BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Note

WEIGHT-LENGTH RELATIONSHIP AND BLOOD CHARACTERISTICS OF SILVER AROWANA, A OSTEOGLOSSIDAE FROM THE STATE OF AMAPÁ (BRAZIL)

Lucas Vasques Tostes[1]
Eliane Tie Oba Yoshioka[2]
Marcos Tavares-Dias[2]

[1]Universidade do Estado do Amapá (UEAP), Avenida Presidente Vargas, 650, Centro, CEP: 68900-070Macapá, AP, Brasil.

[2]Embrapa Amapá, estado do Amapá, Rodovia Juscelino Kubitschek, Km 5, nº 2600, Universidade, CEP: 68903-419, Macapá, Amapá, Brasil. E-mail: marcos.tavares@embrapa.br (corresponding author)

Received: December 11, 2018
Approved: May 12, 2019

ABSTRACT

This study investigated the weight-length relationship, plasma biochemical and hematological parameters in *Osteoglossum bicirrhosum* from the Preto River, in the State of Amapá (Brazil). The weight-length relationship showed a negative allometric growth, with the value of the regression constant (b) equal to 2.539. The relative condition factor (1.00 ± 0.02) indicated good body conditions of fish in their natural environment. In the blood smears of *O. bicirrhosum* (616.2 ± 209.1 g), erythrocytes, thrombocytes and leukocytes were found with morphological characteristics similar to those of other Brazilian fish. Mean glucose levels were: 46.5 ± 14.6 mg dL^{-1} (27.1-95.8); total protein 4.2 ± 0.6 g dL^{-1} (2.3-5.3); total cholesterol 303.9 ± 140.4 mg dL^{-1} (81.6-718.4); triglycerides 244.8 ± 57.5 mg dL^{-1} (76.2-361.9); total erythrocytes 2.75 ± 0.37 x 106 μL^{-1} (1.940-3.350); hemoglobin 8.8 ± 1.2 g dL^{-1} (6.3-13.0); hematocrit 26.6 ± 2.3% (21.0-32.0); mean corpuscular volume 98.6 ± 15.8 fL^{-1} (72.4-149.5); mean corpuscular hemoglobin concentration 32.9 ± 3.9 g dL^{-1} (23.4-44.8); total thrombocytes 90,816 ± 58,888 μL^{-1} (14,760-210,250); total leukocytes 75,287 ± 34,546 μL^{-1} (10,290-191,475); lymphocytes 72,555 ± 33,046 μL^{-1} (10,084-181,901) and 96.5 ± 2.5%(87-100); monocytes 1734 ± 1683 μL^{-1} (0-7.308) and 2.3 ± 1.8% (0-7); neutrophils 915 ± 1435 μL^{-1} (0-6.518) and 1.1 ± 1.5% (0-7), eosinophils 63 ± 225 μL^{-1} (0-960) and 0.1 ± 0.3% (0-1). Therefore, lymphocytes were the predominant leukocytes, while eosinophils were the least frequent. This was the first report of leukocytes and biochemical parameters for *O. bicirrhosum*. Lastly, it was established reference blood values that can be used as interpretative data obtained for this species when under similar environmental conditions.

Key words: growth; freshwater fish; blood; leukocytes.

RELAÇÃO PESO-COMPRIMENTO E CARACTERÍSTICAS SANGUÍNEAS DO ARUANÃ, UM OSTEOGLOSSIDAE DO ESTADO DO AMAPÁ (BRASIL)

RESUMO

Este estudo investigou a relação peso-comprimento, parâmetros bioquímicos plasmáticos e hematológicos em *Osteoglossum bicirrhosum* provenientes do Rio Preto, no estado do Amapá (Brasil). A equação relação peso-comprimento mostrou crescimento do tipo alométrico negativo, com valor da constante de regressão (b) igual 2,539. O fator de condição relativa (1,00 ± 0,02) indicou boas condições corporais dos peixes em seu ambiente natural. Nas extensões sanguíneas de *O. bicirrhosum* (616,2 ± 209,1 g) foram encontrados eritrócitos, trombócitos e leucócitos com características morfológicas similares a de outros peixes teleósteos brasileiros. Os níveis médios de glicose foram: 46,5 ± 14,6 mg dL^{-1} (27,1-95,8); proteína total 4,2 ± 0,6 g dL^{-1} (2,3-5,3); colesterol total 303,9 ± 140,4 mg dL^{-1} (81,6-718,4); triglicerídeos 244,8 ± 57,5 mg dL^{-1} (76,2-361,9); eritrócitos totais 2,75 ± 0,37 x 106 μL^{-1} (1,940-3,350); hemoglobina 8,8 ± 1,2 g dL^{-1} (6,3-13,0); hematócrito 26,6 ± 2,3% (21,0-32,0); volume corpuscular médio 98,6 ± 15,8 fL^{-1} (72,4-149,5); concentração da hemoglobina corpuscular média 32,9 ± 3,9 g dL^{-1} (23,4-44,8); trombócitos totais 90,816 ± 58,888 μL^{-1} (14.760-210.250); leucócitos totais 75,287 ± 34,546 μL^{-1} (10,290-191,475); linfócitos 72 555 ± 33 046 μL^{-1} (10,084-181,901) e 96,5 ± 2,5% (87-100); monócitos 1734 1683 μL^{-1} (0-7,308) e 2,3 ± 1,8% (0-7); neutrófilos 915 ± 1435 μL^{-1} (0-6,518) e 1,1 ± 1,5% (0-7), eosinófilos 63 ± 225 μL^{-1} (0-960) e 0,1 ± 0,3% (0-1). Portanto, linfócitos foram os leucócitos predominantes, enquanto eosinófilos foram os menos frequentes. Esse foi o primeiro relato de parâmetros leucocitários e bioquímicos para *O. bicirrhosum*. Por fim, foram estabelecidos valores sanguíneos de referência que podem ser utilizados como dados interpretativos para esta espécie em condições ambientais semelhantes.

Palavras-chave: crescimento; peixe de água doce; sangue; leucócitos.

INTRODUCTION

Osteoglossum bicirrhosum Cuvier, 1829 (silver arowana) is one of six species of Osteoglossidae, and occurs in the Amazon River Basin and in the Rupununi and Oyapock Rivers (Ferraris-Junior, 2003; Froese and Pauly, 2019). Benthopelagic fish with a sedentary life-style, it can jump out of water to catch prey in nearby branches, which allows this species exploring a variety of arboreal preys, such as insects, arachnids and other small invertebrates (Agudelo-Zamora et al., 2007; Soares et al., 2011). This fish is considered a voracious predator that requires constant feeding throughout the day to maintain its energy requirements (Chaves et al., 2005). It survives in environments with low concentrations of oxygen through aquatic surface respiration (ASR) using the barbels. Reproduction occurs between the end of the dry season (December) and the beginning of the flooding season (January). The females begin the process of sexual maturity at 55 cm out of standard length. This is an important fish species for the fishery because has a relatively large size, which can reach 1.2 m in length and 6 kg in weight, being very used for food by Amazonian riverine populations (Soares et al., 2011; Froese and Pauly, 2019). In addition, it has great potential for aquaculture (Cuaical et al., 2013), and has been used as ornamental fish in aquarium of various part of the world.

The hematological profile of a fish population can indicate its physiological status and health, so that hematology combined with other routine diagnostic methods could be used to identify and assess conditions that cause stress and/or diseases in fish (Tavares-Dias and Moraes, 2004; Ranzani-Paiva et al., 2013; Tavares-Dias, 2015; Bosisio et al., 2017; Barbieri et al., 2017; Fazio, 2019). Hence, blood parameters have been studied in different fish species (Wells et al., 1997; Tavares-Dias et al., 2007; Tavares-Dias, 2015; Val et al., 2016). It is known that the blood component values exhibit genetic and physiological variation. The genetic variation may be due to interspecific factors between species and intraspecific factors within species. Moreover, changes in these blood parameters depend on several factors such as species, temperature, age, photoperiod, nutritional status, cycle of sexual maturity, health condition, water quality, dissolved oxygen changes, gender, lotic or lentic environment, handling stress and transportation, size, feeding and stocking density, and microbial infection and parasitism (Tavares-Dias and Moraes, 2004; Ranzani-Paiva et al., 2013; Val et al., 2016; Fazio, 2019).

Despite the commercial importance of *O. bicirrhosum*, there is still little known on its physiology, especially with regard to hematology. For *O. bicirrhosum* from the Solimões River, in the State of Amazonas, Galdames-Portus et al. (1979) studied the functional properties of hemoglobin, and Johansen et al. (1978) described the hematocrit and hemoglobin. Recently, Val et al. (2016) reported glucose level, hematocrit and hemoglobin of *O. bicirrhosum* from the Negro River, in the State of Amazonas. Since the health status of a fish population can be evaluated through the determination of blood characteristics, as this specifies the ideal physiological condition in a certain population, this study investigated blood and biochemistry parameters of *O. bicirrhosum* from the Preto River, in the State of Amapá (Brazil).

MATERIAL AND METHODS

In October 2012, 38 specimens of *O. bicirrhosum* were collected in Preto River (0o10'38 "S and 051º33'034" W), municipality of Mazagão, state of Amapá (Brazil), for blood analysis. All fish were collected with 40 and 55 mm mesh nets (ICMBIO license: 11884-1). At the time of fish collection, the oxygen concentration dissolved in water (4.3 ± 1.2 mg L^{-1}) and temperature (30.5 ± 1.1 ° C) were determined using digital oximeter (YSI, USA). The pH (5.0 ± 1.1) was measured using a pHmeter (YSI, USA). The Commission of Ethics in the Use of Animals of Embrapa Amapá (Protocol No 002-CEUA/CPAFAP) approved this study.

After mechanical restraint of the fish (without anesthesia), an aliquot of blood was collected by puncturing the caudal vessel using EDTA (10%) syringes. This aliquot was divided in two, and one was used for determining erythrocytes number in Neubauer chamber, concentration of hemoglobin by the cyanmethemoglobin method and the hematocrit through microhematocrit method. With these data, we calculated the hematimetric indexes of Wintrobe: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Blood smears were made and stained panchromatically with the May Grünwald-Giemsa-Wright combination, for differential counting of leukocytes in up to 200 cells of interest, at each blood smear. These blood smears were also used to determine the total number of leucocytes and thrombocytes using indirect method of count (Ranzani-Paiva et al., 2013).

The second aliquot of blood was centrifuged at 75 G for 5 min (Centrifuge Mod. 5424, Hamburg, Germany) to obtain the plasma and determine the concentrations of glucose, total protein, total cholesterol and triglycerides using colorimetric kits (Biotécnica, MG, Brazil) for each one, with the absorbance readings performed in a spectrophotometer (Biospectro SP-220, Curitiba, Brazil).

The body weight (g) and total length (cm) of the fish were used to calculate the length-weight relationship (LWR). The LWR was calculated using the equation $W = aL_t^b$, where W is the total weight in g and TL is the total length in cm, while a is constant and b is the allometric coefficient. The parameters a and b were estimated by linear regression of the transformed equation: $\log W = \log a + b \log TL$ (Le Cren, 1951). The type of growth was verified through the t-test where: H_0: $b = 3$ (isometric growth) and H_1: $b \neq 3$ (allometric growth) ($p = 0.05$) (Zar 2010). The fish weight and length were used to calculate the relative condition factor (Le Cren, 1951), which was compared to a standard value (Kn = 1.00) using the *t*-test.

RESULTS

The equation of the LWR for *O. bicirrhosum* indicated a negative allometric growth (Figure 1).

For *O. bicirrhosum*, body and blood parameters are listed in Table 1, and concentrations of total protein and hemoglobin were the parameters with the lowest ranges.

In *O. bicirrhosum* blood, the number of thrombocytes and leukocytes varied and there was a predominance of lymphocytes (Table 2).

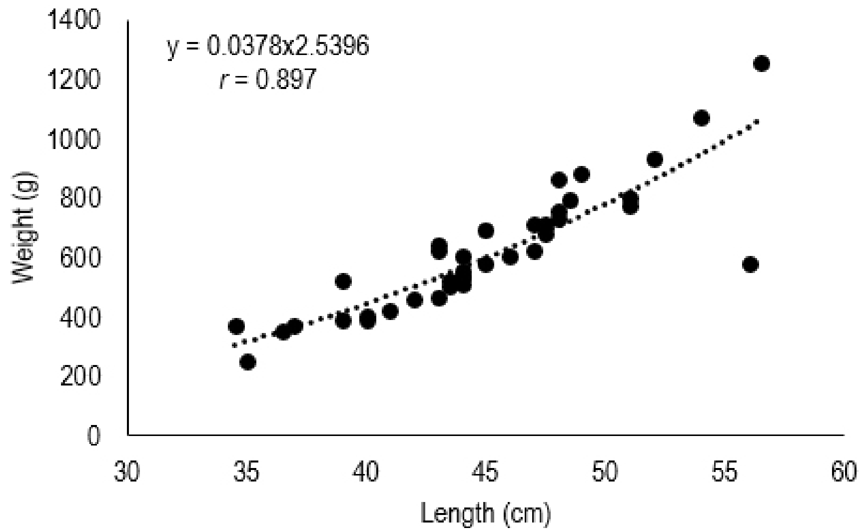

Figure 1. Equation of length-weight relationship for *Osteoglossum bicirrhosum* from the Preto River, State of Amapá (Brazil).

Table 1. Body, biochemical and erythrocytes parameters of *Osteoglossum bicirrhosum* from the Preto River, State of Amapá (Brazil).

Parameters	Mean ± SD	Minimum-Maximum
Weight (g)	616.2 ± 209.1	246-1254
Length (cm)	44.9 ± 5.3	34.5-56.5
Relative condition factor (Kn)	1.00 ± 0.02	0.92-1.04
Glucose (mg dL^{-1})	46.5 ± 14.6	27.1-95.8
Total protein (g dL^{-1})	4.2 ± 0.6	2.3-5.3
Triglycerides (mg dL^{-1})	244.8 ± 57.5	76.2-361.9
Total cholesterol (mg dL^{-1})	303.9 ± 140.4	81.6-718.4
Total erythrocytes (x 10^6 μL^{-1})	2.75 ± 0.37	1.94-3.55
Hemoglobin (g dL^{-1})	8.8 ± 1.2	6.3-13.0
Hematocrit (%)	26.6 ± 2.3	21.0-32.0
MCV (fL^{-1})	98.6 ± 15.8	72.4-149.5
MCHC (g dL^{-1})	32.9 ± 3.9	23.4-44.8

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration.

Table 2. Thrombocytes and leukocytes of *Osteoglossum bicirrhosum* from the Preto River, State of Amapá (Brazil).

Parameters	Mean ± SD	Minimum-Maximum
Thrombocytes (μL^{-1})	90,816 ± 58,888	14,760-210,250
Leukocytes (μL^{-1})	75,287 ± 34,546	10,290-191,475
Lymphocytes (μL^{-1})	72,555 ± 33,046	10,084-181,901
Monocytes (μL^{-1})	1734 ± 1683	0-7308
Neutrophils (μL^{-1})	915 ±1435	0-6518
Eosinophils (μL^{-1})	63 ± 225	0-960
Lymphocytes (%)	96.5 ± 2.5	87-100
Monocytes (%)	2.3 ± 1.8	0-7
Neutrophils (%)	1.1 ± 1.5	0-7
Eosinophils (%)	0.1 ± 0.3	0-1

DISCUSSION

Fish growth is generally measured in the increase of length and weight, and it is the variable used to determine population development. Length-weight relationship can also be used to determine possible differences between separate unit stocks of the same species, provided all units are studied with the same fully standardized sampling methodology and can also provide information on the stock condition. The condition factor of a given fish population generates information on the well-being, based on the assumption that heavier fish population have better body condition (Le Cren, 1951; Oliveira et al., 2018). The relative condition factor of *O. bicirrhosum* was not different (t = –0.008, p = 0.89) from the standard Kn value (Kn = 1.00), indicating good body conditions. Moreover, for *O. bicirrhosum*, growth type was negative allometric, which indicate a relatively higher growth rate in length than in weight, and this growth type is expected for fish species with "elongated" body shape (Oliveira et al., 2018). However, for *O. bicirrhosum* from the Xingu River basin, was reported positive allometric growth (Froese and Pauly, 2019). Such differences could be related to different life stage of this fish species.

The welfare of fish population can be analyzed with the help of some biochemical blood parameters, for example, the glucose, total protein, cholesterol and triglycerides. In addition, the levels of glucose are considered specific indicators of sympathetic activation during stress conditions, and cholesterol, triglycerides and total protein level are important indicators of the biochemical nutrition of fish (Hoseinifar et al., 2011; Rebl and Goldammer, 2018). In wild *O. bicirrhosum*, glucose levels were lower than those reported by Val et al. (2016) for this same fish species, while the total protein, cholesterol and triglycerides levels were similar to those of farmed *Arapaima gigas* (Tavares-Dias et al., 2007). However, the concentrations of these metabolites may vary, in part, influenced by environmental and non-environmental factors such as feeding and mode of life of the fish, particularly related to locomotivity. Thus, the glucose, cholesterol and triglyceride plasma levels differ according to age, general health conditions and fish feeding diet (Tavares-Dias et al., 2007).

Values of blood parameters provide insight into various processes in the fish organism; thereby, are of great importance. Erythrocyte parameters (erythrocyte number, hematocrit and hemoglobin) are particularly recommended on a routine basis to monitor the health of a fish population (Tavares-Dias and Moraes, 2004; Ranzani-Paiva et al., 2013; Fazio, 2019). In O. bicirrhosum, the hematocrit and hemoglobin were similar to the values reported by Johansen et al. (1978) for this same fish species. However, the hemoglobin and MCHC were higher that the values found by Val et al. (2016). The function of hemoglobin is adapted to the metabolic needs of fish and the environment, including intrinsic changes in oxygen affinity, changes in content of allosteric effects and variation in interaction by the successive joints of the oxygen molecules (Galdames-Portus et al., 1979). Thus, environmental problems, such as poor water quality and other stressors, may often contribute to the changes in erythrocyte parameters. *Osteoglossum bicirrhosum*, an air-breathing fish, survives in environments with low concentrations of oxygen through aquatic surface respiration (ASR) using the barbels (Soares et al., 2011). Therefore, *O. bicirrhosum* has a high erythrocyte number, moderate hemoglobin concentration and hence high oxygen carrying capacity, and low blood oxygen affinity.

Since piscine thrombocytes are multifunctional cells, once they are involved in hemostasis and immune defense (Tavares-Dias and Moraes, 2004; Ranzani-Paiva et al., 2013; Fazio, 2019), they are in constant movement in hematopoietic organs and systemic circulation. It has been reported that, in freshwater fish, thrombocytes number ranges from 870.0 to 100, 800 μL^{-1}. These inter-specific variations in thrombocytes number has been attributed to many factors, both biotic (e.g. such as age, sex, seasonality, maturity stage) and abiotic (e.g. water temperature, pH, dissolved oxygen content in water) and in particular to stress (Tavares-Dias and Moraes, 2004). In *O. bicirrhosum*, thrombocytes mean number was 90,816 ± 58,888 μL^{-1}, and therefore is within this range suggested for teleost fish.

In fish, analysis of blood leucocytes is an important tool that can be used as an effective and sensitive index to monitor immunological and pathological changes in animals (Ranzani-Paiva et al., 2013; Tavares-Dias et al., 2007; Tavares-Dias, 2015; Fazio, 2019). The diversity of fishes is reflected in variable leukocyte morphology, indicating a need for additional characterization to better classify these cells prior to clinical evaluations (Fazio, 2019). In the blood smears of *O. bicirrhosum*, there were monocytes, lymphocytes, neutrophils and eosinophils with similar morphological features to those reported for *A. gigas* (Tavares-Dias et al., 2007). The most frequent blood leukocytes in *O. bicirrhosum* was lymphocytes, while eosinophils are rarely observed, but no basophil was found in the blood smears. Similar findings were also reported for *A. gigas* (Tavares-Dias et al., 2007). However, leukocyte number in *O. bicirrhosum* was higher than reported for *A. gigas* (Tavares-Dias et al., 2007). Nevertheless, the piscine leukocytes vary widely among fish species mainly due to the phylogenetic position, sex, season, environmental conditions and age (Tavares-Dias and Moraes, 2004; Tavares-Dias et al., 2007).

In summary, there is an urgent need to make reliable normal databases available for fish species of economic importance. We established basal hematological values for *O. bicirrhosum*, which can be used as interpretative data obtained for this species when under similar environmental conditions. Therefore, other studies have to be lead for knowledge of the blood parameters of *O. bicirrhosum* when in other different environmental conditions.

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