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Hematological and biometric traits of tuvira *Gymnotus inaequilabiatus* (Valenciennes, 1839) (Gymnotiformes: Gymnotidae) from the Brazilian Pantanal

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ABSTRACT

This study describes the hematological and biometric characteristics of male and female *Gymnotus* species from the Pantanal, Mato Grosso do Sul state, Brazil. Fifty adult specimens of *Gymnotus inaequilabiatus* were weighed, measured, and then euthanized. Blood was collected by puncturing the celiac mesenteric vein to determine the hematocrit, hemoglobin content, number of erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, glucose level, absolute value of leukocytes, and relative value of leukocytes and thrombocytes. Body weight and relative condition factor did not differ ($P > 0.05$) between the sexes, as well as erythrogram and the blood glucose values. Hematocrit ranged from 18.0% to 54.0%; hemoglobin from 1.1 to 14.7 g dL^{-1}; number of erythrocytes from 0.2×10^6 to 3.8×10^6 μL^{-1}; MCV from 24.2 to 321.7 fL; and MCHC from 4.2 to 44.5 g dL^{-1}. In the differential count were identified thrombocytes, lymphocytes, neutrophils, monocytes, basophils, immature leukocytes, and PAS-positive granular leukocyte (PAS-GL). Females had a higher percentage of immature leukocytes ($P < 0.05$) than males. Glucose levels, erythrogram, leukogram, and the morphology of defense cells are comparable to other fish species of the Pantanal. Thrombocytes were the most frequent defense cells, followed by lymphocytes and neutrophils.

Key words: blood glucose, erythrogram, leukocytes, sex differences, thrombocytes.

INTRODUCTION

The Gymnotiformes, commonly known as knifefishes, are a small endemic group that constitute approximately 3% of the Neotropical ichthyofauna (Reis et al. 2003). The family Gymnotidae has the widest geographical distribution within the order, ranging from central Argentina to the Mexican southeast (Albert 2001).

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The genus *Gymnotus* is popularly referred to as "tuvira" or "sarapó," and is an important economic resource in the Pantanal region. These fish are caught in the natural environment and used as live bait by tourists and professional fishers. There is thus commercial link involving the exploitation of this species, and substantial efforts have been made to grow them on fish farms (Rotta et al. 2007, Resende et al. 2006). In general, *Gymnotus* spp. are active at night in aquatic macrophyte environments populated by salvinia *Salvinia auriculata,* creeping bladderwort *Utricularia gibba,* South American spongeplant *Limnobium laevigatum,* and mosaic flower *Ludwigia sedoides*. The roots of these plants provide a suitable environment for the fish to feed on insects, their preferred food (Resende et al. 2006).

Due the diversity of the Pantanal ichthyofauna, data on the hematological parameters of wild Pantanal fish are poor; include a basic description and its relation with biotic and abiotic factors (Seriani and Ranzani-Paiva 2012). Therefore, the maintenance of native species will depend upon research that will provide us with a better understanding of biological cycles and their interactions in the environment. In the Pantanal region, these fish experience periodic flooding conditions and may have developed adaptive physiological strategies to survive, especially during the drier aestivation periods. During these periods, many fish species are confined to temporary ponds and are subjected to adverse conditions such as large temperature fluctuations, food scarcity, and parasitism (Britski et al. 1999, Barton et al. 2002, Rios et al. 2005).

Hematology has been used as a tool to study the physiology and health of various fish species, especially in the natural environment (Ranzani-Paiva et al. 2000). Furthermore, the analysis and interpretation of different hematological parameters provides relevant information for the prognosis and diagnosis of many disorders, and helps us to understand the process of physiological adaptation in captivity (Tavares-Dias and Moraes 2004). Additionally, hematological parameters vary depending upon species, age, sexual maturity, health status, and the aquatic biotope (Radu et al. 2009). It is well known that blood comprises 1.3%–7.0% of the total body weight in fish, and it represents, in association with the hematopoietic organs, a critical biomarker for metabolic processes and physiological stress in response to changes in exogenous and endogenous conditions (Morgan and Iwama 1997). The correct evaluation of hematological parameters thus depends upon the availability of reference values. These should be as close as possible to the normal values of various blood components as found in healthy fish under natural conditions (Tavares-Dias and Moraes 2004).

The aim of this study was to establish basal hematological and biometric values for tuvira *Gymnotus inaequilabiatus* (Valenciennes 1839) in the Pantanal of Mato Grosso do Sul State, Brazil. Additionally, we investigated the effects of environmental conditions on a set of variables that could be used as reference standards in future studies of this species.

MATERIALS AND METHODS

Adult specimens of tuvira *G. inaequilabiatus* (26 males and 25 females) from Porto Morrinho, Mato Grosso do Sul (21°41′56″S, 57°52′57″W), were acquired between September 2013 and November 2014 from a specialized live bait supplier in Campo Grande, MS. The fish were transported to the laboratory in oxygenated polyethylene bags, and then were kept at a constant temperature (25.0°C) for 2 h in aquariums supplied with artificial aeration until euthanasia (2-phenoxyethanol, 2 mL L^{-1}). Following euthanasia, the fish were weighed (total weight in grams) and measured (total length in centimeters). These variables were

used to estimate the relative condition factor (*Kn*) according the equation $Kn = W/aL^b$, where *W* is weight of the individual, *L* is the standard length of the individual, and *a* and *b* are the constants from the weight-length relation (Le Cren 1951).

Blood was collected through the celiac mesenteric vein with a syringe and disposable EDTA needles, immediately after opening the abdominal cavity. The hematological parameters measured were hematocrit (%) according (Goldenfarb et al. 1971), hemoglobin content (g dL^{-1}) according (Collier 1944), and erythrocyte count ($\times 10^6$ μL^{-1}) using a Neubauer chamber. Mean corpuscular volume (fL) and mean corpuscular hemoglobin concentration (g dL^{-1}) were calculated from the results obtained for number of erythrocytes, hematocrit, and hemoglobin content following Wintrobe (1934). Cells differential counts (leukocytes and thrombocytes) in blood extensions were stained by the May-Grünwald-Giemsa method using 200 cells per sample (Tavares-Dias et al. 2002). The total count of leukocytes was performed by hemocytometer technique (Ranzani-Paiva et al. 2013). The glucose level (mg dL^{-1}) was measured by an *in vitro* test with a drop of a total blood (Accu-Chek Active; Roche Diagnostics).

The procedures adopted in this study are in accordance with Resolution No. 714 of June 2012, CFMV, certified by the Ethics Committee on Animal Use (CEUA\UFMS protocol 557/2013).

Descriptive statistics calculated included the mean, standard error of the mean, minimum and maximum values, and confidence interval of the mean (CI 95%). The effect of sex was tested by the Mann-Whitney U test (Z, asymptotic significance, 2-tailed). An exploratory factor (dimension reduction) with varimax rotation and principal component analysis (PCA) was also used to assess the data set of biometric and hematological variables. The initial selection of variables was based on commonalities values ≥ 0.60, using a Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy of 0.80 or more and Bartlett's test of sphericity ($P < 0.001$). After selection, a new PCA model adjusted to two factors with normalized correlation was prepared. PCA scores were computed using vector products with the standardized data. SPSS 17.0 (IBM®) and PAST 2.17 (Hammer et al. 2001) software were used in the analysis.

RESULTS

G. inaequilabiatus specimens had a mean weight of 137.0 ± 26.2 g, a mean length of 34.1 ± 2.2 cm, and their condition factor was 1.0 ± 0.02. There was no difference ($P > 0.05$) between the sexes in biometric measurements (Table I). Similarly, the erythrogram and glucose levels did not differ (Table II). Immature leukocytes were more abundant in females than in males, but no other cell categories

TABLE I
Means, standard error of the means (SEM), minimum (Min) and maximum (Max) values and confidence intervals (CI 95%) of biometric variables of male and female tuvira *Gymnotus inaequilabiatus* from Brazilian Pantanal.

Variables	Sex	Mean ± SEM	Min-Max	CI (95%)	P
Weight (g)	Male	137.13 ± 6.00	101.90-218.60	124.51-149.72	
	Female	132.02 ± 5.34	94.60-190.40	120.80-143.22	0.47
Length (cm)	Male	34.93 ± 0.52	29.50-38.50	33.81-36.03	
	Female	33.63 ± 0.51	28.5-38.00	32.55-34.72	0.12
Kn	Male	1.00 ± 0.01	0.94-1.05	0.99-1.03	
	Female	1.00 ± 0.01	0.93-1.04	0.99-1.05	0.89

Kn: Relative condition fator; P: Mann-Whitney U test.

showed any sex differences (P > 0.05). Eosinophils presented less than 1.0% and were not considered in the analysis (Table III).

Figure 1 shows the PCA results. Hematocrit, hemoglobin, MCHV, glucose level, total leukocytes, neutrophils, lymphocytes and PAS-positive granular leukocyte (PAS-GL) were selected from the total of the variables analyzed. Lymphocytes were separated into alone grouping to the neutrophils, PAS-GL and glucose levels. Total leukocytes, MCHV, hemoglobin, and hematocrit maintained a homogeneous group. This result suggested that the innate immunity apparatus could be more explored in this species of fish.

DISCUSSION

The tuvira *G. inaequilabiatus* is a typical species of the lentic environments characteristic of the Pantanal. It is adapted to low oxygen environments and usually lives in small ponds and exhibits little migration (Almeida-Val et al. 1993). There is no sexual dimorphism; sex was determined by necropsy when the specimens were opened for blood collection. This method made it possible to quickly obtain samples suitable for laboratory procedures.

The weight-length ratio did not differ between males and females. In fish, this ratio reflects information about the physiological state of the specimen in relation to its health and describes structural characteristics of individuals within populations (Anderson and Gutreuter 1983). In other wild fish species this ratio is higher in females during the reproductive/spawning season due to added ovarian weight (Tavares-Dias et al. 2004). Whereas the female *Gymnotus* spp. have multiple spawning (Rotta et al. 2007), possibly the number obtained in this study (n = 25) was not enough to reveal significant difference between the sexes. The observed values are in agreement with other species and suggest homogeneity in the study population.

Wild fish from natural environments may exhibit different physiological behaviors related to their survival strategies. The hematological parameters of these species may vary in response to age, sex, water quality, season, stress, infection conditions, capture method, handling, and anesthetic agents (Moraes et al. 2002, Ranzani-

TABLE II

Means, standard error of the means (SEM), minimum and maximum values and confidence interval (CI 95%) of erythrocytic parameters and blood of male and female tuvira *Gymnotus inaequilabiatus* from Brazilian Pantanal.

Variables	Sex	Mean ±SEM	Min-Max	CI (95%)	P
Htc (%)	Male	35.63 ± 1.80	22.00-54.00	31.84-39.42	
	Female	37.50 ± 1.47	18.00-48.00	34.43-40.57	0.73
Hb (g dL^{-1})	Male	8.64 ± 0.71	1.10-14.70	7.15-10.14	
	Female	9.86 ± 0.42	5.5-13.8	8.98-10.74	0.17
Er ($x10^6$ μL^{-1})	Male	1.99 ± 0.15	0.87-3.19	1.66-2.32	
	Female	2.06 ± 0.14	1.15-3.58	1.75-2.37	0.43
MCV (fL)	Male	195.80 ±17.04	113.70-448.30	159.99-231.61	
	Female	194.72 ±12.56	120.10-321.70	168.43-221.02	0.88
MCHV (g dL^{-1})	Male	23.85 ± 1.15	4.20-34.60	20.81-26.90	
	Female	26.62 ± 1.45	21.00-44.50	24.22-29.02	0.17
Glucose (mg dL^{-1})	Male	100.90 ± 6.90	58.00-173.00	85.60-116.19	
	Female	97.15 ± 7.28	43.00-151.00	82.70-111.60	0.69

Htc=Hematocrit; Hb=Hemoglobin; Er= Erythrocytes; MCV=mean corpuscular volume; MCHV=mean corpuscular hemoglobin volume; P: Mann-Whitney U test.

TABLE III
Means, standard error of the means (SEM), minimum (Min) and maximum (Max) values and confidence intervals (CI 95%) of leukocytes and thrombocytes and total leukocytes of male and female tuvira *Gymnotus inaequilabiatus* from Brazilian Pantanal.

	Sex	Mean ±SEM	Min-Max	CI (95%)	P
Lymphocytes (%)	Male	28.24 ± 3.48	8.00-58.00	20.93-35.54	
	Female	22.48 ± 2.65	10.50-57.00	16.92-28.03	0.21
Neutrophil (%)	Male	18.45 ± 2.13	7.50-37.00	13.97-22.92	
	Female	22.33 ± 2.36	6.50-44.5	17.38-27.27	0.88
Monocytes (%)	Male	5.97 ± 0.90	1.00-19.50	4.09-7.86	
	Female	6.50 ± 0.90	0.50-14.00	4.62-8.38	0.63
Thrombocytes (%)	Male	42.18 ± 2.46	19.50-60.00	37.03-47.34	
	Female	41.00 ± 3.26	12.50-66.00	34.18-47.82	0.97
Basophils (%)	Male	1.70 ± 0.44	0.00-6.50	0.85-2.72	
	Female	3.20 ± 0.94	0.00-19.00	1.23-5.18	0.09
Immature leukocytes (%)	Male	2.84 ± 0.41	0.50-7.00	1.98-3.71	
	Female	3.70 ± 0.42	1.50-9.00	2.82-4.58	0.05
PAS-GL	Male	0.53 ± 0.16	0.00-2.00	0.19-0.86	
	Female	0.78 ± 0.30	0.00-6.00	0.15-1.40	0.25
Total leukocytes ($x10^3 \mu L^{-1}$)	Male	6.89 ± 0.47	3.08-10.34	5.90-7.89	
	Female	7.50 ± 0.10	3.30-23.98	5.40-9.60	0.63

PAS-GL: PAS-positive granular leukocyte; P: Mann-Whitney U test.

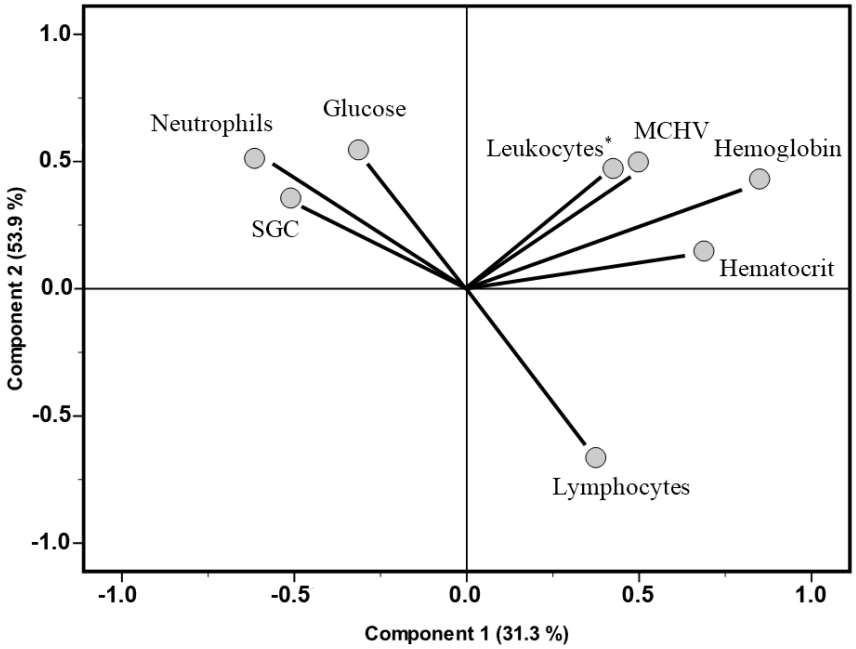

Figure 1 - Principal component biplot of starling hematological data of tuvira *Gymnotus inaequilabiatus* from Brazilian Pantanal, Brazil. MCHV: mean corpuscular hemoglobin concentration; PAS-GL: PAS-positive granular leukocyte; *total count.

Paiva et al. 2005, Drumond et al. 2010, Rio-Zaragoza et al. 2010). Therefore, data evaluation becomes a challenge due the lack of meaningful reference standards. Erythrogram averages found in tuvira *G. inaequilabiatus* were higher than those described in other fish such as jundiá *Rhamdia quelen* (Tavares-Dias et al. 2002). The hematocrit was similar to piauçu *Leporinus macrocephalus* and curimbatá *Prochilodus lineatus*, however the count of erythrocytes and the concentration of hemoglobin were higher, while the mean corpuscular volume and mean corpuscular hemoglobin concentration were lower (Tavares-Dias et al. 2008a). This profile may have relevance to the hematocrit investigation. Fish adapted to lower oxygen levels usually present higher values for erythrocytes and hemoglobin. The facultative air-breathing abilities of *Gymnotus* spp., which have swim bladders that are quite vascularized, are probably involved in this adaptive process, particularly during the dry season (Rotta 2004, Mariano et al. 2011).

The glucose data were comparable to previous studies in other species such as tambaqui *Colossoma macropomum* and pirarucu *Arapaima gigas* according to Tavares-Dias and Sandrim (1998) and Tavares-Dias et al. (2007). However, these values were higher in comparison to species reared in captivity such as pacu *Piaractus mesopotamicus*, matrinxã *Brycon cephalus* and piraputanga *Brycon orbignyanus* (Tavares-Dias and Mataqueiro 2004, M. Tavares-Dias, unpublished data, Tavares-Dias and Moraes 2006). Blood glucose level is a suitable biomarker to evaluate the level of stress in many fish species living in tropical environments. The natural environment of tuvira *Gymnotus* spp. is characterized by low levels of oxygen and limited water movement (Rotta 2004, Resende et al. 2006). Therefore, the ranges found in the present study suggest a physiological adjustment in response to that condition. Increased levels of glucose in the liver and kidney were observed after hypoxia exposure in tuvira *G. carapo* (Moraes et al. 2002).

The morphology of leukocytes and thrombocytes has been studied in the Characiformes (tambaqui *Colossoma macropomum*, traíra *Hoplias malabaricus*, dourado *Salminus maxillosus*, dourado *Salminus brasiliensis*, lambari *Astyanax bimaculatus*), Cypriniformes (carpa *Cyprinus carpio*), Perciformes (oscar *Astronotus ocellatus*), and Siluriformes (abotoado *Oxydoras niger*, jundiá *Rhamdia quelen*) (Tavares-Dias et al. 1999, 2002, Ranzani-Paiva et al. 2003, Tavares-Dias 2006, Pádua et al. 2009, Santos and Tavares-Dias 2010). Subtle variations in cell morphology were observed, although leukocyte classification was still possible. The normal of leukocytes morphology features is an important premise for identification of the changes in the leukogram. In fish, attention should be given to the PAS-GL. This cell is a granulocyte, relatively larger than the neutrophils, with small eccentric nuclei with dense chromatin. The cytoplasm have abundant granules, which are PAS positive. In some species, the presence of white cytoplasmics granules can give the false impression of morphological changes (Ranzani-Paiva et al. 2013). In other studies the PAS-GL was described in 30 teleosts both freshwater and saltwater (Barber and Westermann 1978). In brazilian teleosts, this cell is commonly described in the peripheral blood (Tavares-Dias et al. 2004, Tavares-Dias 2006, Ranzani-Paiva et al. 2013), mainly in fish affected by parasites (Tavares-Dias and Moraes 2004, Ranzani-Paiva et al. 2013, Campos et al. 2014). However, the percentages of some leukocytic forms were more variable than in others studies (Tavares-Dias et al. 2002, Ranzani-Paiva et al. 1999, Pádua et al. 2012, Figueiredo et al. 2014). These differences are difficult to explain due to the variability of biotic and abiotic factors associated with these fish communities. On the other hand, macrophages and granulocytes lack specificity and this allows large numbers of cells to be mobilized quickly to interact with the cells of the specific immune system. Moreover, these cells

can confound the interpretation of results when attempting to make conclusions about the degree of stress present in parasitized fish (Tavares-Dias et al. 2008b).

Usually, the leukocyte percentage is described by mean and standard deviation without considering the relationships among the variables. These relationships may be useful in interpreting the defense response, although many of them do not relate to each other. PCA is a multidimensional tool used to reduce a set of original variables and to extract a small number of latent factors. Furthermore, it helps identify relations among sub-populations with similar profiles (Sparks 2000). In the present study, the lymphocytes split themselves from the neutrophils, PAS-GL and glucose. This arrangement in the opposite side of the graphic (Fig. 1) suggests that many individual fish had high lymphocyte percentages at the moment of the blood collection in comparison to others. This reflects typical variation due to stressful situations, considering the patterns of neutrophils, lymphocytes, and glucose levels (Barton 2002, Adeyemo et al. 2009). The magnitude of the stress response to capture, transport, handling and anesthesia varies substantially among species, and, typically, the effects are apparent for several days after the stress event (Harper and Wolf 2009).

Taking into account the limited information on tuvira *G. inaequilabiatus* in Brazil, and especially in the Pantanal region, these hematological parameters may be used as preliminary reference values. However, further studies are required on other variables related to environmental or biotic factors in the context of commercial rearing. In conclusion, glucose levels, erythrogram, and leukogram results of tuvira *G. inaequilabiatus* correspond to those of other species of the Pantanal, including the morphology of the leukocytes. Furthermore, thrombocytes were the most frequent defense cells in the peripheral blood, followed by lymphocytes and neutrophils.

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