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Hematology and biochemistry of Chelonians from the Amazon region: a literature review

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Abstract

This paper presents a literature review of Amazonian turtles' hematological and biochemical parameters from different habitats. The methodology is based on information in other languages from reliable national and international scientific databases between 2000 and 2021. The results obtained in this study for the hematological and biochemical parameters showed differences between the Amazonian turtle species and other turtle species analyzed in the published works. The hatchlings of *Podocnemis unifilis* (yellow-spotted Amazon River turtle) showed differences in blood parameters among sex ratios. Differences were also observed between recently hatched *Podocnemis erythrocephala* (red-headed river turtle) and adults (sex ratio). The hematocrit (Ht) values of the recently hatched *P. unifilis* (15.1 ± 1.9 %) and *P. erythrocephala* (15.36 ± 2.87 %) groups presented by the authors were lower than those of the adult animals of the same species. In the hematimetric indices, when compared between species, the mean corpuscular volume (MCV) found was higher for *P. unifilis*, *Peltocephalus dumerilianus* and *Podocnemis expansa*. The hematological and biochemical values presented may be influenced by factors such as sex and developmental stage in certain species, as well as by their environment and other aspects of biology. The information gathered can serve as a reference for future research on captive breeding and conservation strategies for freshwater turtles.

Keywords: aquatic organism, blood, clinical pathology, Podocnemididae.

Resumo - Hematologia e bioquímica de quelônios da região amazônica: revisão da literatura

Este artigo apresenta uma revisão bibliográfica sobre parâmetros hematológicos e bioquímicos de tartarugas amazônicas de diferentes habitats. A metodologia é baseada em informações em diferentes idiomas de bancos de dados científicos nacionais e internacionais confiáveis no período entre 2000 e 2021. Os resultados obtidos neste estudo para os parâmetros hematológicos e bioquímicos mostraram diferenças entre as espécies de tartarugas amazônicas e outras espécies de tartarugas analisadas nos trabalhos publicados. Os filhotes de *Podocnemis unifilis* (tracajá) apresentaram diferenças nos parâmetros sanguíneos entre a proporção sexual. Diferenças também foram observadas entre *Podocnemis erythrocephala* (irapuca) recém-eclodidas e adultos (razão sexual). Os valores de hematócrito (Ht) dos grupos *P. unifilis* (15,1 ± 1,9 %) e *P. erythrocephala* (15,36 ± 2,87 %) recém-eclodidos apresentados pelos autores foram menores do que os dos animais adultos da mesma espécie. Nos índices hematimétricos, quando comparados entre as espécies, o volume corpuscular médio (VCM) encontrado foi maior para *P. unifilis*, *Peltocephalus dumerilianus* e *Podocnemis expansa.* Os valores hematológicos e bioquímicos apresentados podem ser influenciados por fatores como sexo e estágio de desenvolvimento em determinadas espécies, bem como pelo seu ambiente e outros aspectos da biologia. As informações coletadas podem servir de referência para pesquisas futuras, para a criação em cativeiro e estratégicas de manejo e conservação de quelônios de água doce.

Palavras-chave: organismo aquático, sangue, patologia clínica, Podocnemididae.

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Resumen - Hematología y bioquímica de los quelonios de la región amazónica: revisión bibliográfica

Este artículo presenta una revisión sobre parámetros hematológicos y bioquímicos de tortugas amazónicas. La metodología se basa en información en diferentes idiomas proveniente de bases de datos científicas en el período comprendido entre 2000 y 2021. Los resultados obtenidos en este estudio para los parámetros hematológicos y bioquímicos mostraron diferencias entre las especies de tortugas amazónicas y otras especies de tortugas analizados en los trabajos publicados. Las crías de *Podocnemis unifilis* (tortuga de río Amazonas con manchas amarillas) mostraron diferencias en los parámetros sanguíneos entre machos y hembras. También se observaron diferencias entre *Podocnemis erythrocephala* (tortuga de río pelirroja) recién nacida y adultos (machos y hembras). Los valores de hematocrito (Ht) de los grupos recién nacidos de *P. unifilis* (15,1 ± 1,9 %) y *P. erythrocephala* (15,36 ± 2,87 %) presentados por los autores fueron inferiores a los de los animales adultos de la misma especie. En los índices hematimétricos, al comparar entre especies, el volumen corpuscular medio (VCM) encontrado fue mayor para *P. unifilis*, para *Peltocephalus dumerilianus*, y *Podocnemis expansa*. Los valores hematológicos y bioquímicos presentados pueden verse influenciados por factores como el sexo y la etapa de desarrollo en ciertas especies, así como por su entorno y otros aspectos de la biología. La información recopilada puede servir como referencia para futuras investigaciones, para la cría en cautividad y estrategias de gestión y conservación de quelonios de agua dulce.

Palabras clave: organismo acuático, sangre, patología clínica, Podocnemididae.

Introduction

For animals from aquatic or terrestrial environments, including reptiles of the order Testudines (Batsch, 1788), under cultivation in captivity for commercialization or in a natural environment, hematology is a crucial tool for assessing the health of the organism (Oliveira *et al.,* 2011, 2015, 2016, 2017, 2017a, 2021; Almosny and Monteiro, 2004). Hematology's benefits are recognized because blood parameters provide rapid identification of stress in the animal (Magro *et al.,* 2015; Aride *et al.,* 2016, 2018, 2020, 2021), which makes it possible to make corrections in management and thus maximize the development of the chelonians (Araújo *et al.*, 2009).

Routine hematological assessment of reptiles includes the determination of hematocrit (Ht) or packed cell volume (PCV), hemoglobin (Hb), erythrocyte (red blood cell) count, leukocyte (white blood cell; global leukocyte) count, differential count of leukocytes (specific leukocyte count) and assessment of blood cell morphology (Nascimento *et al.,* 2020; Castro *et al.,* 2020, 2020a; Nardini *et al.*, 2013). The identification and counting of leukocytes and thrombocytes can be used to assess and control the physiological state of aquatic organisms in captivity, thus standardizing the ideal conditions for their rearing (Bussons *et al.,* 2021; Liebl *et al.,* 2021, 2022; Tavares-Dias *et al.*, 2007). In the blood count, as in other tests, the analyses may be influenced by factors such as sexual dimorphism, species, stage of development, type of environment, population genetics, maturity, migratory conditions, diet, stress levels, seasonality, presence of ectoparasites and environmental conditions (Polese *et al.,* 2022; Ribeiro *et al.,* 2024; Stacy *et al.*, 2011). In addition, factors determined by human interference, such as handling, storage, sample processing, collection location, anticoagulant, dye, and cell counting method used, also influence these parameters (Santos *et al.,* 2024; Marcon *et al.*, 2008).

According to Silveira *et al.* (2017), blood biochemical profiles are used to assess the physiological status of reptiles and should consider the environmental conditions and physiological parameters, such as nutritional status, sex, age, season, and physiological status. Biochemical analyses are performed with the plasma or serum of the blood collected, and the substances produced by the animal's organs, which regulate the body's endocrine system, are quantified. As in biochemical analyses with animals of other genera, in chelonians, the blood samples collected must be centrifuged for plasma separation, processed as soon as possible, stored under refrigeration or frozen until compounds such as glucose, triglycerides, cholesterol and proteins are analyzed (Innis and Knotek, 2020; Batista, 2021).

Chelonians (Testudines) of the Podocnemididae family include three genera of freshwater turtles: *Erymnochelys* (Baur, 1888), *Peltocephalus* (Duméril and Bibron, 1835), and *Podocnemis* (Wagler, 1830). The last one includes wild species from the Amazon, which for centuries have been exploited for trade, human consumption and other purposes. The main *Podocnemis* species that are exploited are *Podocnemis expansa* (Schweigger, 1812), *Podocnemis unifilis* (Troschel, 1848) and *Podocnemis sextuberculata* (Cornalia, 1849) (Andrade, 2008; Van Dijk *et al.*, 2014; Ferrara *et al.*, 2017).

The South American river turtle (*P. expansa*), the yellow-spotted river turtle (*P. unifilis*), the six-tubercled river turtle (*P. sextuberculata*), and the red-headed river turtle (*Podocnemis erythrocephala*; Spix, 1824) are inhabitants of the Solimões River basin and its tributaries, in addition to other white, black and clearwater rivers (Ríos-Villamizar *et al.*, 2020). These reptiles are found in terrestrial or aquatic environments, and some reptiles are classified as semiaquatic species using land and water at separate times of the year (Ferrara *et al.*, 2017).

The life strategy of Chelonians involves producing many offspring during each reproductive cycle, and they have a high birth rate. They directly contribute to the ecological functions of where they live by being part of the food chain and recycling nutrients. Through their diet, which is generally omnivorous, they transform organic matter into animal protein by ingesting abundantly living or dead matter, which is essential for cleaning rivers and lakes (Ferrara *et al.*, 2017). Therefore, this work aims to present, through a literature review, the hematological and biochemical parameters of Chelonians from the Amazon.

MATERIAL AND METHODS

For the preparation of this literature review, information was collected from national and international scientific literature databases, including Google Scholar (https://scholar.google.com.br/), Digital Library of Thesis (http://bdtd.ibict.br/vufind/), Scopus (https://www.scopus.com/), and CAPES Journals (https://www-periodicos-capes-gov-br), using studies available in Portuguese, English, and Spanish. The following keywords were used: Chelonians, Amazonian testudines, hematology, biochemistry, Amazon turtle and the publication date of the works published was delimited between 2000 and 2024.

After reviewing the titles and abstracts related to the researched topic and excluding those not containing relevant information, the ten publications with the highest citation index in the databases and the easiest access were selected. These publications were tabulated and organized according to species, blood parameters, locality, developmental stage, sexual dimorphism, and authorship of the articles, before being qualitatively analyzed in a descriptive manner.

RESULTS AND DISCUSSION

The studies presented in this literature review demonstrate significant differences in all analyzed parameters for the species *Peltocephalus dumerilianus, P. erythrocephala, P. expansa, P. unifilis, and P. sextuberculata*, when compared across environments (captive production, natural environment, laboratory environment), sexual dimorphism (sex ratio), and developmental stages (recently hatched, juvenile, and adult).

The hatchlings of *P. unifilis* (tracajás) described in the studies by Yoshioka *et al.* (2017) showed differences in blood parameters compared to adult chelonians (sex ratio) studied by Pamphilio Júnior (2017) and Tavares-Dias *et al.* (2012) (Table 1). For the species *P. erythrocephala* (irapuca), there were differences between recently hatched and adults (sex ratio) described in the work by Santos (2011). The author emphasizes that the statistical differences revealed that the developmental stage is an important factor in establishing reference hematological values for groups of Chelonians.

The hematocrit (Ht) values of the *P. unifilis* hatchling group (15.1 ± 1.9 %) and *P. erythrocephala* recently hatched (15.36 ± 2.87 %) presented by the authors in Table 1 were lower than those of adult animals of the same species, even though they were collected in different environments (natural environment and laboratory environment). This suggests that the developmental stage may significantly influence hematocrit percentage more than the environment for these species. However, these tabulated values are higher when compared to the work of Lopes (2016) with chelonians of the species *Phrynops hilarii* (Duméril and Bibron, 1835; cágados-de-barbelas) with Ht between 10.57 % and 13.13 % occurring in the natural environment of the Pampa region, southern Brazil, with different weights and developmental stages.

Hematocrit values below 20 %, as occurred in the chelonians in the studies by Yoshioka *et al.* (2017), Tavares-Dias *et al.* (2012), and Santos (2011) (Table 1), as well as in the work of Lopes (2016), suggest that the animal may be in a condition of anemia (Morselli *et al.*, 2016). However, considering the amount of data analyzed in this review and the fact that hematological parameters of chelonians tend to have significant

Table 1. Erythrogram of chelonians in the State of Amazon (AM), Brazil.

Species	Ht (%)	Hb (g dL^{-1})	RBC (millions μL^{-1})	MCV (fL)	MCH (%)	MCHC (%)	Phase/sexual dimorphism*	Location	References
Genus *Peltocephalus*									
P. dumerilianus	20.6 ± 3.7	7.9 ± 2.4	0.34 ± 0.08	637.2 ± 71.2	247.0 ± 37.1	39.0 ± 4.9	Adult/M	AM/CP	Anselmo et al. (2021)
P. dumerilianus	20.5 ± 3.6	9.0 ± 2.7	0.29 ± 0.05	716.0 ± 173.1	285.3 ± 69.6	44.2 ± 12.1	Adult/F	AM/CP	Anselmo et al. (2021)
Genus *Podocnemis*									
P. erythrocephala	21.5 ± 0.4	6.30 ± 1.32	0.44 ± 0.08	485.5 ± 90.28	142.1 ± 26.6	29.11 ± 3.5	Adult/M	Negro River, AM/NE	Santos (2011)
P. erythrocephala	20.77 ± 0.38	5.73 ± 11	0.41 ± 0.08	477.2 ± 117.8	130.5 ± 32.6	27.6 ± 3.6	Adult/F	Negro River, AM/NE	Santos (2011)
P. erythrocephala	15.36 ± 2.87	4.71 ± 1.16	0.38 ± 0.12	433.78 ± 151.40	121.58 ± 39.7	28.58 ± 2.68	Recently hatched	Negro River, AM/NE	Santos (2011)
P. expansa	25.1 ± 6.9	6.5 ± 1.21.3	20.28 ± 0.07	922.3 ± 150.2	-	26.2 ± 5.4	Adult/ M and F	AM/CP	Oliveira-Júnior et al. (2009)
P. expansa	21.8 ± 6.6	5.1 ± 2.3	0.28 ± 0.08	851.4 ± 281.1	-	22.3 ± 5.5	Adult/ M and F	Purus River, AM/NE	Tavares-Dias et al. (2012)
P. expansa	29.7 ± 4.2	16.3 ± 7.3	-	852.7 ± 245.6	-	55.3 ± 23.3	Recently hatched	Purus River, AM/NE	Tavares-Dias et al. (2012)
P. expansa	23.9 ± 1.8	6.7 ± 0.4	-	859.5. ± 27.3	241.1 ± 7.4	28.1 ± 0.7	Adult/ M and F	AM/CP	Marcon et al. (2008)
P. unifilis	23.9 ± 4.7	7.0 ± 1.6	0.18 ± 0.08	14251 ± 448.3	-	30.1 ± 3.3	Adult/ M and F	Purus River, AM/NE	Tavares-Dias et al. (2012)
P. unifilis	15.1 ± 1.9	6.03 ± 0.84	1.80 ± 4.35	1005.5 4 ± 250	361.27 ± 775	39.98 ± 93	Hatchlings	AP/LE	Yoshioka et al. (2017)
P. unifilis	22.30 ± 3.43	6.25 ± 1.56	0.20 ± 0.09	1446.4 ± 769.53	35.50 ± 14.26	27.77 ± 7.69	Adult/ M and F	AP/LE	Pamphilio Júnior (2017)
P. sextuberculata	17.6 ± 5.4	4.7 ± 1.2	0.16 ± 0.05	1.291.3 ± 652.3	-	27.3 ± 4.2	Adult/ M and F	Purus River, AM/NE	Tavares-Dias et al. (2012)

Ht= Hematocrit; **Hb=** Hemoglobin; **RBC=** red blood cells; **MCV=** Mean corpuscular volume; **MCH=** Mean Corpuscular Hemoglobin; **MCHC=** Mean corpuscular hemoglobin concentration; ***Development phase; **M=** male; **F=** female; **CP=** Captive Production; **NE=** Natural Environment; **LE=** Laboratory Environment. AM= Amazonas; AP= Amapá.

variations between developmental stages, sex ratio, sexual dimorphism, and environments (Lopes, 2016), the condition of anemia could not be confirmed.

Hematocrit values above 40 % indicate hemoconcentration or polycythemia (Morselli *et al.*, 2016). The reviewed studies did not present values exceeding this percentage (Ht> 40 %) in this study. Therefore, the hematocrit data for the Chelonians presented (Table 1) are within the stipulated range for reptiles, which varies from 20 to 40 % (Stacy *et al.*, 2011).

In animals, hemoglobin concentration is related to activity level, being higher in more active animals (Lopes, 2016). Santos (2011), in his research on irapuca (*P. erythrocephala*), observed hemoglobin

concentrations that varied across all developmental stages (Table 1) and were higher in mature chelonians and lower in recently hatched ones. In the hemoglobin (Hb) analyses presented in Table 1, males of the studied species exhibited higher values than females. However, according to Pessoa (2015), there is no significant difference between the sexes for turtles. As previously discussed in this literature review regarding hematocrit, hemoglobin also tends to show higher values with increasing age.

Medeiros et al. (2012) used red-eared sliders (Trachemys scripta elegans; Wied, 1838), and hemoglobin values averaged 7.1 %, close to the average values shown in Table 1. Stacy et al. (2011) highlight that most clinically healthy reptiles have hemoglobin percentages between 5.5 and 12 g dL^{-1}. Therefore, the results of the cited studies are within the average hemoglobin range for reptiles.

Santos (2011) presented RBC and MCH (Red Blood Cells and Mean Corpuscular Hemoglobin, respectively) data with lower values for recently hatched *P. erythrocephala* compared to mature animals (*P. dumerilianus* and *P. erythrocephala*). In hematimetric indices, when compared between species, the Mean Corpuscular Volume (MCV) was higher in the *P. unifilis* group with 1425.1 ± 448.3 fL (Tavares-Dias *et al.*, 2012), the *P. dumerilianus* group with 716.0 ± 173.1 fL (Anselmo *et al.*, 2021), and the *P. expansa* group with 92.3 ± 150.2 fL (Oliveira-Júnior *et al.*, 2009), all adult chelonians (Table 1).

The lowest MCV concentrations were observed by Santos (2011) in recently hatched *P. erythrocephala* (433.78 ± 151.40 fL) (Table 1). According to the references established by Campbell (2006) for reptiles, the MCV varies between 200 and 1200 fL. Therefore, the analyzed MCV values are by the reference, except for those of *P. sextuberculata* (1291.3 ± 652.3 fL) and *P. unifilis* (1425.1 ± 448.3 fL) adult sex ratio. The high MCV value verified by Tavares-Dias *et al.* (2012) suggests macrocytosis (increased erythrocyte volume), possibly due to erythropoiesis influenced by hypoxia during the diving stress tested by the authors (Costa *et al.*, 2020). Other relevant factors when analyzing increases in MCV in reptiles are electrolyte balance disturbances in the organism, such as abnormal water loss and the time of year (seasonal), considering animals in a natural environment (Marcon *et al.*, 2008). Only adult female *P. dumerilianus* in captive production and recently hatched *P. expansa* in a natural environment (Anselmo *et al.*, 2021; Tavares-Dias *et al.*, 2012) presented MCHC (Mean Corpuscular Hemoglobin Concentration) values above 41 %, which is the maximum percentage determined by Campbell (2006) for reptiles.

The quantification of thrombocytes and leukocytes is essential for understanding the immune defenses of chelonian organisms (Tavares-Dias and Moraes, 2007). Chelonian thrombocytes are nucleated, elliptical, and spindle-shaped cells with coagulation (hemostasis) and immune defense functions, with a problematic estimation due to the aggregates they form (Oliveira-Júnior *et al.*, 2009; Pamphilio Júnior, 2017). The thrombocyte levels presented for the adult sex ratio of *P. unifilis* and for recently hatched *P. sextuberculata* and *P. expansa* were higher compared to the values obtained for *P. expansa* and *Phrynops geoffroanus* (Schweigger, 1812; Geoffroy's side-necked turtle) presented by Pessoa (2015) (Figure 1). The chelonians analyzed by Pessoa (2015) were tested for hemoparasitosis and the occurrence of thrombocytopenia (decreased number of thrombocytes) in debilitated or stressed turtles (Manire *et al.*, 2017).

Leukocytes protect vital processes from infectious disturbances and stress, making them indicators of animal health (Silveira *et al.*, 2017). Considering all the Chelonian species analyzed, the data presented by Tavares-Dias *et al.* (2012) in Figure 1 reveal that the number of circulating leukocytes was higher for adult *P. expansa* and *P. unifilis* (respectively, 8007 ± 3271 µL and 6661 ± 3741 µL) compared to adult *P. sextuberculata* (6022 ± 2197 µL).

The lowest result for adults (Figure 1) was observed by Tavares-Dias *et al.* (2012) in their study of adult *P. unifilis* (6661 ± 3741 µL). Among the hatchlings, *P. unifilis* had the lowest leukocyte concentration (4325 ± 1199 µL), while *P. sextuberculata* had the highest result (10408 ± 7165 µL), with both species being studied by Tavares-Dias *et al.* (2012).

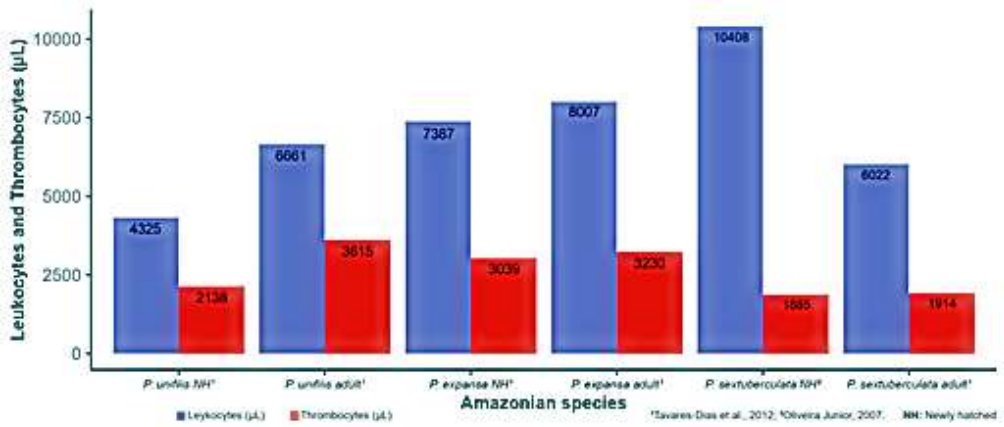

Figure 1. Leukocytes and thrombocytes of Amazonian chelonians (*Podocnemis* genus).

According to Pires *et al.* (2008), the maximum leukocyte concentration for chelonians in a natural environment is 6200 µL. Therefore, the concentration observed for *P. sextuberculata* (Tavares-Dias *et al.*, 2012) can be classified as leukocytosis (high leukocyte concentration; Figure 1). In addition to the species above, *P. expansa* in both the hatchling stage (7387 ± 2925 µL) and adult stage (8007 ± 3271 µL), studied in the natural environment (Rio Purus – AM; Figure 2) by Tavares-Dias *et al.* (2012), also showed leukocytosis. Leukocytosis may occur as a physiological response to stress conditions, such as diving, which the authors tested, and infectious processes (Santos, 2011).

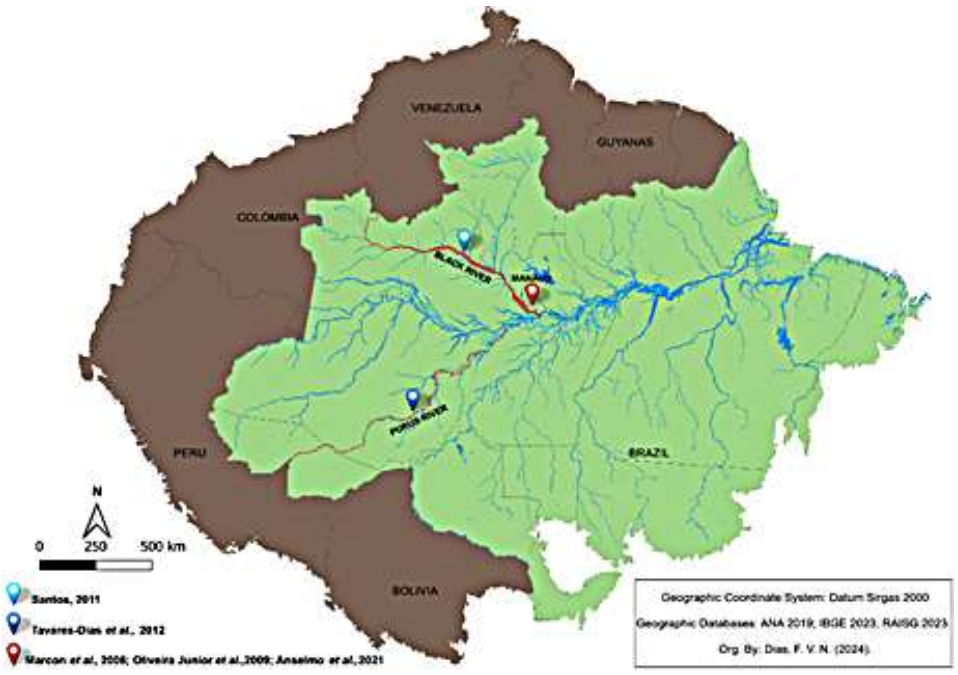

Figure 2. This literature review analyzed infographics of research sites (natural environment, production, and laboratory) of Amazonian Chelonians, according to each author.

In the differentiation of leukocytes, the relative and absolute counts of eosinophils in the studies analyzed (Table 2) showed similar numbers compared to those reported by Pires et al. (2008) (468.86 ± 302.04 µL; 15.55 ± 6.52 %). Eosinophil values may increase in cases of parasitism and nonspecific immune stimulation, and the significance of eosinopenia in reptiles remains unknown (Silveira *et al.*, 2017).

Heterophils are round granulocytes with a rounded nucleus and cytoplasm filled with spindle-shaped eosinophilic granules that function in phagocytosis and innate immunity (Manire *et al.*, 2017). An increase in heterophils may indicate compromised animal health, as these cells suggest a severe inflammatory response to bacterial infection and other pathological processes. In studies by Pires *et al.* (2006), Pires *et al.* (2009), and Pamphilio Júnior (2017) on chelonians, the values presented were 59.37 %, 53.77 %, and 27.00 %, respectively. The current literature review found lower values for the species *P. unifilis, P. expansa,* and *P. sextuberculata*, with counts of 3262 ± 2165 µL, 4820 ± 1767 µL, 2883 ± 1064 µL, and 6518 ± 4667 µL,

corresponding to 48.97 %, 65.22 %, 33.4 %, and 62.62 %, respectively (Table 2). The increase in heterophils observed is known as heterophilia and may indicate a response to inflammatory disease (Batista, 2021).

Table 2. Differentiation of leukocyte granulocytes and agranulocytes of Amazonian Chelonians (*Podocnemis* genus).

Species	Lymphocyte (µL)	Eosinophils (µL)	Heterophiles (µL)	Basophils (µL)	*Phase/sexual dimorphism	Location	References
P. unifilis	537 ± 245	434 ± 353	69.4 ± 8.4	237 ± 94	Recently hatched	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. unifilis	1235 ± 1005	1416 ± 778	3262 ± 2165	322 ± 188	Adult/ M and F	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. expansa	1159 ± 561	718 ± 532	4820 ± 1767	332 ± 256	Recently hatched	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. expansa	2009 ± 912	2423 ± 1216	2623 ± 1554	511 ± 459	Adult/ M and F	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. expansa	-	1373.0 ± 658	2883.0 ± 1064	491.0 ± 337	Adult/ M and F	AM/NE	Oliveira-Júnior *et al.* (2009)
P. sextuberculata	1502 ± 1358	1049 ± 547	6518 ± 4667	682 ± 480	Recently hatched	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. sextuberculata	654 ± 284	749 ± 278.0	409 ± 266	4024 ± 1498	Adult/ M and F	Purus River, AM/NE	Tavares-Dias *et al.* (2012)

P. = Genus *Podocnemis*; *Development phase; **M=** male; **F=** female; **CP=** Captive Production; **NE=** Natural Environment; **LE=** Laboratory Environment; **AM**=Amazonas.

Lymphocytes account for 15 % to 85 % of the leukocyte count in reptiles, according to Stacy *et al.* (2011). In a study conducted by Pires *et al.* (2006) on *Caretta caretta* (Linnaeus, 1758) raised in captivity in Bahia, northeastern Brazil, the lymphocyte values obtained were 2009 ± 912 µL, representing 29.5 % of the total leukocytes, while Pamphilio Júnior (2017) reported values of 54.50 % in his studies on *Chelonoidis carbonarius* (Spix, 1824). The values reported by the cited authors fall within the reference percentage for lymphocytes in chelonians and are similar to those found in the current studies (Table 2).

Basophils are small, rounded cells characterized by metachromatic basophilic cytoplasmic granules, which can obscure the nucleus. Although the function of basophils in reptiles is not yet fully understood, an increase in their percentage is reported in certain hemoparasitic and viral infections (Silveira *et al.*, 2017). The basophil counts described in studies by Stein *et al.* (2015) ranged from 500.28 to 2159 µL, similar to those found in adult and hatchling *P. sextuberculata* and *P. expansa* in this review (Table 2). Compared to other genera, Chelonians exhibit a high circulation of basophils in the bloodstream, potentially accounting for up to 60 % of leukocytes. However, when this percentage is exceeded, it may indicate the presence of viral infections (Stacy *et al.*, 2011; Batista, 2021).

Analyzing metabolites allows for evaluating the nutritional status in wild or captive animals (Pamphilio Júnior, 2017). As shown in Table 3, the levels of total proteins vary between species and developmental stages within each study analyzed.

In the study by Tavares-Dias *et al.* (2012), total protein levels were higher (Table 3) in adult sex ratio chelonians of the species *P. expansa* and *P. unifilis* (2.4 ± 0.7 g dL^{-1} and 3.8 ± 0.7 g dL^{-1}, respectively).

Table 3. Plasma biochemical parameters of Amazonian Chelonians.

Species	Total Proteins (g dL^{-1})	Glucose (mg dL^{-1})	Total Cholesterol (mg dL^{-1})	Triglycerides (g dL^{-1})	*Phase/ sexual dimorphism	Location	References
Genus *Peltocephalus*							
P. dumerilianus[1]	3.6 ± 1.2	76.4 ± 8.1	86.7 ± 10.9	379.1 ± 31.2	Adult/M	AM/CP	Anselmo *et al.* (2021)
P. dumerilianus[1]	4.4 ± 2.1	83.4 ± 8.0	73.8 ± 27.6	379.5 ± 31.2	Adult/F	AM/CP	Anselmo *et al.* (2021)
Genus *Podocnemis*							
P. unifilis[2]	3.31 ± 0.48	35.82 ± 8.07	82.27 ± 28.33	80.84 ± 52.13	Adult/ M and F	AM/LE	Pamphilio Júnior (2017)
P. unifilis[2]	2.3 ± 3.5	89.7 ± 18.4	800.4 ± 324.1	58.6 ± 40.7	Recently hatched	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. unifilis[2]	3.8 ± 0.7	149.9 ± 66.5	125.0 ± 55.2	27.8 ± 8.3	Adult/ M and F	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. unifilis[2]	3.81 ± 0.67	49.65 ± 8.59	95.51 ± 16.47	151.83 ± 34.46	Hatchlings	AM/LE	Yoskioka *et al.* (2012)
P. erythrocephala[2]	20.84 ± 3.41	6.49	1.84 ± 0.53	0.69 ± 0.17	Adult/ M	Negro River, AM/NE	Santos (2011)
P. erythrocephala[2]	20.82 ± 4.28	6.53	2.09 ± 0.66	0.77 ± 0.37	Adult/ F	Negro River, AM/NE	Santos (2011)
P. erythrocephala[2]	14.67 ± 1.89	7.81	2.81 ± 0.83	0.47	Recently hatched	Negro River, AM/NE	Santos (2011)
P. expansa[2]	2.1 ± 0.5	89.7 ± 18.4	677.7 ± 162.8	64.9 ± 16.8	Recently hatched	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. expansa[2]	2.4 ± 0.7	92.7 ± 22.2	38.3 ± 26.5	18.9 ± 7.0	Adult/ M and F	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. expansa[2]	3.5 ± 1.3	91.3 ± 17.7	58.1 ± 18.3	35.4 ± 19.7	Adult/ M and F	AM/NE	Oliveira-Júnior *et al.* (2009)
P. expansa[2]	2.8 ± 0.7	94.9 ± 30.6	38.3 ± 38.6	31.3 ± 20.9	Adult/ M and F	AM/CP	Marcon *et al.* (2008)
P. sextuberculata[2]	1.9 ± 0.5	92.1 ± 19.8	945.8 ± 240.7	83.6 ± 61.4	Recently hatched	Purus River, AM/NE	Tavares-Dias *et al.* (2012)
P. sextuberculata[2]	1.6 ± 0.3	127.1 ± 42.2	117.3 ± 51.4	37.7 ± 9.9	Adult/ M and F	Purus River, AM/NE	Tavares-Dias *et al.* (2012)

*Development phase; **M=** male; **F=** female; **CP=** Captive Production; **NE=** Natural Environment; **LE=** Laboratory Enviroment; AM= Amazonas; AP= Amapá.

A similar pattern was observed in the study by Santos (2011) for the species *P. erythrocephala*, where total protein levels were significantly higher in adults (males: 20.84 ± 3.41 g dL^{-1}; females: 20.82 ± 4.28 g dL^{-1}) compared to hatchlings (14.67 ± 1.89 g dL^{-1}) (Table 3). The increase in total protein levels in females is related to maturation and the accumulation of vitellogenic protein. In males, the higher protein concentration in the bloodstream is associated with elevated testosterone levels (Kakizoe *et al.,* 2007). Therefore, the increase in total protein observed by the authors may be related to the sex ratios analyzed and the collected animals' maturation or adult life stage.

In contrast, Tavares-Dias *et al.* (2012) reported higher protein levels in hatchlings (1.9 ± 0.5 g dL^{-1}) compared to adults of the species *P. sextuberculata*, which may be related to the sex of the collected animals, the high metabolism typical of early life stages, or the diet of the adult animals. Omnivorous species tend to be carnivorous when younger and become herbivorous when adults, highlighting the influence of diet on plasma levels in chelonians (Andrade, 2008).

In reptiles, total protein levels in healthy animals range from 3 to 7 g dL^{-1}. When exceeding 7 g dL^{-1}, it is considered hyperproteinemia, often occurring alongside dehydration and associated with chronic inflammatory disease (Campbell, 2006). In the studies by Oliveira-Júnior *et al.* (2009) on the species *P. expansa*, the total protein levels were higher than those found by Marcon *et al.* (2008) and Tavares-Dias *et al.* (2012), which may reflect differences in the animals' origin (captive production in Amazonas State versus the natural environment of the Purus River - AM, respectively), as well as environmental and nutritional conditions (Marcon *et al.*, 2008).

The glucose and cholesterol levels reported by Santos *et al.* (2011) for *P. erythrocephala* (7.81 mg dL^{-1} and 2.81 ± 0.83 mg dL^{-1}, respectively) were higher in hatchlings compared to adults, similar to the values found for *P. expansa* and *P. sextuberculata* by Tavares-Dias *et al.* (2012) (Table 3). Elevated total cholesterol and glucose concentrations in hatchlings indicate high energy demands due to the accelerated metabolism at this stage of development, which may be met by the mobilization of these compounds from yolk residues (Santos, 2011).

Triglyceride levels for *P. unifilis, P. expansa,* and *P. sextuberculata* reported by Tavares-Dias *et al.* (2012) were higher in hatchlings (Table 3). Generally, turtles exhibit different growth rates throughout their lives, and like cholesterol levels, triglycerides are mobilized to meet the energy demands of the high metabolic activity in hatchlings. The triglyceride levels (22.9 ± 9.8 mg dL^{-1}) reported by Fonseca *et al.* (2016) for captive *P. expansa* were similar to the values observed in the species of this study. In animals with diabetes mellitus, biliary obstructions, pancreatitis, hypothyroidism, or those on diets rich in carbohydrates and fats, serum cholesterol levels may be elevated (Fonseca *et al.*, 2016). The studies by Anselmo *et al.* (2021), Pamphilio Júnior (2017), and Yoshioka *et al.* (2017) presented the highest triglyceride levels, which may be related to the diets provided to the animals, considering they were in commercial and laboratory production environments. The variation in triglycerides and cholesterol (hyperlipidemia) may respond to physiological or pathological processes (Marcon *et al.*, 2008).

Conclusion

The hematological and biochemical values observed in this study vary according to sex, developmental stage, and species and are also influenced by environmental factors, lifestyle, seasonal changes, and other biological aspects.

Certain factors to which the animals were exposed may have directly contributed to the elevated levels observed in hematology and biochemistry, potentially caused by stress during the collection process, which could have impacted the results. The probable anemia noted may be linked to leukocytosis and could indicate parasitic infestation, especially in captive animals where crowding facilitates infection.

The hematological and biochemical parameters of the species studied by various authors and compiled in this study can inform and improve management practices in Chelonian farming and serve as a reference for future research.

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