http://dx.doi.org/10.1590/1678-4162-11994

M.O. Pereira
https://orcid.org/0000-0003-1339-9543
A.L. Preto
https://orcid.org/0000-0002-6185-8989
A.V. Moraes
https://orcid.org/0000-0003-0946-7320
M.F. Bittencourt
https://orcid.org/0000-0003-1467-3356
J.D. Hess
https://orcid.org/0000-0001-8275-6212
J.I.A. Andrade
https://orcid.org/0000-0002-9907-9948
A. Jatobá
https://orcid.org/0000-0002-9470-4775

Arq. Bras. Med. Vet. Zootec., v.72, n.6, p.2186-2192, 2020

Sex disparities determined by biometric and hematological measurements in *Astyanax bimaculatus*

[*Disparidades sexuais determinadas por medições biométricas e hematológicas em Astyanax bimaculatus*]

M.O. *Pereira*, A.L. *Preto*, A.V. *Moraes*, M.F. *Bittencourt*, J.D. *Hess*, J.I.A. *Andrade*[*], A. *Jatobá*

Laboratório de Aquicultura - Instituto Federal Catarinense - Araquari, SC

ABSTRACT

This study aimed to compare the body indexes and hematological characteristics between *Astyanax bimaculatus* males and females. Four hundred fish were randomly distributed into four polyethylene tanks (100 fish/unit) in a recirculation system and fed four times a day (3% of biomass). After 90 days, ten fish (five ♀ and five ♂) were removed to perform blood tests and to measure weight, height, total length, height/length ratio, condition factor and index determination: vicerosomatic (VSI), hepatosomatic (HSI), and gonadosomatic (GSI). The results showed a higher average weight (g) in females (12.32±0.71) compared to males (6.98±0.75), the same happened to height (cm) = (3.01±0.07) and (2.40±0.05), total length (cm) = (3.01±0.07) and (2.40±0.05), VSI (%) = (11.43±0.81) and (3.55±1.05), HSI (%) = (0.72±0.08) and (0.30±0.04), respectively. Mean corpuscular hemoglobin (pg) was higher in females (3.72±1.20) than in males (2.99±1.51). Regarding the number of thrombocytes ($10^3.\mu L^{-1}$), there was an increase in males (25.71±3.91) compared to females (17.40±6.40).

Keywords: yellowtail lambari, growth performance, hemogram, sexual dimorphism

RESUMO

O objetivo deste trabalho foi comparar os índices corporais e as características hematológicas entre machos e fêmeas de Astyanax bimaculatus. *Quatrocentos peixes foram distribuídos aleatoriamente em quatro caixas de polietileno (100 peixes/unidade), em sistema de recirculação, e alimentados quatro vezes ao dia (3% da biomassa). Após 90 dias, 10 peixes (cinco ♀ e cinco ♂) foram retirados para realização das análises sanguíneas e para mensuração do peso, da altura, do comprimento total, da relação altura/comprimento, do fator de condição e da determinação dos índices: viscerossomático (IVS), hepatossomático (IHS) e gonadossomático (IGS). Os resultados mostraram um maior peso médio (g) nas fêmeas (12,32±0,71) em relação aos machos (6,98±0,75); o mesmo aconteceu para altura (cm) = (3,01± 0,07) e (2,40± 0,05), comprimento total (cm) = (3,01±0,07) e (2,40±0,05), IVS (%) = (11,43±0,81) e (3,55±1,05), IHS (%) = (0,72±0,08) e (0,30±0,04), respectivamente. Hemoglobina corpuscular média (pg) foi maior nas fêmeas (3,72±1,20) que nos machos (2,99±1,51). Em relação ao número de trombócitos ($10^3/\mu L$), houve um aumento nos machos (25,71± 3,91) em relação às fêmeas (17,40±6,40).*

Palavras-chave: lambari-do-rabo-amarelo, desempenho zootécnico, hemograma, dimorfismo sexual

INTRODUCTION

Aquaculture is practiced all over the world as the main fish-producing activity owing to the rapid growth of the world's population, while the production of fish from fisheries has declined (The State of World Fisheries and Aquaculture, 2016). This requires the development of more efficient aquaculture systems, aiming to increase the supply and quality of fish. Yellowtail lambari belongs to the order Characiformes, family Characidae and subfamily Tetragonopterinae, with more than 75 species and subspecies (Garutti and Britski, 2000). They are naturally occurring fish in Brazilian rivers and their small size and

Recebido em 3 de abril de 2020
Aceito em 17 de julho de 2020
*Autor para correspondência (*corresponding author*)
E-mail: jaqueline.andrade@ifc.edu.br

ease of handling make them an interesting option for Brazilian fish farmers (Baldisserotto *et al*., 2014). This fish can be easily reared in different types of ponds with the use of artificial feeding at all life stages. Its tolerance to temperature variations contributes to its rusticity in commercial production systems (Garutti, 2003), besides supporting high stocking densities (e.g. more than six fish per liter) (Jatobá and Silva, 2015).

The occurrence of anatomical differences between the sexes is very common in the animal kingdom. However, considering the commercial production of any species, these differences can interfere with the standardization of the final product (Navarro *et al*., 2003). For fish, Bye and Lincoln (1986) consider sexual maturation one of the biological processes that most affect the yield of intensive systems, essentially because, during this period, energy for somatic growth is redirected to gonadal development and the formation of gametes, resulting in sexual dimorphism. These same authors also highlight the risk of overpopulation in ponds/tanks due to uncontrolled spawning. At the reproductive stages of the fish life cycle, energy is spent in the production of gametes, which generally interferes with their growth (Vazzoler, 1996). In tilapia, females have reduced growth, making the male economically more attractive (Meurer *et al*., 2005). While lambari females show a more satisfactory growth compared to males (Porto-Foresti *et al*., 2005).

Hematological parameters have been used as physiological indicators of organic dysfunction in aquaculture and are commonly applied as tools for prognosis and diagnosis of fish health status (Burgos-Aceves *et al*., 2019). However, there are intrinsic and extrinsic factors that can cause changes in these parameters, for example, age of the animal, reproductive period, sex and environment in which they are exposed (Fazio, 2019). Therefore, it is important to know whether *A. bimaculatus* males and femals of the same age and in the same environment, have a different hematological profile. Thus, hematology can be used as a tool to monitor changes in homeostasis and the health of the animals created.

Although females tend to grow more than males, therefore, allowing the production of monosex lots, this technique is not applied commercially, and both sexes of *A. bimaculatus* are created together. However, it is important to determine the characteristics between the sexes before a monosex commercial production (Antoniutti *et al*., 1985; Peterson and Davis, 2012). Accordingly, this study aimed to compare the biometry, body indexes, and hematological characteristics of the yellowtail lambari males and females (*A. bimaculatus*).

MATERIAL AND METHODS

This study was carried out at the Laboratório de Aquicultura (LAQ), Federal Institute of Santa Catarina (IFC), Campus Araquari/SC, Brazil (Protocol number 104/2015 approved by the Animal Ethics Committee). The experimental design was completely randomized, and four hundred fish from the same spawning, with a mean weight 0.27 g, were distributed into four experimental units (800 L polyethylene tanks) of 100 fish each and equipped with a recirculation aquaculture system (RAS) and biological filter (Figure 1). The fish were fed four times a day with 3% of their biomass, according to (Moraes *et al*., 2018).

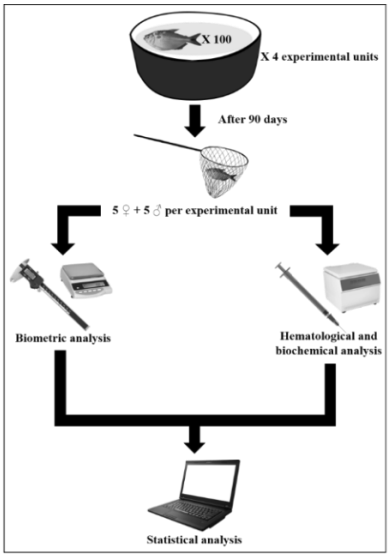

Figure 1. Experimental design, biometric and hematological analyzes.

During the experimental period, dissolved oxygen (3.15±1.52 mg.L-1) and temperature (21.78±2.59°C morning; 23.51±2.37°C afternoon), were measured twice a day. Whereas, the pH (7.32±0.11) and total dissolved ammonia (0.09±0.06mg.L-1) were measured twice a week.

The water parameters were considered suitable for rearing this species (Porto-Foresti *et al*., 2005). After the 90-day experimental period followed by 24 h of starvation, ten animals (five ♀ and five ♂) per experimental unit were anesthetized with eugenol (75mg.L^{-1}) for hematological analysis; and then euthanized by a cerebral concussion to the calculus of the hepatosomatic, vicerosomatic and gonadosomatic indexes (Figure 1). Sex identification was done by checking whether males would have spines on the anal fin and whether the females would have the width of the abdomen equal to or greater than the width of the back (Garutti, 2003).

The biometry of the animals was performed, being analyzed weight (g), height (cm), total length (cm), height/length (cm) ratio and condition factor (k). A longitudinal incision was made in the ventral region in order to remove and weigh the liver, viscera and gonads to the calculate hepatosomatic index (HSI = liver weight/body weight), viscerosomatic fat index (VSI = viscerosomatic fat weight/body weight) and gonadosomatic index (GSI = gonad weight/body weight).

For hematological and biochemical analysis, approximately 0.2ml of blood was withdrawn from the caudal vein with syringes containing EDTA 10% and used for blood smears stained with May Grunwald/Giemsa/Wright solution for white blood cell (WBC) count using an indirect method by counting the total leukocytes number (WBC) in 2000 erythrocytes in the smears (Ishikawa *et al*., 2008) and the total number of leukocytes (WBC) were calculated by the indirect method (Ranzani-Paiva *et al*., 2013). Hematocrit percentage was measured by the microhematocrit method and red blood cell count (RBC) in a Neubauer chamber after dilution 1:200 in the Dacie solution.

Hemoglobin concentration was determined by spectrophotometry using a commercial kit (LabTest, Minas Gerais, Brazil). The hematimetric indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Ranzani-Paiva *et al*., (2013), and glucose (G-TECH free, Accumed-Glicomed, Brazil). For each identified cell, length, width, and area were measured using Olympus cellSens Imaging Software (version 1.7) on a computer linked to an optical microscope coupled to a digital camera. The measures were compared between the different sexes.

Data were submitted to the Kolmogorov-Smirnov test and Levene's test to evaluate normality and homoscedasticity, respectively. Data approved from these tests were assessed by Student's *t*-test to observe differences between males and females. The remaining data were assessed by the Mann Whitney test. All tests recognized 5% significance.

RESULTS AND DISCUSSION

Differences in growth performance between sexes in fish may interfere with commercial profitability (Navarro *et al*., 2006). From this perspective, females showed greater total weight, height, and length (Table 1), similar to that described by Reidel *et al*. (2004), reporting on Curimbatá *(Prochilodus lineatus)* and Piavuçu *(Leporinus macrocephalus*). However, Souza, *et al.* (1999) observed better growth in the male Nile tilapia (*Oreochromis niloticus*). The height/length ratio and condition factor did not differ between sexes (Table 1). The height/length ratio suggests that both sexes present similar conformation of fillet. Thus, this piece of information is very important for the industrial processing standardization of fish (Reidel *et al*., 2004).

Table 1. Mean values±standard deviation total weight, height, total length, height / length ratio and condition factor after 90 days of experiment with yellowtail lambari (*A. bimaculatus*)

Indexes	Male	Female	Significance (*P*)
Total weight (g)	6.98±0.75	12.32±0.71*	0.00
Height (cm)	2.40± 0.05	3.01± 0.07*	0.00
Total length (cm)	2.40±0.05	3.01±0.07*	0.00
Height/length ratio (cm)	7.35±0.36	8.88±0.26	0.21
Condition Factor	1.02±0.09	0.99±0.04	0.29

*Indicates significant difference between sexes in the Student's *t* test.

The reproductive cycle may influence the weight of gonads, liver, and fat (Vazzoler, 1996). During this period, the liver synthesizes and secret vitellogenin, which is transported to the gonads by the bloodstream (Baldisserotto *et al*., 2014). Females showed higher HSI (Table 2), and this increase could be associated with the production of vitellogenin. These data corroborate the findings of Pereira Filho *et al*. (2011), who reported the increase of HSI in *Astyanax scabripinnis* females during oocyte maturation. Males had lower VSI values (Table 2), similar to the data obtained in *Leporinus copelandii* by Costa *et al*. (2005), likely owing to energy mobilization during the maturation of gonads, suggesting better body condition during the reproductive cycle.

Table 2. Averaged values±standard deviation of viscerosomatic index (VSI), hepatosomatic index (HSI) and gonadosomatic index (GSI) of yellowtail lambari (*A. bimaculatus*) reared for 90 days

Index	Male	Female	Significance (*P*)
VSI (%)	3.55±1.05	11.43±0.81*****	0.00
HSI (%)	0.30±0.04	0.72±0.08******	0.02
GSI (%)	LS	7.78±0.92**	0.02

*****Indicates significant difference between sexes in the Student's *t*-test. ** Indicates significant difference between sexes in the Mann Whitney test. LS: lost sample.

The GSI is normally used as an important reproductive parameter for females; however, for males, it does not always represent the current reproductive condition (Pereira Filho *et al*., 2011). The analysis of GSI corresponds to the reproductive phase in which females are found. Navarro *et al*. (2006) observed higher GSI in *A. scabripinnis* females reared together with and separate from the males. Barbieri *et al*. (1982) also observed a superior GSI in *A. fasciatus* and *A. bimaculatus* females. These results corroborate the data in the present work.

Fish hematology is an important tool for identifying changes in homeostasis, as well as the appearance of diseases and standardizing ideal reared conditions (Fazio, 2019). The hematological parameters of male and female *A. bimaculatus* did not show significant differences, except in HCM (Table 3), while other studies reported values close to those found in this experiment related to the number of erythrocytes, hematocrit, hemoglobin, glucose, MCV, MCHC and MCH in *A. bimaculatus* submitted to environmental conditions similar to this study (Jatobá *et al*., 2018; Moraes *et al*., 2018).

Motlagh *et al*. (2012) did not observe significant differences in hematological parameters between *Betta splendens* males and females, corroborating the study carried out with *A. bimaculatus*, except for MCH. Karimi *et al*. (2013) who evaluated the influence of sex on hematological parameters of *Acanthopagrus latus* and observed that the number of erythrocytes was higher in males than in females; although, other parameters such as hematocrit, MCV, MCH, and MCHC did not show a significant difference between the sexes.

Table 3. Averaged values±standard deviation of hematological variables of yellowtail lambari (*A. bimaculatus*) reared for 90 days

Hematological variables	Male	Female	Significance (*P*)
Erythrocytes (10^6µL^{-1})	2.85±0.50	2.47±0.30	0.09
Hematocrit (%)	33.01±3.01	34.20±4.10	0.66
Hemoglobin (g.dL^{-1})	8.46±4.30	9.56±3.20	0.21
Glucose (mg.dL^{-1})	156.01±48.01	149.00±28.01	0.83
Mean corpuscular volume (fL)	9.70±4.20	10.00±6,70	0.36
Mean corpuscular hemoglobin concentration (g.dL^{-1})	2.79±1.20	3.02±8.80	0.09
Mean corpuscular hemoglobin (pg)	2.99±1.51	3.72±1.20*	0.04

*Indicates significant difference between sexes in the Student's *t*-test.

A. bimaculatus females showed a greater significant increase in the MHC than males (Table 3), different from that observed in *A. latus* (Karimi *et al*., 2013) and in the rainbow trout (Ranzani-Paiva, *et al.* 1998). The increase in MHC in females could be related to the stage of gonadal

maturation, because the developed gonads (final stages of maturation) increase the demand for oxygen due to the incorporation of nutrients in the oocytes (Vazzoller, 1982). Leukocytes are often used as indicators of health status in fish because they are part of the innate immune defense and are involved in the regulation of immune function in organisms (Burgos-Aceves *et al*., 2019; Stenberg *et al*., 2019). The leukogram consists of neutrophils, lymphocytes, basophils, monocytes, eosinophils, special granulocytic cells, and thrombocytes.

In this study, males had a higher number of circulating thrombocytes than females (Table 4). This increase in the number of thrombocytes could be caused by a hemorrhage due to the difficulty in drawing blood from males, as they are smaller than females. This is related to the function of thrombocytes, which in addition to defense mechanisms and role in homeostasis, also participate in blood clotting processes (Ranzani-Paiva *et al*., 2013). No differences were observed between the sexes for other leukocytes (Table 4), suggesting that males and females have the same amount and proportion of circulating blood cells in the same environmental conditions, according to the results found for *A. latus* (Karimi *et al*., 2013).

No morphological differences were observed in lymphocytes and thrombocytes between the sexes (Table 5). These cells have the highest occurrence in the blood and are, therefore, used for morphological characterization.

Table 4. Leukogram (averaged values±standard deviation) of yellowtail lambari (*A. bimaculatus*) reared for 90 days

Cells (x 10^3.µL^{-1})	Male	Female	Significance (*P*)
Leukocytes	28.50±9.30	28.60±8.80	0.49
Thrombocytes	25.71±3.91*	17.40±6.40	0.03
Basophils	0.10±0.20	0.10±0.00	0.47
Monocytes	1.00±0.50	1.30±0.80	0.27
Lymphocytes	27.31±8.10	27.00±8.50	0.48
Neutrophils	0.10±0.10	0.10±0.10	0.48

*Indicates significant difference between sexes in the Student's *t*-test.

Table 5. Measurements (mean±standard deviation) of lymphocytes and thrombocytes of yellowtail lambari males and females (*A. bimaculatus*) reared for 90 days

Cellular dimensions	Male	Female	Significance (*P*)
	Lymphocytes		
Length (µm)	96.13±10.99	102.56±18.96	0.12
Width (µm)	87.51±11.56	91.75±12.74	0.19
Area (µm²)	7.443.06±1.307.90	7.751.48±1.543.82	0.41
	Thrombocytes		
Length (µm)	134.51±22.04	144.85±18.55	0.36
Width (µm)	72.03±9.06	76.26±11.18	0.46
Area (µm²)	7.548.48±1.461.61	7.868.84±1.057.85	0.64

Data were submitted to the Student's *t* test.

In conclusion, *A. bimaculatus* females have better growth performance, and this genus is indicated for higher yield (biomass). However, male body indexes suggest higher carcass yield. Thus, the choice of a monossex production must be planned according to the way the final product will be marketed (live, fresh, or processed). While for health or research purposes, the hematological profile of both sexes can be used to represent a population.

REFERENCES

ANTONIUTTI, D.M.; RANZANI-PAIVA, M.J.T.; GODINHO, H.M.; PAIVA, P. Morfologia das gônadas, escala de maturidade e fator de condição de *Plecostomus albopunctatus* Regan, 1908 (*Osteichthyes, Loricariidae*) do rio Jaguari, São Paulo, Brasil. *Bol. Inst. Pesca*, v.12, p.87-103, 1985.

BALDISSEROTTO, B.; CYRINO, J.E.P.; URBINATI, E.C. *Biologia e fisiologia de peixes neotropicais de água doce*. Jaboticabal: Funep. 2014. 336p.

BARBIERI, G.; SANTOS, M.V.R.; SANTOS, J.M. Época de reprodução e relação peso/comprimento de duas espécies de A*styanax* (*Pisces, Characidae*). *Pesqui. Agropecu. Bras.*, v.17, p.1057-1065, 1982.

BURGOS-ACEVES, M.A.; LIONETTI, L.; FAGGIO, C. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. *Sci Total Environ*., v.670, p.1170–1183, 2019.

BYE, V.J.; LINCOLN, R.F. Commercial methods for the control of sexual maturation in rainbow trout (*Salmo gairdneri* R.). *Aquaculture*, v.57, p.299-309, 1986.

COSTA, A.P.R.; ANDRADE, D.R.; VIDAL JUNIOR, M.V.; SOUZA, G. Indicadores quantitativos da biologia reprodutiva de fêmeas de piau-vermelho no Rio Paraíba do Sul. *Pesqui. Agropecu. Bras.*, v.40, p.789-795, 2005.

FAZIO, F. Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*, v.500, p.237-242, 2019.

FAO - Food and Agriculture Organization. The State of World Fisheries and Aquaculture: contributing to food security and nutrition for all. Rome. 2016.

GARUTTI, V.; BRITSKI, H.A. Descrição de uma espécie nova de *Astyanax* (*Teleostei, Characidae*), com mancha umeral horizontalmente ovalada, da bacia do rio Guaporé, Amazônia. *Papeis Avulsos Zool.*, v.40, p.217-229, 2000.

GARUTTI, V. *Piscicultura ecológica*. São Paulo: UNESP. 2003. 333p.

ISHIKAWA, N.M.; RANZANI-PAIVA, M.J.T.; LOMBARDI, J.V. Metodologia para quantificação de leucócitos totais em peixe, *Oreochromis niloticus*. *Arch Vet Sci.*, v.13, p.54-63, 2008.

JATOBÁ, A.; SILVA, B.C. Stocking density in the juvenile production of two characins species in a recirculating system. *Arq. Bras. Med. Vet. Zootec.*, v.67, p.1469-1474, 2015.

JATOBÁ, A.; MORAES, K.N.; RODRIGUES, E.F. *et al*. Frequency in the supply of *Lactobacillus* influence its probiotic effect for yellowtail lambari. *Cienc. Rural*, v.48, n.10, 2018.

KARIMI, S.; KOCHINIAN, P.; SALATI, A.P. The effect of sexuality on some haematological parameters of the yellowfin seabream, *Acanthopagrus latus* in Persian Gulf Iran. *J. Vet. Res.*, v.4, p.65-68, 2013.

MEURER, F.; HAYASHI, C.; BOSCOLO, W.R. *et*. Fontes protéicas suplementadas com aminoácidos e minerais para a tilápia do Nilo durante a reversão sexual. *Rev. Bras. Zootec.*, v.34, p.1-6, 2005.

MORAES, A.V.; OLIVEIRA, M.P.; MORAES, K.N. *et al*. Autochthonous probiotic as growth promoter and immunomodulator for *Astyanax bimaculatus* cultured in water recirculation system. *Aquacul. Res*., v.49, p.1-7, 2018.

MOTLAGH, S.P.; ZAREJABAD, A.M.; NASRABADI, R. G.; AHMADIFAR, E.; MOLAEE, M. Haematology, morphology and blood cells characteristics of male and female Siamese fighting fish (*Betta splendens*). *Comp Clin Pathol*., v.21, p.15-21, 2012.

NAVARRO, R.D.; FILHO, O.P.R.; SILVA, R.F. *et*. Influência do sexo no desempenho de lambari prata (*Astyanax scabripinnis* Jenyns, 1842). *Zootec. Trop.*, v.21, p.359-369, 2003.

NAVARRO, R.D.; SILVA, R.F.; FILHO, O.P.R. *et*. Comparação morfometrica e índices somáticos de machos e fêmeas do lambari prata (*Astayanax scabripinnis* Jerenyns, 1842) em diferente sistema de cultivo. *Zootec. Trop.*, v.24, p.165-176, 2006.

PEREIRA FILHO, H.P.; ANDRADE, D.R.; TONINI, W.C.T.; VIDAL JUNIOR, M.V. Biologia reprodutiva de fêmeas de lambari-prata, *Astyanax scabripinnis* Jenyns, 1842 (Characidae; Tetragonopterinae; Teleostei) em condições de cativeiro. *Cienc. Anim. Bras.*, v.12, p.625-634, 2011.

PETERSON, B.C.; DAVIS, K.B. Effects of gender and sex hormones on disease susceptibility of channel catfish to *Edwardsiella ictaluri*. *J. World Aquacul. Soc*., v.43, p.733-738, 2012.

PORTO-FORESTI, F.; CASTILHO-ALMEIDA, R.B.; FORESTI, F. Biologia e criação do lambari-do-rabo-amarelo *(Astyanax altiparanae)*. In: BALDISSEROTTO, B.; GOMES, L.C. (Orgs.). *Espécies nativas para piscicultura no Brasil*. Santa Maria: UFMS, 2005. 468p.

RANZANI-PAIVA, M.J.T.; TABATA, Y.A.; EIRAS, A.C. Hematologia comparada entre diplóides e triplóides de truta arco-íris, *Oncorhynchus mykiss* Walbaum (*Pisces, Salmonidae). Rev. Bras. Zool.*, v.15, p.1093-1102, 1998.

RANZANI-PAIVA, M.J.T.; PÁDUA, S.B.; TAVARES-DIAS, M.; EGAMI, M.I. *Métodos para análise hematológica em peixes.* Maringá: EDUEM. 2013. 135p.

REIDEL, A.; OLIVEIRA, L.G.; PIANA, P.A. *et al*. Avaliação de rendimento e características morfométricas do curimbatá *Prochilodus lineatus* (Valenciennes, 1836), e do piavuçu *Leporinus macrochephalus* (Garavello & Britski, 1988) machos e fêmeas. *Rev. Varia Sci.*, v.4, p.71-78, 2004.

SOUZA, M.L.R.; MACEDO-VIEGAS, E.M.; KRONKA, S.D. Influência do método de filetagem e categorias de peso sobre rendimento de carcaça, filé e pele da tilápia-do-Nilo *(Oreochromis niloticus)*. *Rev. Bras. Zootec.*, v.28, p.1-6, 1999.

STENBERG, O.K.; HOLEN, E.; PIEMONTESE, L. *et al*. Effect of dietary replacement of fish meal with insect meal on *in vitro* bacterial and viral induced gene response in Atlantic salmon (*Salmo salar*) head kidney leukocytes. *Fish Shellfish Immunol*., v.91, p.223-232, 2019.

THE STATE of world fisheries and aquaculture: contributing to food security and nutrition for all. Rome: FAO, 2016.

VAZZOLER, A.E.A.M. *Manual de métodos para estudos biológicos de populações de peixes*: reprodução e crescimento. CNPq, 1982.

VAZZOLER, A.E.A.M. Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: EDUEM. 1996.