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SHORT COMMUNICATION

Notes on the hematology of free-living *Phrynops geoffroanus* (Testudines: Chelidae) in polluted rivers of Southeastern Brazil

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ABSTRACT. *Phrynops geoffroanus* (Schweigger, 1812) is the freshwater turtle with the widest geographical distribution in South America. During 2006, physical examination and hematological evaluation were performed on free-ranging turtles from two polluted rivers, the Piracicaba River (n = 51) and its tributary Piracicamirim (n = 42), in southeastern Brazil. Red blood cell and thrombocyte counts, mean corpuscular volume and mean corpuscular hemoglobin levels differed in turtles from the two water courses. Although free-ranging turtles showed ectoparasites and boat propeler lesions, animals apparently had no signs of clinical disease. In spite of our results, further monitoring of the demography and health status of *Phrynops geoffroanus* in anthropogenically altered environments is recommended.

KEY WORDS. Hemogram; lesion; pollution; turtle.

Geoffroy's side-necked turtle, *Phrynops geoffroanus* (Schweigger, 1812) is the freshwater turtle with the widest geographical distribution in South America, ranging from the Colombian Amazon to southern Brazil and northern Argentina, living in rivers, streams and lakes (Pritchard & Trebbau 1984, Ernst & Barbour 1989). Recently, the species has been found inhabiting polluted urban rivers in Brazil (Souza & Abe 2000, Brites & Rantin 2004), exhibiting values of population density and biomass among the highest recorded for neotropical freshwater turtles (Souza & Abe 2000).

Hematologic values are important for monitoring health of free-ranging turtle populations (Bolten & Bjorndal 1992, Metin *et al.* 2006). Most of hematological studies in free-ranging and captive turtles are reported as baseline values in endangered species (Marks & Citino 1990, Bolten & Bjorndal 1992, Christopher *et al.* 1999, Brenner *et al.* 2002, Deem *et al.* 2006, Metin *et al.* 2006, Kakizoe *et al.* 2007) or in diseases related to anthropogenic causes (Jacobson *et al.* 1991, Work *et al.* 2001, Christopher *et al.* 2003). Investigation on animals living in disturbed habitats is crucial, since man-made alterations in the environment, such as habitat loss, degradation or pollution, are the main reasons for population declines of reptiles (Gibbons *et al.* 2000, Rodrigues 2005) and can negatively influence animal health (Christopher *et al.* 1999). Anemia is one of the disorders that can be detected in species living in polluted habitats (Keller *et al.* 2004, Belskii *et al.* 2005). Our goal in this investigation was to describe hematological range values of *P. geoffroanus* from two disturbed sites in Southeastern Brazil.

From May through October 2006, free-living turtles were captured in the Piracicaba River (PIRA) and its tributary, Piracicamirim (PISCA), located in Piracicaba River basin (PRB), a highly developed basin (12.400 km^2), with industries and a high human population density (Krusche *et al.* 1997, Martinelli *et al.* 1999). PRB is located in the State of São Paulo, Southeastern Brazil. The main industrial activities include textile, paper and pulp, sugar and ethanol, metallurgic, food crops, tanning, chemical and fuel refineries (Cetesb 2001). Sugar cane and citrus are the main agricultural products (Krusche *et al.* 1997). The densest human population and industries are concentrated in the central part of the basin. PISCA is considered the most polluted tributary of PIRA. The main sources of pollution are non- treated domestic sewage and fertilizers used in sugar cane crops (Ometo *et al.* 2004). Its micro basin (133 km^2) encompasses portions of three counties: Piracicaba, Rio das Pedras, and Saltinho (Ometo *et al.* 2000).

Turtles were captured in PIRA by active search using a motor boat and hand dip nets, in a region known as Monte Alegre (22º41'75"S, 47º33'58"W), located in the central area of the basin, in the suburban area of the city of Piracicaba. We captured turtles in PISCA close to its largest urbanized area on the University of São Paulo campus (22º42'51"S, 47º37'36"W). We used four gill nets (nylon; mesh size 3-5 cm; 1.5-2 m deep; 15 m long) extended perpendicularly between the river banks (Souza & Abe 2000) and checked every three hours. The animals were captured under Ibama license (Proc. 02010.000005/05-61).

Immediately after capture, turtles were transported in plastic containers to the laboratory in Piracicaba. Three ml of blood were collected from the external jugular vein (Rogers & Booth 2004). Immediately after blood collection, two blood smears were prepared for each turtle for estimation of thrombocytes and white cell differential counts. The blood smears were air-dried and placed in cardboard holders. The remaining blood was placed in lithium-heparin tubes, chilled (4C), and processed at the Veterinary School of Medicine of the University of São Paulo (FMVZ/USP) within 5-10 h of collection.

Packed cell volume was determined with microhematocrit capillary tubes filled from the lithim-heparin tubes and centrifuged at 10,000 rpm for five minutes in a microcentrifuge (Mader 2000). Plasma protein concentration was measured with a refractometer (Anderson *et al.* 1997). Hemoglobin concentration was determined by the cyanmethemoglobin method (Campbell 1996). White and red blood cell counts were determined using the standardized recount system in a modified Neubauer chamber, with Natt-Herrick solution as diluent (Frye 1991). White cell differential and thrombocytes were manually determined from modified May-Grunwald-Giemsa stained blood smears (Rosenfeld 1947). A hundred leukocytes were examined on each slide and were categorized as lymphocytes, eosinophils, heterophils, basophils and monocytes (Sypek & Borysenko 1988, Brenner *et al.* 2002, Deem *et al.* 2006). Thrombocytes were counted every 1,000 red blood cells on the blood smear. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the packed cell volume, hemoglobin concentration, and red blood cell count (Campbell 1996).

After blood sampling, animals were sexed based on their external morphological characteristics (Ernst & Barbour 1989, Souza & Abe 2000), measured at the straight-line carapace length, weighed and had a physical examination performed. The health status of each turtle was based on general body condition (Deem *et al.* 2006), searching for clinical signs as sunken eyes, cachexia, lethargy and emaciation.

Descriptive statistics (mean, standard deviation, minimum and maximum) were performed. Data were tested for normality. Analysis of variance (General linear model) and analysis of mean (Anom) (Minitab for Windows 15) were performed for comparison between PIRA and PISCA turtles' blood values, where sex, sampling site and its interaction were considered as grouping factors.

A total of 51 adult turtles (29 males and 22 females) from PIRA and 42 adults (24 males and 18 females) from PISCA were captured. Mean straight-line carapace lengths were 288 ± 27 mm (range: 236-348 mm) and 291 ± 32 mm (range: 221-365 mm) from PIRA and PISCA respectively. Body mass was 2280 ± 675 g (range: 1340-4100 g) and 2444 ± 814 g (range: 950-5200 g) from PIRA and PISCA respectively. Fourteen animals (six males and eight females) from PIRA and eight (two males and six females) from PISCA had carapace injuries, probably caused by boat propeller strikes as we have noticed during the field work in PIRA. The traumatic lesions were completely healed in 12 of these turtles from PIRA and seven from PISCA. Ectoparasites (leeches) were observed on the skin and carapace in most of the animals from PIRA and PISCA, but this information was not registered systematically. Wild turtles were not cachectic or lethargic. They were in good body condition and apparently had no clinical signs of disease.

Results of *P. geoffroanus* hematology are listed in table I. Sex and "site by sex" interaction did not influence turtle blood values from both PIRA and PISCA ($p > 0.05$, Tab. I). Statistical differences occurred in 4 of 13 parameters analysed, due to location ($p < 0.05$, Tab. I). PIRA turtles had higher red blood cell and thrombocytes counts than PISCA turtles. However, PISCA turtles had higher mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). There were no differences in white blood cell and white cell differential counts between sites. Lymphocytes and heterophils were the most common white cells in PISCA turtles. For PIRA animals, lymphocytes were the most common in white cell differential counts (Tab. I).

The data presented herein provide hematological values for free-ranging *P. geoffroanus*. Even though free-ranging turtles were captured in disturbed and polluted sites, animals showed no detectable evidence of clinical disease. The shape of the lesions on the cracked and fractured carapaces is consistent with damage caused by motorboat strikes and show no signs of being caused by pathological agents, as recorded for other freshwater turtles (Garner *et al.* 1997, Knotkova *et al.* 2005).

Comparing the hematological values from PIRA turtles and its tributary PISCA, there was no variation due to sex or "site by sex" interaction. Rather, the site where animals were captured was responsible for the variation. Turtles from PIRA had a higher red blood cell count when compared with turtles from PISCA, what could be related to the river hydrodynamics. PIRA is larger and has a stronger water flow (Martinelli *et al.* 1999). Consequently, turtles in this site may have to swim more, thus consuming more oxygen, what could result in a higher number of erythrocytes. Although red blood cell count was higher in PIRA turtles, animals did not show differences in hemoglobin concentration and packed cell volume. PISCA turtles had a higher MCH and MCV than PIRA turtles. However, these variables are calculated values (Campbell 1996), and probably are being influenced by the red blood cell count.

Table I. Hematology of *P. geoffroanus* from anthropogenically affected environments, southeastern Brazil. Piracicaba River (PIRA) and Piracamirim stream (PISCA). Mean, standard deviation and range values in parentheses.

Measure	Females PIRA (n = 22)	Males PIRA (n = 29)	Females PISCA (n = 18)	Males PISCA (n = 24)
Packed cell volume%	21.1 ± 5.4 (13-32)	22 ± 4.5 (14-30)	21.1 ± 4.6 (15-32)	22.6 ± 5.6 (12-32)
Plasma protein concentration g dl/1	5.91 ± 1.29 (3.8-8.4)	6.2 ± 1.47 (3.4-9.2)	5.96 ± 1.18 (3.9-7.8)	6.81 ± 2.15 (3.6-11.2)
Hemogloblin g/dl	5.1 ± 1.38 (3.2-7.8)	5.68 ± 1.61 (2.8-9.5)	4.86 ± 1.06 (3.3-7.1)	5.38 ± 1.95 (2.9-11.2)
Red blood cell (x 10^3/µl) *	484 ± 178 (210-870) [a]	482 ± 140 (250-860) [a]	364 ± 146 (100-660) [b]	384 ± 99.7 (240-660) [b]
White blood cell (x 10^3/µl)	5.9 ± 3.5 (1.5-15)	5.7 ± 3.1 (1.5-12)	6.7 ± 3.0 (1.5-15)	6.6 ± 3.3 (3-16)
Absolute heterophils (x 10^3/µl)	1.9 ± 2.0 (0.2-7.5)	1.9 ± 1.5 (0.3-7.2)	2.5 ± 2.1 (0.5-8.7)	2.0 ± 1.8 (0.4-7)
Absolute eosinophils (x 10^3/µl)	0.89 ± 0.47 (0.2-1.8)	0.99 ± 0.78 (0.1-2.9)	0.93 ± 0.64 (0.2-2.5)	1.1 ± 0.8 (0.1-3.3)
Absolute basophils (x 10^3/µl)	0.43 ± 0.3 (0.07-1.0)	0.37 ± 0.3 (0.05-1.2)	0.48 ± 0.41 (0.05-1.8)	0.47 ± 0.34 (0-1.1)
Absolute lymphocytes (x 10^3/µl)	2.4 ± 1.4 (0.8-6.2)	2.3 ± 1.7 (0.7-7)	2.43 ± 1.5 (0.5-5.6)	2.8 ± 1.6 (0.6-6.4)
Absolute monocytes (x 10^3/µl)	0.17 ± 0.22 (0-0.7)	0.14 ± 0.11 (0-0.4)	0.26 ± 0.3 (0-1.2)	0.20 ± 0.29 (0-1.4)
Thrombocytes (x 10^3/µl) *	25 ± 28 (7.2-148) [a]	19 ± 11 (7.8-59) [a]	12 ± 7.8 (1.7-28) [b]	12.6 ± 7.4 (2.1-29) [b]
MCV fl *	469 ± 122(241.3-628.5)[a]	487 ± 141 (215.1-756.7) [a]	650 ± 246 (418-1500) [b]	601.2 ± 140.7 (400-1000) [b]
MCH pg *	112.9 ± 31.3(74.6-214)[a]	122.4 ± 37.6(68.2-243.5) [a]	149.9 ± 55.7 (90.9-330) [b]	144.7 ± 58.3 (93.9-329.4) [b]
MCHC g/dl	24.7 ± 6.1 (19.6-39)	26.7 ± 9.6 (13.3-51.1)	23 ± 1.9 (19.5-26.6)	23.6 ± 4.7 (17.8-41.4)

*Values statistically different ($p < 0.05$) between turtles from Piracicaba River and Piracamirim stream.

Published data of freshwater turtles suffering from necrotic shell diseases (Garner *et al.* 1997, Knotkova *et al.* 2005) showed higher values of white blood cells, lymphocytes, heterophils and basophils than those obtained for the free-ranging *P. geoffroanus* in this study. However it is difficult to decide whether the hematological values obtained for *P. geoffroanus*, in this investigation, are low or normal, since there are no reference range values for free-ranging *P. geoffroanus* inhabiting unpolluted rivers. Future studies should focus on the hematology of *P. geoffroanus* from unpolluted environments for comparison with the data presented here.

Despite the fact that the turtles from PISCA and PIRA were apparently healthy, subclinical infections can occur. We recommend monitoring the health status of these turtles,with the addition of other techniques such as biochemistry analysis, necropsies, histological examination, and population size monitoring on a long term basis.

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