0.28 ± 0.07                | 0.28 ± 0.08                 | 0.18 ± 0.08                 |
|                   | *P. unifilis*             | 637.2 ± 71.2      | 716.2 ± 173.1       | 0.278 | 485.5 ± 90.3      | 477.2 ± 117.8         | 922.3 ± 150.2              | 851.4 ± 282.1               | 1425.1 ± 448.3              |
|                   | *P. sextuberculata*       | 247.8 ± 285.3     | 285.3 ± 69.6        | 0.226 | 29.11 ± 3.4       | 27.6 ± 3.6            | 26.2 ± 5.4                 | -                           | -                           |
|                   |                          | 39.0 ± 4.9        | 44.2 ± 12.1         | 0.306 | 142.1 ± 26.6      | 130.5 ± 32.6          | -                           | 22.3 ± 5.5                  | 30.1 ± 3.3                  |
| **Biochemical**   |                          |                   |                     |    |                   |                      |                             |                             |                             |
| Glucose (mg dL⁻¹) | *P. dumerilianus*         | 76.4 ± 8.1        | 83.4 ± 8.0          | 0.138 | 116.4             | 116.4                | 91.3 ± 17.7                | 92.7 ± 22.2                 | 149.9 ± 66.5                |
|                   | *P. erythrocephala*       | 3.6 ± 1.2         | 4.4 ± 2.1           | 0.471 | 20.8 ± 3.4        | 20.8 ± 4.3            | 3.5 ± 1.3                  | 2.4 ± 0.7                   | 3.8 ± 0.7                   |
|                   | *P. expansa*              | 379.1 ± 46.5      | 397.5 ± 31.2        | 0.377 | 12.72 ± 3.6       | 14.5 ± 7.3            | 35.4 ± 19.7                | 18.9 ± 7.0                  | 27.8 ± 8.3                  |
|                   | *P. unifilis*             | 86.7 ± 10.9       | 73.8 ± 27.6         | 0.192 | 32.7 ± 9.1        | 38.2 ± 12.7           | 58.1 ± 18.3                | 38.3 ± 26.5                 | 125.0 ± 55.2                |
|                   | *P. sextuberculata*       | 73.8 ± 27.6       | 73.8 ± 27.6         | 0.192 | 32.7 ± 9.1        | 38.2 ± 12.7           | 58.1 ± 18.3                | 38.3 ± 26.5                 | 125.0 ± 55.2                |
| Total proteins (g dL⁻¹) | *P. dumerilianus*         | 22.3 ± 5.5        | 30.1 ± 3.3          | 0.413 | 22.3 ± 5.5        | 30.1 ± 3.3            | 27.3 ± 4.2                 | -                           | -                           |
|                  | *P. erythrocephala*       | 150.2 ± 55.2      | 125.0 ± 55.2        | 0.384 | 125.0 ± 55.2      | 117.3 ± 51.4          | -                           | -                           | -                           |

*Student's t test.*
According to De La Ossa-V et al. (2011), the big-headed turtle is classified as an omnivorous species, and carnivorous and omnivorous individuals tend to have plasma biochemistry values superior to herbivorous species.

The results of the erythrogram (Ht, Hb, RBC, MCV, MCH, MCHC) help to determine the type of anemia, volume, dehydration, and responses associated with parasite levels in chelonian individuals reared in both natural and cultivated environments (Marcon et al., 2008; Oliveira-Junior et al., 2009; Oliveira et al., 2011; Morselli et al., 2016). The study by Tavares-Dias et al. (2012) establishes erythrogram values for the Amazonian freshwater turtles P. expansa, P. unifilis (Troschel, 1848), and Podocnemis sextuberculata (Cornali, 1849) considering ontogeny, fishing tools, and forced diving. The Ht, RBC, and MCV values for P. dumerilianus are similar to the values of captive P. expansa individuals (Oliveira-Júnior et al., 2009; Tavares-Dias et al., 2012), which indicates an oxygen uptake similar to the described Testudine species. For Hb, MCH, and MCHC, the values were closer to those related to the P. unifilis. However, it is noteworthy that these studies did not investigate issues associated with sexual dimorphism. Thus, according to the erythrogram, P. dumerilianus has intermediate biological characteristics that fall between P. expansa, and P. unifilis.

In the biochemical analysis, no statistically significant differences were found between males and females about plasma glucose, total protein, triglyceride, and total cholesterol levels, which is similar to the results described for P. expansa, P. unifilis and P. sextuberculata (Tavares-Dias et al., 2012). In the present study, we observed that the glucose values were similar to those described by Oliveira-Júnior et al. (2009) and Tavares-Dias et al. (2012) for P. expansa. These indices indicate normoglycemia in the P. dumerilianus when compared to other species sampled from the natural environment (Tavares-Dias et al., 2012). Total protein levels for captive animals are generally lower than those in wild animals (Norton, 1990; Christopher et al., 2003), and this variable is associated with food management. Thus, the total protein levels of P. dumerilianus were similar to those described for P. expansa (Marcon et al., 2008; Oliveira-Júnior et al., 2009; Tavares-Dias et al., 2012), but were lower than those described for P. unifilis and P. sextuberculata (Tavares-Dias et al., 2012).

For triglyceride and total cholesterol levels, the values found were higher than those described for P. expansa (Marcon et al., 2008; Oliveira-Júnior et al., 2009; Tavares-Dias et al., 2012). Cholesterol levels were lower than those shown for P. unifilis and P. sextuberculata (Tavares-Dias et al., 2012). Thus, we can infer that, for the big-headed turtle, there is possibly a case of a lipid metabolism disorder. This can be measured by changes in cholesterol, which is secreted from the liver as bile acids (Swimmer, 2000). These changes may occur due to the diet provided, which presents high-fat values and promotes an increase of triglycerides and cholesterol. Also, factors such as lack of exercise in a confined condition promote increased blood fat levels.

CONCLUSIONS

Based on the erythrogram data, we can infer that the big-headed turtle is a species with intermediate characteristics that fall between the species P. expansa and P. unifilis. In the plasma biochemistry, glucose and total protein values are similar to P. expansa. Total cholesterol and triglyceride levels were high,
certainly resulting from inadequate nutrition management, as well as the size of the tanks to promote greater swimming activity for this Amazonian Testudine species.

The application of reference hematological intervals is fundamental for the monitoring of species in both natural and farm environments since they serve as a basis for actions related to the sustainable use of these resources. The hematological parameters for the *P. dumeriliana* determined in the present study can be used in actions aimed at species management, conservation, and captive breeding strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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