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ZOOLOGY

Haematological assessment of four amazonian ornamental armoured catfish (Teleostei, Loricariidae)

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ABSTRACT. The aim of this study was to characterize the haematological profile of four Amazonian ornamental freshwater armoured catfish: acari-bola (*Peckoltia oligospila* - L06), acari-pleco (*Cochliodon sp*. - L145), acari-canoa (*Lasiancistrus saetiger* - L323) and acari-assacu (*Pseudocanthicus spinosus* - L160). The highest blood glucose levels (72.47 ± 28.7 mg dL^{-1}) and erythrocyte counts (0.51 ± 0.2 x 10^6 cel. μL^{-1}) were recorded for acari-canoa. The acari-bola and acari-pleco presented similar concentrations of total plasma protein (TPP) (7.96 ± 1.8 and 7.93 ± 1.8 g dL^{-1}, respectively) against lower TPP concentrations observed in acari-canoa (4.87 ± 1.5 g dL^{-1}) and acari-assacu (6.55 ± 1.5 g dL^{-1}). The acari-assacu had lower total haemoglobin concentration (5.88 ± 1.7 g dL^{-1}) and haematocrit (12.66 ± 4.6%). No interspecific differences were observed in mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). The acari-pleco and acari-assacu presented the highest counts for neutrophils (4142.42 ± 3280.1 cel. μL^{-1}) and thrombocytes (4778.33 ± 1224.8 cel. μL^{-1}), respectively. The haematological profiles were similar to those reported in the literature for freshwater fish and the interspecific differences observed were discussed.

Keywords: blood profile; leukocytes; fish; pleco.

Avaliação hematológica em quatro acaris ornamentais amazônicos (Teleostei, Loricariidae)

RESUMO. O objetivo deste estudo foi caracterizar os perfis hematológicos de quatro espécies de acaris ornamentais da região amazônica: acari-bola (*Peckoltia oligospila* - L06), acari-pleco (*Cochliodon* sp. - L145), acari-canoa (*Lasiancistrus saetiger* - L323) e acari-assacu (*Pseudocanthicus spinosus* - L160). Os maiores níveis de glicose (72,47 ± 28,7 mg dL^{-1}) e contagem de eritrócitos (0,51 ± 0,2 x 10^6 cel. μL^{-1}) foram encontrados no acari-canoa. O acari-bola e acari-pleco apresentaram concentrações semelhantes de proteína plasmática total (PPT) (7,96 ± 1,8 e 7,93 ± 1,8 g dL^{-1}, respectivamente) em oposição às menores concentrações de PPT no acari-canoa (4,87 ± 1,5 g dL^{-1}) e acari-assacu (6,55 ± 1,5 g dL^{-1}). Essa última espécie apresentou menor concentração total de hemoglobina (5,88 ± 1,7 g dL^{-1}) e hematócrito (12,66 ± 4,6%). Não foram observadas diferenças significativas entre as quatro espécies nos parâmetros volume corpuscular médio (VCM) e concentração de hemoglobina corpuscular média (CHCM). O acari-pleco e o acari-assacu apresentaram os maiores valores de neutrófilos (4142,42 ± 3280,1 cel. μL^{-1}) e de trombócitos (4778,33 ± 1224,8 cel. μL^{-1}), respectivamente. Os perfis hematológicos foram semelhantes aos relatados na literatura para peixes de água doce e as diferenças interespecíficas observadas foram discutidas.

Palavras-chave: perfil sanguíneo; leucócitos; peixes; pleco.

Introduction

Amazon ornamental fish are of keen interest to the aquarium trade, notably to European and Asian markets. Armoured catfish, popularly known as suckermouth catfish or acari in Portuguese, stand out among marketed species (Loricariidae) (Ramos, Araújo, Prang, & Fujimoto, 2015; Araújo, Santos, Rebello, & Issac, 2017). In the Brazilian Amazon, namely in the State of Pará, Loricariids rank among the top five most commercialized and exported ornamental fish species, representing 56% of the annual income for families that are largely dependent on this type of activity (Torres, Giarizzo, & Carvalho Jr., 2008). Due to the economic importance of suckermouth catfish, further studies on their biological and haematological features are necessary for ecological and physiological evaluation, and to provide a better understanding of their phylogeny, habitat, environment adaptability and temporal trends in blood variables. Different species of fish, even those of the same genus or species, have been found to present differences in the number, size and volume of erythrocytes, haemoglobin concentration and haematocrit (Tavares-Dias & Moraes, 2004).

Along with ecological studies, haematology is an essential tool to help understand the connections between fish and the environment they inhabit and interact with (Tavares-Dias & Moraes, 2004). Blood analysis allows for the assessment of health and potential pathological disorders that can affect the homeostasis of the fish (Narra, Rajender, Reddy, Murty, & Begum, 2017; Nandi, Banerjee, Dan, Ghosh, & Ray, 2017). It is important to characterize patterns of immune blood cells for each teleost species under normal conditions to allow inferences of how cell populations will behave when disease processes unfold. This information is relevant to guarantee the quality of marketed fish, since the knowledge of osmoregulation changes can improve the right health management of these animals, reducing mortality rates, resulting in less pressure on natural stocks (Palikova et al., 2017; Neves et al., 2016).

Therefore, due to the economic importance of Loricariids as ornamental fish, and the lack of basic knowledge concerning the haematological profile of these fish, it is indispensable to make a database available for retrieval of elementary information and permit future comparisons. This study aimed to describe the haematological profiles of four Amazonian ornamental armoured catfish species: acari-bola (*Peckoltia oligospila*), acari-pleco (*Cochliodon sp*.), acari-canoa (*Lasiancistrus saetiger*) and acari-assacu (*Pseudocanthicus spinosus* - L160) captured from the Guamá River Basin - Northeast of Pará State, in order to facilitate future endeavours in the evaluation of abnormal haematology of these species.

Material and methods

In order to determine their haematological profile, 40 acari-bola (*Peckoltia oligospila* - L06), 37 acari-pleco (*Cochliodon* sp. - L145), 57 acari-canoa (*Lasiancistrus saetiger* - L323) and 38 acari-assacu (*Pseudocanthicus spinosus* - L160) juveniles, irrespective of sex, were captured from middle Guamá river, Northeast of Pará, Brazil, with the help of a local fisherman. Needles and syringes coated with EDTA (10%) were used for blood sampling (300 to 500 μL of blood taken from caudal vasculature) immediately after capture. For harvesting of blood aliquots, fish were submitted to short-term handling and the time for each sample collection did not exceed 40 s.

Blood samples of each fish were collected in the field for determination of glycaemia (mg dL^{-1}) using a Prestige® IQ 50 automatic metre; total plasma protein (TPP, g dL^{-1}) using a refractometer (Quimis®); haemoglobin (g dL^{-1}) using a CELM (CELM® 500/550); haematocrit (%) by the microhaematocrit method (Goldenfarb, Bowyer, Hall, & Brosious, 1971); total erythrocyte count (μL) was determined in a Neubauer chamber; blood smears were prepared for differential leukocyte counts using panchromic staining (Rosenfeld, 1947), determining the red blood cell, erythroblast and thrombocyte indices. Further, red blood cell indices including: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Vallada (1999). After blood sampling, each fish was weighed and measured (standard and total length) and observed for the presence of superficial injuries and/or ectoparasites. Water quality measurements were carried out in three different sections of the river, within the capture period, and before the blood sampling using a HANNA® HI 93715 multiparameter probe. Mean and standard deviations were as follows: Temperature 26.4 ± 1.2°C and dissolved oxygen 6.5 ± 0.8 mg L^{-1} (LT Lutron® DO-5519), conductivity 22.1 ± 0.2 mS cm^{-1} (EC-PCSTestr35®), pH 6.6 ± 0.4 (Quimis Q-400BC/BD®) and ammonia 0.0 ± 0.0 mg L^{-1} (HANNA® HI 93715). After blood sampling fish were kept in aerated tanks for 24 hours prior to being returned to the river. Data were tested using a one-way ANOVA and when F-value was significant ($p < 0.05$) a Tukey test was used for pair-wise comparison of means ($p < 0.05$). The Shapiro-wilk normality test was used to identify and eliminate outliers.

Results and discusion

No mortalities as a consequence of handling for blood collection were observed by the time fish were released back to the river. Haematological profiles of the four species are summarised in Table 1. Variations in blood count composition among different fish species is expected, some differences even observable between conspecifics. This is a reflection of the ecological niche that is occupied. The niche induces adaptations in physiology, between sexes, during gonadal development, and in relation to nutrition and seasonal variations (Campbell & Murru, 1990; Sharma, Akhtar, Pandey, Singh & Singh, 2017).

Table 1. Biometric data and haematology in acari-bola (*Peckoltia oligospila*), acari-pleco (*Cochliodon sp.*), acari-canoa (*Lasiancistrus saetiger*) and acari-assacu (*Pseudocanthicus spinosus*). Values are presented as means ± SD.

	Bola (*P. oligospila*) n = 40	Pleco (*Cochliodon*sp.) n = 37	Canoa (*L.saetiger*) n = 57	Assacu (*P. spinosus*) n = 38
Weight (g)	34.07 ± 10.0	25.49 ± 11.6	39.29 ± 13.9	30.70 ± 20.7
Standard Length (cm)	10.69 ± 1.2ab	9.42 ± 1.8b	11.22 ± 1.4a	11.08 ± 2.7a
Total Length (cm)	13.20 ± 1.2b	12.85 ± 1.8b	13.96 ± 2.0ab	14.53 ± 3.1a
Glucose (mg dL^{-1})	50.28 ± 8.8b	64.17 ± 26.9ab	72.47 ± 28.7a	52.92 ± 15.5b
Haematocrit (%)	24.87 ± 3.9a	22.11 ± 9.1a	23.77 ± 8.7a	12.66 ± 4.6b
TPP (g dL^{-1})	7.96 ± 1.8a	7.93 ± 1.8a	4.87 ± 1.5c	6.55 ± 1.5b
Haemoglobin (g dL^{-1})	8.65 ± 2.1a	9.90 ± 4.7a	10.60 ± 4.7a	5.88 ± 1.7b
MCV (fL)	557.89 ± 132.3	523.43 ± 389.6	470.27 ± 402.0	399.87 ± 588.6
MCH (ρg)	207.04 ± 58.9a	247.95 ± 111.03a	193.37 ± 103.2a	123.14 ± 42.7b
MCHC (g dL^{-1})	35.96 ± 9.4	45.39 ± 20.2	44.01 ± 14.1	51.13 ± 19.4
RBC* (x 10^6 μL^{-1})	0.47 ± 0.1ab	0.38 ± 0.2b	0.51 ± 0.2a	0.44 ± 0.1ab
Erythroblasts (µL)	1234.37 ± 562.7a	1335.79 ± 812.2a	673.17 ± 302.9b	889.53 ± 382.4ab
Thrombocytes (µL)	3458.30 ± 1543.2ab	2149.10 ± 1465.9c	3356.70 ± 2048.9bc	4778.33 ± 1224.8a
Leukocyte (µL)	9401.19 ± 2694.7	11533.00 ± 8327.3	10829.60 ± 4912.9	10261.00 ± 3619.3
Lymphocytes (µL)	6622.14 ± 2070.0	6121.40 ± 4625.7	9096.78 ± 4200.4	7178.28 ± 1754.4
Neutrophils (µL)	1917.94 ± 655.1bc	4142.42 ± 3280.1a	945.51 ± 731.1c	3337.11 ± 1032.3ab
Monocytes (µL)	598.15 ± 270.5	442.33 ± 398.4	328.64 ± 288.7	444.57 ± 241.7

*RBC = red blood cell. Values followed by distinct letters in rows are significantly different after ANOVA and Tukey test ($p < 0.05$).

Acari-canoa had higher blood glucose levels compared to the other acaris. Blood glucose concentration is regarded as a reliable indicator of physiological stress in fish (Neves et al., 2016; Chagas et al., 2016), and is directly related to the mode of life and metabolic activity of a fish (Ranzani-Paiva & Silva-Souza, 2004). Acari-canoa appears to have a more active swimming behaviour, and presumably, requires greater energy reserves to supply its metabolism compared to the other three studied species.

Regarding total plasma protein (TPP) content, similar concentrations were observed between acari-bola and acari-pleco (7.93 and 7.96 g dL^{-1}, respectively) as opposed to lower TPP concentrations in acari-assacu (6.55 g dL^{-1}) and acari-canoa (4.87 g dL^{-1}). In fact, higher concentrations of TPP have been reported for other loricarids including: *Leporacanthicus galaxias* (8.6 ± 3.4 g dL^{-1}), *Hypostomus* sp. (8.2 ± 2.18 g dL^{-1}) and *Rineloricaria* cf. *lanceolata* (9.0 ± 2.00 g dL^{-1}) (Fujimoto et al., 2013), also captured from natural environments. In order to maintain homeostasis, plasma proteins are involved in fundamental processes such as the transport of metabolites, blood coagulation and humoral defense (Satake, Ishikawa, Hisano, Pádua, & Tavares-Dias, 2009).

Acari-assacu had lower total haemoglobin concentration, haematocrit and MCH, compared to the other species. Acari-canoa had higher erythrocyte counts and lower erythroblast counts and total plasma protein, whereas the opposite was found for acari-pleco. With regards to MCV and MCHC, no significant differences among the four species were observed. According to Carvalho, Seibert, Coelho, and Marques (2009) the blood haemoglobin values in *Hypostomus emarginatus* (= *Squaliforma emarginata*, Loricariidae) are lower than those found in other species due to its accessory breathing system. However, these changes were not observed in any species in the present study, suggesting that they make up for the absence of accessory breathing by increasing the concentration of haemoglobin in their erythrocytes. These findings corroborate those of Fujimoto et al. (2013) and Neves et al. (2016) for *Ancistrus* sp., *Hypostomus* sp. and *Rineloricaria* cf. *lanceolata*.

Acari-canoa showed a higher erythrocyte count (0.51 x 10^6 μL^{-1}) and haemoglobin weight (MCH = 193.37 pg). These results resemble those described by Carvalho et al. (2009) for *H. emarginatus* (= *S. emarginata*, Loricariidae) erythrocyte count of 0.56 x 10^6 μL^{-1} and MCH = 103.20 pg. These erythrogram patterns reflect a strategy for improving gas exchange through development of greater membranes, or otherwise, are related to metabolic activity or the presence of less efficient haemoglobin. Acari-assacu had the lowest ($p < 0.05$) haematocrit (12.66%) in this study. Haematocrit in acari-bola (24.87%), acari-pleco (22.11%) and acari-canoa (23.77%) were undistinguishable ($p > 0.05$) and similar to those described in *Hypostomus emarginatus* (21.44%) (Carvalho et al., 2009) and *H. plecostomus* (24.9%) (Graham, 1997).

Differential leukocyte count showed the presence of lymphocytes, neutrophils and monocytes, in that order of frequency. Neither eosinophils, basophils nor PAS positive granulocytes were observed among the four species. Cells exhibited morphological and quantitative patterns comparable to those described in literature, with the highest average counts of neutrophils observed in acari-pleco. Neutrophils are considered important leukocytes for inflammation and phagocytosis

(Tavares-Dias & Moraes, 2004). Thus, it can be assumed that when affected by infectious disease challenges and/ or contact with xenobiotics, acari-pleco may trigger a faster and more efficient response to cope with the unfavourable conditions.

Similarly to our findings, the absence of basophils, eosinophils and PAS positive granulocytes was also reported for *Hypostomus emarginatus* (Carvalho et al., 2009), *Oreochromis niloticus* (Azevedo et al., 2016) and *Tilapia rendalli* (Tavares-Dias & Moraes, 2004). The absence and/or low frequency of these leukocytes have been reported in many species of fish, and when present at high frequencies, are often related to parasitism and allergic processes (Tavares-Dias & Moraes, 2004). Fish in this study were apparently healthy, ectoparasite-free specimens and had no detectable superficial lesions.

Acari-pleco and acari-bola showed higher erythroblast counts compared to the other species. The presence of immature cells in the peripheral circulation of fish is a frequent occurrence, and may be necessary to allow for a more effective red blood cell replacement to ensure adequate transport of oxygen (Carvalho et al., 2009). Thrombocytes were more frequent in vascular blood of acari-assacu compared to acari-pleco and acari-canoa. Phagocytic activity is ascribed to these cell morphotypes and their capacity to provide enhanced immune responses in fish has been previously reported (Ranzani-Paiva & Silva-Souza, 2004).

Conclusion

In conclusion, armoured catfish in this study presented similar haematological profiles when compared to other freshwater fish species reported in the literature. Nevertheless, interspecific haematological variability was observed and should not be overlooked, for it can lead to diverse physiological responses which can affect competence to cope with disease processes or common stressors such as fishing, stocking or transportation of these species.

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