http://dx.doi.org/10.1590/1678-4162-12726

Arq. Bras. Med. Vet. Zootec., v.74, n.5, p.841-852, 2022

The influence of season, environment, sex, and body mass on biochemical profile of the freshwater turtle *Phrynops geoffroanus* (Schweigger, 1812)

[*Influência da estação, do ambiente, do sexo e da massa corporal no perfil bioquímico do cágado* Phrynops geoffroanus (*Schweigger, 1812*)]

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ABSTRACT

We analyzed the influence of the season, the environment, and the sex, as well as the relation of body mass (BM) in the serum albumin (ALB), aspartate aminotransferase (AST), creatinine (C), creatine kinase (CK), phosphorus (P), total calcium (tCa), total protein (TP), urea (U), uric acid (UA), calcium:phosphorus ratio (Ca:P), and the globulin value (GV) of thirty individuals of *Phrynops geoffroanus* of the urban area of Cuiabá, Mato Grosso, Brazil. The modeling of biochemical parameters was performed using the Generalized Additive Models for Location, Scale and Shape (GAMLSS) to verify the influence of variables considered in this study on each of the biochemical parameters analyzed. The season influenced AST, CK, C, tCa, Ca:P and UA. The environment influenced tCa, Ca:P, U and UA. On the other hand, CK, tCa, P, Ca:P and U differed significantly between males and females. Regarding the BM, a relationship of this variable was observed with CK, C, tCa, P, U, UA and Ca:P. We concluded that the season, environment, sex, and body mass can influence the biochemical parameters of *P. geoffroanus*, and these factors should be routinely considered in the interpretation of laboratory results.

Keywords: Chelidae, chelonians, serum chemistry, side-necked turtle, Testudines

RESUMO

Analisou-se a influência da estação, do ambiente e do sexo, assim como a relação da massa corporal (BM) nos níveis sorológicos de albumina (ALB), aspartato aminotransferase (AST), creatinina (C), creatina quinase (CK), fósforo (P), cálcio total (tCa), sólidos totais (TS), ureia (U), ácido úrico (UA), relação cálcio:fósforo (Ca:P), e do valor da globulina (GV) de Phrynops geoffroanus *da área urbana de Cuiabá, Mato Grosso, Brasil. A modelagem dos parâmetros bioquímicos foi realizada utilizando-se os modelos aditivos generalizados para locação, escala e forma (GAMLSS) para verificar a influência das variáveis consideradas neste estudo em cada um dos parâmetros bioquímicos analisados. A estação sazonal influenciou os níveis de AST, CK, C, tCa, Ca:P e UA. O ambiente foi capaz de influenciar tCa, Ca:P, U e UA. Por outro lado, CK, tCa, P, Ca:P e U diferiram significativamente entre machos e fêmeas. Em relação à BM, observou-se relação dessa variável com CK, C, tCa, P, U, UA e Ca:P. Concluiu-se que a estação sazonal, o ambiente, o sexo e a massa corporal são capazes de influenciar os parâmetros bioquímicos de* P. geoffroanus *e que esses fatores devem ser rotineiramente considerados na interpretação dos resultados laboratoriais.*

Palavras-chave: cágado-de-barbicha, Chelidae, química sérica, quelônios, Testudines

INTRODUCTION

Brazil occupies the third place in the world in abundance of reptiles (848 species), the Testudines order being represented by eight families and 38 species (Costa, Guedes and Bérnils, 2021; Uetz *et al.*, 2022). In Mato Grosso, this order is represented by 12 species, of which 10 are freshwater turtles (Costa, Guedes and Bérnils, 2021; Moura *et al.*, 2021).

Phrynops geoffroanus (Chelidae) is a chelonian known as “Geoffroy’s side-necked turtle”. It has wide geographical distribution in South America

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Submitted: March 17, 2022. Accepted: July 7, 2022.

(Brazil, Colombia, Ecuador, Peru, Bolivia, Venezuela, Paraguay, Uruguay and far northeast Argentina), and is found in the most diverse environments in Brazil, with influence from the Cerrado, Amazon Rainforest and Caatinga and with different degrees of anthropization (Ferrara *et al.*, 2017; Müller *et al.*, 2019). Populations above 200 individuals/ha have already been recorded in urban environments and this is probably the result of the abundance of sewage and organic waste produced by humans, the absence of predators, and increased availability of nesting areas (Souza and Abe, 2000). In captivity, this species is the most frequent of the Chelidae family, living in zoos and screening centers in South America (Molina, 1999; Murphy, 2016).

P. geoffroanus has generalist feeding habits and adapts to anthropized urban areas such as polluted streams, whose low levels of water oxygenation compromise the development of its main food component (Chironomidae larvae) (Souza and Abe, 2000), as well as channeling of streams that lead to trampling when the animals move in search of food or nesting areas (Müller *et al.*, 2019). Despite these unfavorable factors to the life of urban populations, the species is classified as Least Concern (LC) by the IUCN (Rhodin *et al.*, 2021).

Thus, medical evaluation of animals, either free-living or in captivity, is essential as a diagnostic tool for their conservation (Almosny, 2014). Because they are ectothermic animals, whose clinical signs may not be evident, differently from what occurs in mammals, biochemical tests are recommended, once they may indicate physiological, hepatic or renal alterations resulting from lesions and/or diseases. These tests, in short, help us to evaluate the individual's health status and the better treatment, besides providing a more assertive prognosis (Ryser-Degiorgis, 2013; Innis and Knotek, 2020).

In reptiles, the interpretation of laboratory tests results is a challenge (Innis and Knotek, 2020) because profound physiologic adaptations may occur in response to a number of intrinsic and extrinsic factors (Campbell, 2022). Intrinsic factors (including sex, age, and the physiologic status of the reptile) and extrinsic factors (such as season, temperature, habitat, diet, and disease) can result in different biochemical parameters, even within the same species (Campbell, 2022). In addition, because they are ectothermic, they are still exposed to variables associated with the environment in which they live (Campbell, 2022) such as, for example, the climate, which, in turn, is in close relationship with the location (Mikolajewicz *et al.*, 2018).

Although the influences of these factors are cited in the literature, only a few of them are considered when establishing reference values (Brites, 2002; Hidalgo-Vila *et al.*, 2007; Hernandez *et al.*, 2017; Almonacid, 2020; Erazo and Calderón, 2020). Consequently, the reported reference intervals are wide and their diagnostic efficacy is limited (Scope *et al.*, 2013; Campbell, 2022). In addition, extrapolation of the values already established for other species may lead to erroneous conclusions about the health status of the reptile being evaluated (Ryser-Degiorgis, 2013).

In this paper, we present biochemical parameters for a sample of *P. geoffroanus* composed of individuals captured in different seasons (rainy and dry) and environments (urban stream and captivity), and from different sexes and body mass. We also analyzed the influence of all these factors on the levels of each analyte evaluated.

MATERIALS AND METHODS

This research was authorized by the state (Sema/MT n°. 758/2018) and federal (Sisbio no. 60092) environmental agencies and approved by the Ethics Committee of the Federal University of Mato Grosso (n°. 23108.232109/2017-68).

Blood samples were obtained from males (n = 16) and females (n = 14) *P. geoffroanus* from January 2017 to December 2018 (Table 1). The animals were from an urban stream (n = 14) or kept in captivity (n = 16) in the Medicine and Research Center of Wild Animals of the Federal University of Mato Grosso. These same samples were obtained during two seasons: rainy, from October to April (n=20) and dry, from May to September (n=10), as described by Tarifa (2011) for the State of Mato Grosso.

The animals from urban stream were returned to the capture sites immediately after blood samples had been collected. They were captured in funnel traps (1.20 m of length and 0.80 m of length, both of plastic mesh of 25 mm) (Balestra *et al.*, 2016) installed in a urban stream called "Córrego do Barbado" (15°36'26.9"S, 56°03'58.1"W), tributary of the Cuiabá river, upper Paraguay river basin. The traps were installed in two different points of the stream with tuna or sardine baits and were inspected twice a day. This urban stream is located within the Campus of the Federal University of Mato Grosso. The area does not have access to the human population, it presents flow of domestic effluents and degraded riparian forest. Despite this, native animals (e.g., capybaras and caimans) are often sighted in the area, demonstrating that it still provides resources for the fauna.

Table 1. Number of blood samples collected from both male and female *Phrynops geoffroanus* living in two different environments and during different seasons

	Urban stream	Captivity	Rainy season	Dry season
Male (n = 16)	n = 8	n = 8	n = 12	n = 4
Female (n = 14)	n = 6	n = 8	n = 8	n = 6
Total	14	16	20	10

Blood samples were obtained immediately after capturing to reduce stress. Maximum volume of 1% of the body mass of each individual was obtained by puncture of the post-occipital venous sinus, packed in a tube containing gel and clot activator. After centrifugation, the serum was kept under refrigeration until the moment of processing, not exceeding 24 hours. All analyses were performed in an automatic biochemistry analyzer (Wiener Lab®, CM250 model) using the respective commercial kits. The serum volume determined the number of analytes dosed for each individual, and it was not possible to verify, in the same animal, all parameters.

The biochemical parameters analyzed (and, inside the parentheses, their abbreviations, measuring units, and sample size, respectively) were: albumin (ALB, g/dL, *n* = 28); aspartate aminotransferase (AST, UI/L, *n* = 28); creatine kinase (CK, UI/L, *n* = 21); creatinine (C, mg/dL, *n* = 27); phosphorus (P, mg/dL, *n* = 26); total calcium (tCa, mg/dL, *n* = 25); total protein (TP, g/dL, *n* = 28), urea (U, mg/dL, *n* = 28), and uric acid (UA, mg/dL, *n* = 28). Calcium:phosphorus ratio (Ca:P, *n* = 25) and globulin value (GV, g/dL, *n* = 28) were calculated (Meuten and Sample, 2022).

The body mass of each individual and sexing were performed prior to clinical examination (Flanagan, 2015), and evidence of changes determined the exclusion of seven individuals from the samples to be analyzed. The turtles from the urban stream were returned to the same capture site.

The normality of the residues was verified using the Shapiro-Wilk test (Royston, 1982), and the homogeneity of variances by the Bartlett (Bartlett, 1937) and Breusch-Pagan (Breush and Pagan, 1979) tests. The modeling of biochemical parameters was performed using the Generalized Additive Models for Location, Scale and Shape (GAMLSS) (Rigby and Stasinopoulos, 2005). For biochemical parameters that presented heterogeneity of variances, in addition to the mean (μ), regression in the variability parameter (σ) was also assigned. Thus, the normal distribution for ALB, C, GV, TS, tCa and Ca:P, and gamma distribution for AST, CK, P, U and UA were assumed. The influence of the variables season, environment, and sex on biochemical parameters was considered significant when P < 0.05.

Since the variable body mass has a nonlinear relationship with biochemical parameters, the cubic spline smoothing function, represented by the cs(·) function, implemented in the GAMLSS package (Stasinopoulos and Rigby, 2007) was used. Finally, to verify the quality of adjustment, we used the wp(·) function that provides the worm plot (Van Buuren and Fredriks, 2001). All analyses were performed using the R software (R Core team®, version 4.0.5, 2021).

RESULTS

The results showed that the season influenced six analytes: AST, C, CK, tCa, Ca:P, and UA, in which AST, CK, tCa and Ca:P ratio were higher during the dry season, while C and UA were higher in the rainy season (Tab. 2 and 5); the environment influenced four analytes: tCa, U, UA and Ca:P that were higher in urban stream turtles (Tab. 3 and 5) while females of *P. geoffroanus* presented higher levels of CK, tCa, P, Ca:P, and U (Table 4 and 5). There was no influence of the variables season, environment, and sex in ALB, GV, and TP (Table 5). Through Fig. 1 and 2, it is possible to observe a relation between the body mass and the biochemical parameters CK (Fig. 1-D), C (Fig. 1-C), tCa (Fig. 2-A), P (Fig. 1-E), and Ca:P ratio (Fig. 2-D), U (Fig. 2-B), and UA (Fig. 2-C), in which the black line of the figure represents the estimated smooth curve that can be interpreted as the average behavior of the biochemical parameter as the animal gains weight, and the shading in blue represents the 95% confidence bands for the smoothing curve that was estimated.

Table 2. Mean ($\overline{X}$), standard deviation (SD), and minimum (Min) and maximum (Max) values of the biochemical parameters of *Phrynops geoffroanus*, during the rainy and dry seasons

Biochemical parameters	Rainy season (n=20)		Dry season (n=10)	
	$\overline{X}$±SD	Min-Max	$\overline{X}$±SD	Min-Max
Albumin (g/dL)	2.22±0.79	0.2 – 3.5	2.22±0.74	0.8 – 3.0
AST (UI/L)*	80.52±40.16	39 - 193	145.33±86.63	27 – 297
Creatinine (mg/dL)*	0.47±0.20	0.1 – 0.8	0.20±0.19	0.03 – 0.6
CK (UI/L)*	1124.14 ±1101.45	128 - 3382	2289.5±1506.96	207 – 3811
P (mg/dL)	4.03±2.50	1.90 – 12.8	4.62±2.22	1.5 – 9.5
TP (g/dL)	4.17±1.31	1.1 – 6.2	4.79±1.26	2.1 – 6.4
tCa (mg/dL)*	9.10±4.24	4.3 – 20.7	12.30±5.93	4.6 – 25.2
Urea (mg/dL)	25.62±21.00	2 - 63	29.559±10.07	20 – 46
UA (mg/dL)*	1.58±1.04	0.4 – 4.2	1.06±0.85	0.1 – 3.0
Ca:P*	2.39±1.03	0.92 – 5.17	2.85±0.41	2.21 – 3.48
GV (g/dL)	2.06±0.88	0.90 – 3.80	2.53±0.76	1.3 – 3.8

AST, aspartate aminotransferase; tCa, total calcium; Ca:P, calcium:phosphorus ratio; CK, creatine kinase; GV, globulin value; P, phosphorus; TP, total protein; UA, uric acid. *Shows significant differences between the seasons.

Table 3. Mean ($\overline{X}$), standard deviation (SD), and minimum (Min) and maximum (Max) values of the biochemical parameters of *Phrynops geoffroanus*, from two different environments: urban stream and captivity

Biochemical parameters	Environment			
	Urban Stream (n=14)		Captivity (n=16)	
	$\overline{X}$±SD	Min-Max	$\overline{X}$±SD	Min-Max
Albumin (g/dL)	1.95 ±0.87	0.2 – 3.0	2.46±0.59	1.5 – 3.5
AST (UI/L)	107.15±72.03	27 - 297	98.76 ±63.14	39 – 256
Creatinine (mg/dL)	0.42±0.22	0.1 – 0.8	0.35± 0.25	0.03 – 0.8
CK (UI/L)	1344.16 ±1320.92	207 - 3811	1402.58 ±1281.74	128 – 3382
P (mg/dL)	4.10±2.11	1.5 – 9.5	4.37±2.61	1.9 – 12.8
TP (g/dL)	4.33±1.49	1.1 – 6.1	4.44±1.17	2.5 – 6.4
tCa (mg/dL)*	11.82±6.59	4.3 – 25.2	9.02±3.13	4.7 – 14.7
Urea (mg/dL)*	35.81 ±17.79	11 - 63	20.14±14.77	2 – 59
UA (mg/dL)*	1.65± 1.13	0.4 – 4.2	1.51 ±0.80	0.1 – 3.5
Ca:P*	2.93±1.02	1.79 – 5.17	2.26±0.63	0.92 – 3.21
GV (g/dL)	2.36±0.71	0.9 – 3.1	2.10±0.96	1.00 – 3.80

AST, aspartate aminotransferase; tCa, total calcium; Ca:P, calcium:phosphorus ratio; CK, creatine kinase; GV, globulin value; P, phosphorus; TP, total protein; UA, uric acid. *Shows significant differences between the environments.

Table 4. Mean ($\overline{X}$), standard deviation (SD), and minimum (Min) and maximum (Max) of the biochemical parameters of *Phrynops geoffroanus*, females and males

Biochemical parameters	Sex			
	Females (n=14)		Males (n=16)	
	$\overline{X}$±SD	Min-Max	$\overline{X}$±SD	Min-Max
Albumin (g/dL)	2.18±0.89	0.2 – 3.5	2.25±0.68	0.8 – 3.2
AST (UI/L)	108.76 ±61.08	27 - 256	97.15± 73.55	39 – 297
Creatinine (mg/dL)	0.31±0.25	0.03 – 0.8	0.45±0.20	0.05 – 0.8
CK (UI/L)*	1841.66 ±1475.47	207 – 3382	1153.83 ±1129.43	128 –3811
P (mg/dL)*	5.47±3.08	3 – 12.8	3.37±1.12	1.5 – 5.2
TP (g/dL)	4.52±1.37	1.10 – 6.40	4.28±1.28	2.1 – 6.1
tCa (mg/dL)*	13.01±5.93	4.7 – 25.2	8.42±3.46	4.3 – 16.7
Urea (mg/dL)*	27.70±15.92	4 - 61	26.6±19.31	2 – 63
UA (mg/dL)	1.15±0.70	0.1 – 2.7	1.58±1.15	0.4 – 4.2
Ca:P*	2.73±1.14	0.92 – 5.17	2.45±0.67	1.53 – 4.09
GV (g/dL)	2.29±0.82	0.90– 3.80	2.16±0.90	1 – 3.80

AST, aspartate aminotransferase; tCa, total calcium; Ca:P, calcium:phosphorus ratio; CK, creatine kinase; GV, globulin value; P, phosphorus; TP, total protein; UA, uric acid. *Shows significant differences between the sexes.

During the analyses, we did not observe hemolysis or lipemia in any of the samples. Finally, to verify the quality of adjustment, the worm plot was considered, in the context of GAMLSS models. Moreover, in all models it was possible to conclude that the distributions used for each of the biochemical parameters were a good choice for data analysis and that the obtained results are reliable, since no trend (vertical displacement, slope) was observed.

Table 5. Result of the estimation for the adjusted regression of the biochemical parameters of *Phrynops geoffroanus*

Effect	Estimate	Standard error	P-value	Estimate	Standard error	P-value
		Albumin (g/dL)			**Creatinine (mg/dL)**	
Intercept	1.98	0.55	0.17	0.55	0.17	<0.01
Sex (M)	0.32	0.01	0.10	0.01	0.10	0.87
Season(D)	0.03	-0.28	0.07	-0.28	0.07	**<0.01**
Envir (FL)	-0.51	0.04	0.06	0.04	0.06	0.50
		Phosphorus (mg/dL)			**Globulin (g/dL)**	
Intercept	1.67	0.39	<0.01	2.25	0.79	<0.01
Sex (M)	-0.61	0.21	**0.01**	-0.47	0.45	0.30
Season(D)	0.07	0.15	0.64	0.28	0.32	0.37
Envir (FL)	0.06	0.12	0.64	0.35	0.26	0.20
		TP (g/dL)			**Uric acid (mg/dL)**	
Intercept	0.40	1.25	<0.01	1.85	0.61	<0.01
Sex (M)	-0.11	0.72	0.87	-0.60	0.35	0.10
Season(D)	0.57	0.49	0.26	-0.73	0.24	**<0.01**
Envir (FL)	-0.07	0.41	0.81	0.42	0.20	**<0.04**
		CK (UI/L)				
Intercept	1.16	1.55	0.47			
Sex (M)	2.93	0.86	**<0.01**			
Season(D)	1.18	0.48	**0.03**			
Envir (FL)	0.42	0.43	0.35			
		Total calcium (mg/dL)			**Ca:P ratio**	
Intercept	0.19	0.91	<0.01	2.16	0.19	<0.01
Sex (M)	-8.92	0.34	**<0.01**	-2.04	0.08	**0.03**
Season(D)	-1.16	0.83	0.18	0.50	0.17	**0.01**
Envir (FL)	6.27	0.60	**<0.01**	1.85	0.12	0.15
Intercept	0.94	0.18	<0.01	-0.63	0.19	<0.01
Season(D)	-2.19	0.53	**0.01**	-2.04	0.54	**<0.01**
Envir (FL)	1.50	0.51	**0.01**	1.32	0.50	**0.02**
		AST (UI/L)			**Urea (mg/dL)**	
Intercept	4.12	0.50	<0.01	3.69	0.26	<0.01
Sex (M)	0.07	0.29	0.79	-0.37	0.11	**<0.01**
Season(D)	0.64	0.24	**0.01**	-0.04	0.21	0.86
Envir (US)	0.15	0.14	0.29	0.50	0.10	**<0.01**
Intercept	1.16	0.17	<0.01	-0.20	0.16	0.21
Season(D)	0.63	0.28	**0.04**	-1.79	0.28	**<0.01**

AST, aspartate aminotransferase; Ca:P, calcium:phosphorus ratio; CK, creatine kinase; D, dry; Envir, environment; M, male; TP, total protein; US, urban stream. Significant values are in bold.

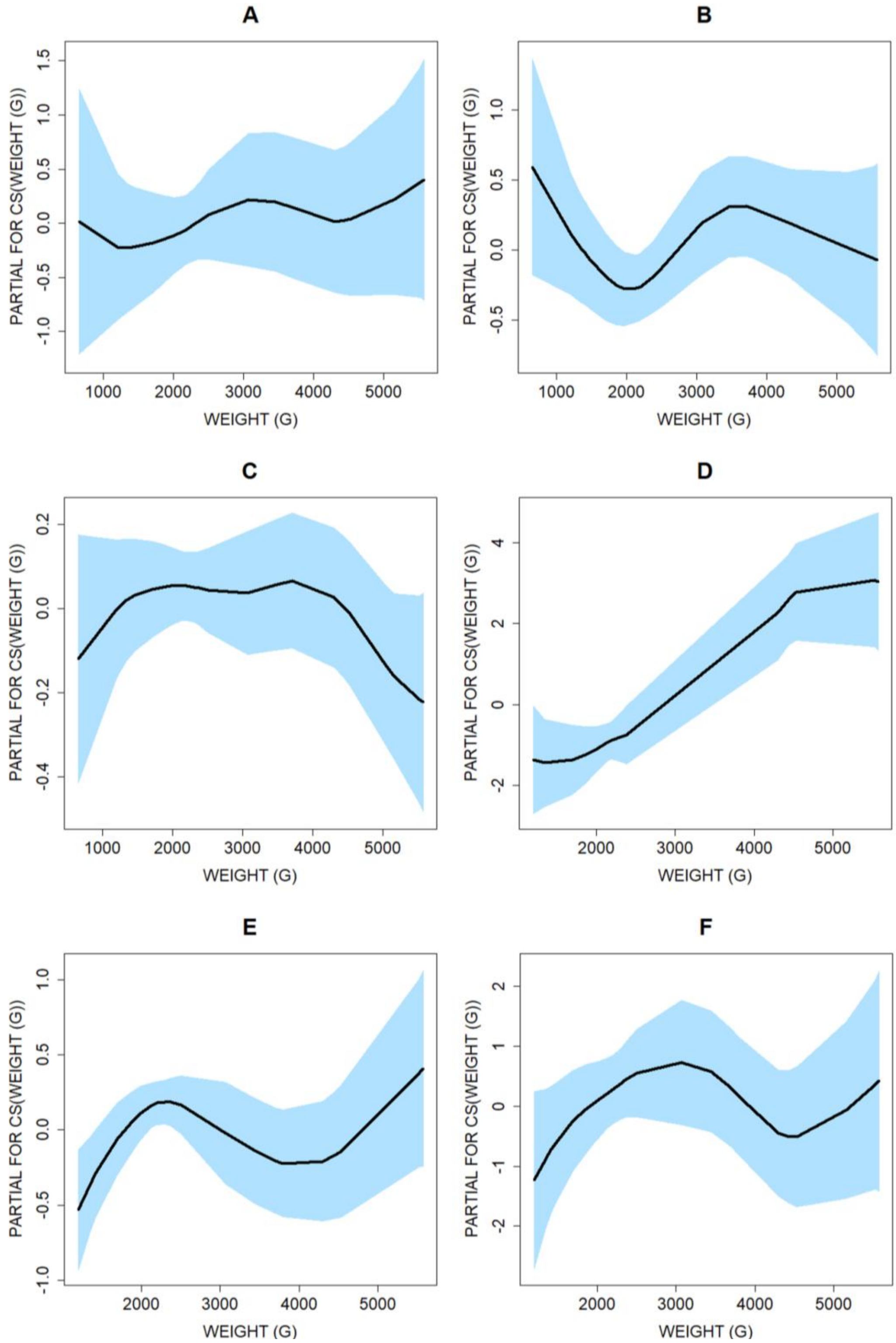

Figure 1. Shapes of smooth curves adjusted for the explanatory variable body mass of biochemical parameters of *Phrynops geoffroanus.* A. Albumin. B. Aspartate aminotransferase. C. Creatinine. D. Creatine kinase. E. Phosphorus. F. Total protein.

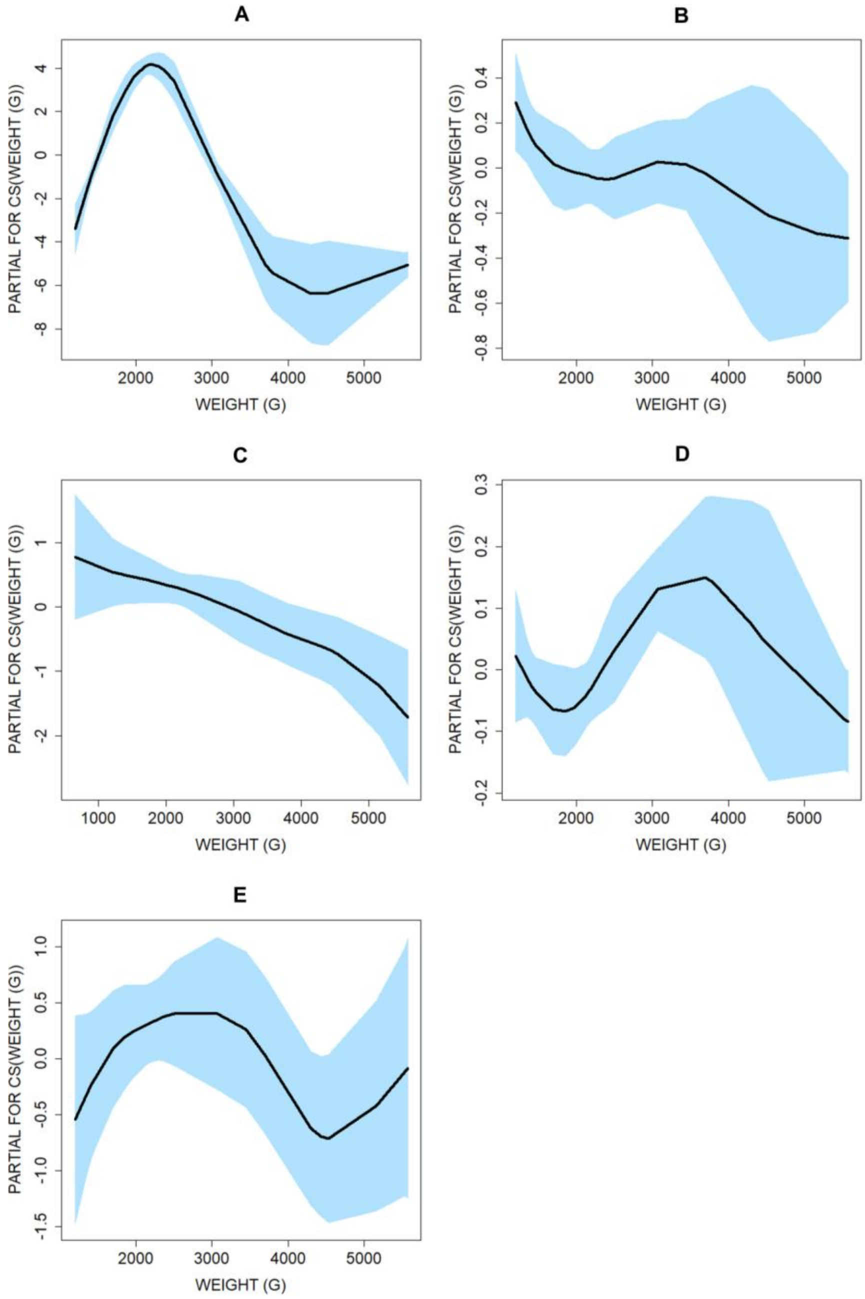

Figure 2. Shapes of smooth curves adjusted for the explanatory variable body mass of biochemical parameters of *Phrynops geoffroanus.* A. Total calcium. B. Urea. C. Uric acid. D. Calcium:phosphorus ratio. E. Globulin value.

DISCUSSION

In *P. geoffroanus* from this research, higher levels of AST were observed in the dry season, also presenting greater variability in this period, similarly to what was observed for CK, which was also higher in females.

AST and CK are found mainly in striated muscles and in the liver (Eatwell *et al.*, 2014). In chelonians, the increase of these enzymes may result from increased muscle activity such as excessive exertion, injuries from fights in the breeding season, intramuscular injections or systemic infections (Campbell, 2022).

In *Cuora flavomarginata* from Taiwan, the concentrations of AST peaked in the summer when compared with the winter. CK and AST values in males reached their higher levels in summer and fall. These seasonal differences are potentially due to an increase in fighting and copulation behavior during this period (Yang *et al.*, 2014). The same was observed in *Pelodiscus sinensis* from China that showed higher levels of AST and CK during the mating period. The authors suggest that stress and tissue injuries may also increase the level of such analytes (Vistro *et al.*, 2020). Moreover, increased AST and CK levels in *P. sinensis* during the non-hibernation period may show that, in this period, the animals are more active, having higher metabolic activities (Vistro *et al.*, 2020). In a study with females of the sea turtle *Eretmochelys imbricata* from the Persian Gulf, the authors also found significant higher values of CK during the nesting season (March-April), when compared with the foraging season (Ehsanpour *et al.*, 2014), just like in females of *Caretta caretta* from Turkey, AST and the isoenzyme CK-MB were also higher during t nesting period (Sözbilen and Kaska, 2018). As presented in the literature, the reproductive period seems to influence the levels of AST and CK, possibly due to intense muscle activity, as occurs in *P. geoffroanus* whose mating period occurs during the dry season (autumn and winter) (Molina, 1992; Souza, 2004; Silva and Vilela, 2008; Moura *et al.*, 2012; Ferrara *et al.*, 2017; Abrantes *et al.*, 2021).

Higher CK averages in females were also described in *Glyptemys muhlenbergii* from USA (Brenner *et al.*, 2002) and *P. geoffroanus* from Brazil (Brites, 2002). CK levels also showed a positive relationship with BM in *P. geoffroanus*, in which the values were higher with the increase of BM, until reaching stability in animals with more than 4000g (Fig. 1-D). It is possibly that the increase in muscle mass volume results from a higher average of this enzyme in females, since they weigh more than males (Balestra *et al.*, 2016).

Creatinine levels in *P. geoffroanus* found in this study were higher in the rainy season. In reptiles, the concentration of C in the blood is not usually tested to evaluate kidney disease (Campbell, 2022), but its dosage may be useful in the evaluation of the animal, because this protein is part of the muscles, being used to store energy (Meuten and Sample, 2022). Thus, C concentrations in reptiles are naturally lower, indicating only nutritional and hydration status (Soltanian *et al.*, 2021; Campbell, 2022). Therefore, the increase in serum C levels may have occurred due to the greater supply of food in this period, when there is an increase in appetite, to provide energy for copulation and egg production (Yang *et al.*, 2014).

In Fig. 1-C, we observe that the concentration of C showed a small increase in animals with more than 1000g, remaining stable until approximately 4000g. In *Trachemys dorbigni* in captivity, a positive relationship was observed between C concentration and BM (Gradela *et al.*, 2020). Thus, C levels are likely to be influenced not only by the reproductive activity and diet of the animals, but also by the increase in body mass volume.

Regarding the minerals analyzed, tCa and Ca:P ratio were higher in the dry season and in urban stream turtles, and the mean values of tCa, P and the Ca:P ratio were significantly higher in females. In addition, tCa showed greater variability in the dry season and in urban stream turtles, while data from Ca:P had greater variation in the rainy season and in urban stream animals.

The Ca:P ratio is one of the most useful early indicators of kidney disease in reptiles. Most chelonians have a Ca:P ratio higher than 1,5 and, in cases of kidney disease, this ratio will be lower due to increased blood P (Campbell, 2022). P levels are generally related to tCa, the first being usually eliminated through the kidneys (Eatwell *et al.*, 2014). Thus, any reduction in renal blood flow often results in hyperphosphatemia (Eatwell *et al.*, 2014). Increased P levels can also be observed in young animals or diet rich in P and low in tCa (Eatwell *et al.*, 2014). Its decrease may also indicate a period of anorexia (Eatwell, Hedley and Barron, 2014; Innis and Knotek, 2020; Campbell, 2022).

Higher averages of tCa, P and Ca:P in females have been described in freshwater turtles (Hidalgo-Vila *et al.*, 2007; Rangel-Mendoza *et al.*, 2009; Vistro *et al.*, 2020) and this is because, during egg development, hypercalcemia occurs in response to estrogen and reproductive activity. On the other hand, the greatest variability of tCa levels and Ca:P ratio in the rainy season and also in urban stream turtles may occur due to variations in the diet or reproductive activity of the sampled animals (Innis and Knotek, 2020; Campbell, 2022).

In connection with the BM, a pattern was observed in the levels of tCa, P and Ca:P. tCa and P peaked in their concentrations in individuals between 2000 and 3000g. As the analysis of the BM includes all males and females in both seasons, and females showed significant higher values of this biochemical parameters, it is possible that this peak is due to females, since most females sampled weight between 2000 and 3000g and their reproductive process requires a higher demand of these ions, leading to an increase in them. In addition, this fluctuation in the levels of tCa, P and Ca:P may also be indicative of a diet rich in P and poor in tCa (Campbell, 2022).

Females and urban stream individuals presented higher mean urea values than males and those in captivity, also showing a greater variation of the data obtained in the rainy season. In freshwater turtles, U is the main final product of protein metabolism, its elimination depending on glomerular filtration (Campbell, 2022). The increase in U concentrations may indicate a high-protein diet (Tavares-Dias *et al.*, 2009; Campbell, 2022), but may also indicate loss of kidney filtration capacity during events of low perfusion or dehydration (Innis and Knotek, 2020).

In Brazil, free-living females of *P. geoffroanus* were found to be predominantly carnivorous, while males are omnivorous, a fact that have been related to the higher nutritional demand of the female for egg development (Ribeiro *et al.*, 2017). Thus, we understand that the increase in U in urban stream turtles and females could be related to the difference in diets.

BM also influenced U levels, as they decreased slightly in larger individuals. This negative relationship can be explained by the feeding habits of *P. geoffroanus*, whose juveniles ingest a greater amount of protein than adults (Souza, 2004), resulting in lower values of this analyte in the heavier individuals.

We have observed statistically significant differences in uric acid levels between seasons and environments, being higher in the rainy season and in urban stream turtles. Like U, UA is one of the products excreted in protein metabolism in many species of turtles. Clinically, UA monitoring is important for the evaluation of renal function, however this analyte is affected by many factors (Innis and Knotek, 2020). Among them, feeding habits, as we can observe in carnivorous species which seem to have higher levels of UA than herbivores (Eatwell *et al.*, 2014).

Although it is an omnivorous species, *P. geoffroanus* has a preferentially carnivorous and insectivorous habit (Pereira *et al.*, 2018), especially during the rainy season, which coincides with the greater food supply (Malheiros, 2016). In fact, the seasonality of UA in chelonians results from the availability and quality of food components.

UA levels, as well as U, decrease as individuals' BM increases, with slight fluctuation in those weighing around 3000 g. Thus, there is a relationship between BM and the levels of U and UA of *P. geoffroanus*, this analytes, as previously mentioned, is part of protein metabolism (Innis and Knotek, 2020), and, as juveniles have more carnivorous habits than adults, these values tend to be higher in the former. It is possible that other variables may influence this result, such as the season, in which, during the flood period, there is an increase in food supply and consequently a higher protein intake (Souza and Abe, 2001), justifying this small fluctuation in UA and U values.

The parameters TP, ALB and GV were not influenced by the variables season, environment, sex, and BM. Despite differences between species and types of experiments, the absence of influence on these biochemical parameters was also described by other authors (season: Yang *et al.*, 2014; sex: Omonona *et al.*, 2011; Ninette, 2014; Almonacid, 2020; body mass: Gradela *et* *al.*, 2020). On the other hand, in *Podocnemis expansa* submitted to a diet in which the protein source was added only once a week, it was observed a decrease in TP levels when compared to the group receiving it daily (Tavares-Dias *et al.*, 2009), becoming evident that dietary protein seems to influence the serum levels of TP, ALB and GV.

Information about the composition of *P. geoffroanus* diet and its variation throughout the year is limited, and it is not possible either to infer about the protein levels of this diet in urban stream turtles. However, it is known that *P. geoffroanus* is a generalist omnivorous species (Souza and Abe, 2000; Pereira *et al.*, 2018) and adapts to anthropic environments, such as urban streams, rich in food resources from organic waste generated by humans (Souza and Abe, 2000), as observed at the collection site of the animals of this experiment, with abundant presence of aquatic larvae and fish of various sizes. Because the animals are omnivorous and resistant, it is possible that environmental changes may not present changes in serum levels of these proteins, even when there is variation in the diet, or these variations were not enough to influence these parameters.

Our results indicate that season, environment, sex, and body mass can consistently influence the biochemical parameters of *P. geoffroanus* and all of these extrinsic and intrinsic factors have potential to significantly alter biochemical measures and need to be considered when evaluating health. Thereof, the biochemical parameters described in this study can be used as baseline for biochemical tests in *P. geoffroanus* in similar conditions to those presented in this work.

CONCLUSIONS

The analyses of the biochemical profile of *P. geoffroanus* showed that AST, CK, tCa and Ca:P ratio were higher during the dry season, while the serum levels of C and UA were higher in the rainy season; the urban stream individuals showed higher concentrations of UA, U, Ca^{2+} and Ca:P; while females of *P. geoffroanus* presented higher levels of CK, tCa, P, and Ca:P ratio, and U than males. Season, environment, and sex did not influence TP, ALB, and GV.

ACKNOWLEDGEMENTS

The authors would sincerely like to thank Cempas (Center for Medicine and Research of Wild Animals), BPMAVG (Battalion of the Military Environmental Police of Várzea Grande) and Sema (Mato Grosso State Environment Department). Without the cooperation of these government agencies, we would hardly have been able to complete our research, because, in addition to giving us the samples, they helped us in the physical restraint of the animals and in the collection of these samples.

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