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Influence of seasonality on hematological parameters of farmed streaked prochilod (*Prochilodus lineatus)*

Influência da sazonalidade nos parâmetros hematológicos do curimatã (*Prochilodus lineatus*) em cultivo

Influencia de la estacionalidad en los parámetros hematológicos de sábalo (*Prochilodus lineatus*) en cultivo

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Abstract

The objective of the present study was to evaluate the influence of seasonality on the hematological aspects of juvenile farmed *Prochilodus lineatus*, to highlight the changes caused by seasonal variations and to develop reference intervals appropriate for each season. The study of the hematological parameters of farmed species has showed the mechanisms of adaptation to environmental variations, both in the natural environment and in production systems, allowing discernment of the differences between physiological and pathological reactions. Among the environmental factors, temperature and day length are recognized as a key factor in promoting changes in fish physiology, which can be reflected in hematological parameters. The present study analyzed the hematological parameters of juveniles of farmed streaked prochilod (*Prochilodus lineatus*) considering seasonal features such as temperature and day length, to highlight the changes caused by seasonality and the mechanisms involved in adaptation, and thus, elaborate reference intervals for each season. We found that seasonality has a significant impact on the hematological parameters of juvenile

specimens of farmed streaked prochilod (*Prochilodus lineatus*), as it promotes changes in hematimetry, hemoglobinometry, white blood cells count and in the prevalence of leukocyte types. These findings demonstrate the importance of establishing reference intervals for farmed species, considering the factors responsible for physiological modifications, especially seasonal features.
Keywords: *Prochilodus lineatus;* Hematology; Seasonal variation; Reference interval.

Resumo
O objetivo do presente estudo foi avaliar a influência da sazonalidade sobre os aspectos hematológicos de juvenis de Prochilodus lineatus em cultivo, para evidenciar as mudanças causadas pelas variações estacionais e elaborar intervalos de referência adequados à cada estação. O estudo dos parâmetros hematológicos das espécies de peixes de cultivo tem demonstrado os mecanismos de adaptação às variações ambientais, tanto no habitat natural quanto nos sistemas de produção, possibilitando o discernimento das diferenças entre as reações fisiológicas e as reações patológicas. Dentre os fatores ambientais, a temperatura e o fotoperíodo são reconhecidos como fatores chave a promoverem mudanças na fisiologia dos peixes, as quais podem ser refletidas nos parâmetros hematológicos. O presente estudo analisou os parâmetros hematológicos de juvenis de curimatã (*Prochilodus lineatus*) em cultivo, considerando as características sazonais como temperatura e fotoperíodo, para evidenciar as mudanças causadas pela sazonalidade e os mecanismos envolvidos na adaptação, com a finalidade de elaborar intervalos de referência para cada estação. Verificamos que a sazonalidade tem impacto significativo nos parâmetros hematológicos dos juvenis de curimatã (*Prochilodus lineatus*), pois promove mudanças na hematimetria, hemoglobinometria, leucometria global e na prevalência dos tipos leucocitários. Estes achados demonstram a importância do estabelecimento de intervalos de referência para as espécies de peixes cultivadas, considerando os fatores responsáveis pelas modificações fisiológicas, em especial as características sazonais.
Palavras-chave: *Prochilodus lineatus;* Hematologia; Variação sazonal; Intervalo de referência.

Resumen
El objetivo del presente estudio fue evaluar la influencia de la estacionalidad en los aspectos hematológicos de los juveniles de Prochilodus lineatus en cultivo, poner de manifiesto los cambios provocados por las variaciones estacionales y desarrollar intervalos de referencia adecuados para cada estación. El estudio de los parámetros hematológicos de las especies de peces de piscifactoría ha demostrado los mecanismos de adaptación a las variaciones ambientales, tanto en el hábitat natural como en los sistemas de producción, lo que permite discernir las diferencias entre las reacciones fisiológicas y las patológicas. Entre los factores ambientales, la temperatura y el fotoperiodo son reconocidos como factores clave para promover cambios en la fisiología de los peces, que pueden reflejarse en los parámetros hematológicos. En el presente estudio se analizaron los parámetros hematológicos de los juveniles de sábalo (*Prochilodus lineatus*) en cultivo, considerando características estacionales como la temperatura y el fotoperiodo, con el fin de destacar los cambios provocados por la estacionalidad y los mecanismos implicados en la adaptación, con el propósito de elaborar intervalos de referencia para cada estación. Comprobamos que la estacionalidad tiene un impacto significativo en los parámetros hematológicos de los juveniles de sábalo (*Prochilodus lineatus*), ya que promueve cambios en la hematimetría, la hemoglobinometría, la leucometría global y la prevalencia de los tipos de leucocitos. Estos resultados demuestran la importancia de establecer intervalos de referencia para las especies de peces cultivados, teniendo en cuenta los factores responsables de los cambios fisiológicos, especialmente las características estacionales.
Palabras clave: *Prochilodus lineatus*; Hematología; Variación estacional; Intervalo de referencia.

1. Introduction

By 2030, aquaculture will be the main source of fish supply, surpassing extractive activities. Thus, it is essential to monitor the health status of fish stocks and investigate physiological responses to environmental factors such as temperature, day lenght and dissolved oxygen (Fazio, 2019). Fish physiology is greatly affected by environmental factors, and temperature is a key factor to promote significant chances in metabolism, activity level, reproduction, appetite, weight gain and immune defenses (Pascoli et al. 2011). As for hematological aspects, temperature is the main factor for seasonal changes in blood parameters such as hematimetry, hematocrit and hemoglobinometry in teleosts (Valenzuela et al. 2008). Even when temperatures do not vary significantly over seasons, some hematological parameters, as the hematimetric values, show differences between seasons, suggesting photoperiod is also a key factor for those variations (Zapata et al. 1992). Therefore, hematological analysis of farmed species is important, both for understanding the relationships between blood parameters and habitat, as well as adaptability to the environment and its mechanisms (Acharya & Mohanty, 2019). Moreover, hematological parameters assist to monitor the health status of fish stocks by revealing the changes that occur in response to various disease-causing factors (metabolic,

infectious, nutritional deficiencies, stress) before they cause symptoms and damages to yield (Satheeshkumar et al. 2011). For a proper interpretation of this parameters, it is necessary to discern between physiological modifications (in adaptation to the environment) and pathological responses; thus, reference intervals for the hematological parameters of the farmed species should be developed, considering factors such as water temperature, photoperiod, diet and reproductive stage (Grant, 2015).

The streaked prochilod (*Prochilodus lineatus*) is a teleost of the Curimatidae family, subfamily Prochilodontidae (ITIS, 2020), bentopelagic, neotropical and potamodromous, native to South America and occurring in Brazil, Argentina, Bolivia and Uruguay (Castro & Vari, 2003). The species has gained importance in aquaculture of native Brazilian species (Boscolo et al. 2011) and was introduced in Asian aquaculture, being farmed in China and Vietnam (Kalous et al. 2011).

The present study aimed to evaluate the hematological aspects of juvenile streaked prochilod (*Prochilodus lineatus*) considering seasonality, to highlight the influence of water temperature and daylength on the physiological parameters of the species, and thus establish seasonal reference intervals.

2. Methodology

2.1 Approval by the Ethics Committee

The present study was approved by the Ethics Committee for the Use of Animals of Federal Fluminense University (protocol number 888) and by the Ethics Committee for the Use of Animals of Foundation Institute for Fishing of the State of Rio de Janeiro (protocol number 08/2017).

2.2 Management of the specimens

120 specimens of *Prochilodus lineatus* reared in fish farming in the city of Cordeiro, state of Rio de Janeiro, Brazil (latitude 22°01'43"S, longitude 42°21'39"W), were kept in an excavated tank and fed twice a day (9am and 4pm) with a commercial feed formulated with 40g/kg of crude protein, 100g/kg of ethereal extract, 100g/kg of moisture, 130g/kg of mineral matter, 45g/kg of crude fiber, 25g/kg of calcium, 10mg/kg of phosphorus and 600mg/kg of vitamin C (levels guaranteed by the manufacturer). Feeding was suspended 24 hours before the blood sampling procedure.

Water quality was monitored daily during the experimental period, accordingly to Kubitza (1998), with assessment of physical and chemical parameters (temperature, pH, dissolved and saturated oxygen, ammonia, nitrite, nitrate, phosphorus, phosphate and transparency), carried out using a multiparameter photometer (HI83203-01 Hanna®), oximeter (HI9156-04 Hanna®), multiparameter meter (HI98130 Hanna®) and Secchi disk. The excavated tank had an open circulation system, with constant water inlet and outlet of water, at a flow of at least 0,5L/minute.

2.3 Blood sampling and hematological analysis

In the spring/summer period, sixty specimens were used, with average size of 17,72±1,54cm and average weight of 66,38±17,99g. Blood samples collected every 45 days, between September and March, at an average temperature of 26±1,42ºC (minimum 24ºC, maximum 28ºC), average day length of 764,92 minutes (12,74h or 12h 44,4min) (Sunrise and Sunset, 2020) and average content of dissolved oxygen in water of 6,39±0,67mg/L (average saturated oxygen of 71,85±6,27%).

In the autumn/winter period, sixty specimens were used, with average size of 18,26±0,86cm and average weight of 67,19±8,49g. Blood samples collected every 45 days, between April and August, at an average temperature of 21,5±1,4ºC (minimum 19ºC, maximum 23ºC), average day length of 666,82 minutes (11,11h or 11h 6,6min) (Sunrise and Sunset, 2020) and average content of dissolved oxygen in water of 7,57±0,41mg/L (average saturated oxygen of 74,42±2,91%).

The specimens were captured with a net and anesthetized with eugenol solution (50mg/L) (Medeiros et al. 2019) until the loss of balance and swimming. Then, peripheral blood was taken from the caudal vein with a 20X0,55mm needle and 3mL

syringe (Witeska et al. 2022). The sample was transferred to microtainer tube (0,5mL) containing EDTA, and a drop of blood was spread on glass slide to produce smears for hematological analysis. The specimens were euthanized after blood sampling, by anesthetic overdose (Eugenol solution 150mg/L), and a necropsy was performed, with assessment of internal and external organs, for evaluation of their health status.

The hematological parameters analyzed were hematocrit, hematimetry, hemoglobinometry, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), thrombocyte count, global leukometry (WBC) and differential leukocyte count.

Hematimetry, number of thrombocytes, global count of leukocytes and differential count of leukocytes were performed manually according to Almosny and Santos (2001). The erythrocytes were counted in an improved Neubauer chamber (Global Glass®) using Natt Herrich solution (Dinâmica®) as diluent in 1:100 ratio, in five squares of the central square, and the final count was established by the product between the number of cells counted, the number of squares counted, the dilution used and the depth of the Neubauer chamber. Lymphocytes and thrombocytes were counted together, in all squares of the central square, and this count was used to determine the number of thrombocytes and global leukometry in the blood smears. The blood smears were stained with Giemsa (Merck Millipore®) (incubation period of 25 minutes), and global count of leukocytes, thrombocyte count and differential count of leukocyte were performed in optical microscope (Leica® DM750) with 1000X magnification. The number of thrombocytes and the global leukometry were calculated by counting 100 cells in random fields, which was used as a proportion to the count obtained in the Neubauer chamber to establish the individual count of these cells. The differential count of leukocytes was performed by counting 100 WBCs in random fields, using the global leukometry count to establish the absolute and relative counts of the WBC populations.

Hematocrit determination was done according to Almosny and Santos (2001) using the the microhematocrit method, in which capillary tubes are filled with blood up to 2/3 of its capacity and subsequently sealed; then, centrifuged in a microhematocrit centrifuge (Fanem®) at 10.000 rpm speed for 5 minutes. After centrifugation, hematocrit determination was done by measuring the red cell column placing the capillary tubes onto a microhematocrit reader card (VIN®). Total plasma proteins were determined by the refractometric method, in which a part of the plasma contained in the capillary tubes was transferred to a portable refractometer (Global Equipamentos®) for dosage in mg/dL.

Hemoglobin dosage (hemoglobinometry) was done according to Almosny and Santos (2001), and was determined by the cyanmethemoglobin method, in which 20uL of the blood sample was added to 5mL of Drabkin solution (Dinâmica®) for 10 minutes and centrifuged in macrocentrifuge (Centrilab®) to remove cell debris produced by the hemolysis. The supernatant was analyzed in spectrophotometer (Kasvi®) at 540nm, resulting in absorbance used in the formula to calculate hemoglobin concentration in g/dL.

Hematimetric values were calculated accordingly to Almosny and Santos (2001), in which MCV was determined by hematocrit x10 / hematimetry ratio; MCHC, obtained from the hemoglobinometry x 100 / hematocrit ratio, and MCH, obtained by the ratio between hemoglobinometry and hematimetry.

2.4 Statistical analysis

Exploratory analysis of the collected data was performed using Kolmogorov-Smirnov and Shapiro-Wilk normality tests, with a significance level of 5%, and Kruskal-Wallis and Wilcoxon tests, a significance level of 2% for comparison between seasons. These tests and the 95% confidence intervals were performed using the statistical software R®.

3. Results

Data on water quality during the period of this experiment is shown in Table 1.

Table 1. Mean values and standard deviation of the water quality parameters analyzed during the period of the experiment, divided among the year seasons.

Parameters	Spring/Summer	Autumn/Winter
Total ammonia (mg/L)	0.08 ± 0.04	0.11 ± 0.03
Nitrite (mg/L)	6.14 ± 4.01	6.79 ± 2.44
Nitrate (mg/L)	7.17 ± 3.90	12.62 ± 1.51
Phosphorus (mg/L)	0.08 ± 0.01	0.10 ± 0.04
Phosphates (mg/L)	0.05 ± 0.01	0.05 ± 0.04
Temperature (ºC)	26 ± 1.42	21.5 ± 1.41
Oxygen (mg/L)	6.39 ± 0.67	7.57 ± 0.41
Saturated oxygen (%)	71.85 ± 6.27	74.42 ± 2.91
pH	7.43 ± 0.33	7.17 ± 0.33
Transparency (cm)	18.46 ± 1.26	17.16 ± 1.62

Source: Authors.

All specimens used in this study were healthy at the time of the blood sample proceedings, with no evidence of external or internal lesions, nor the presence of macroscopic ectoparasites or endoparasites.

Hematoscopy showed the presence of erythrocytes, thrombocytes, lymphocytes, neutrophils, heterophils, monocytes, eosinophils, heterophils and PAS-positive granulocytes. In all analyzed seasons, the most prevalent leukocyte was the lymphocyte, followed by the neutrophil. In the spring/summer period, heterophils and eosinophils were significant more prevalent ($p<0,05$) compared to monocyes. In the autumn/winter period, monocytes were significantly more prevalent ($p<0,05$) compared to eosinophils and heterophils. PAS-positive granulocyte appeared with very low frequency in all analyzed seasons.

Comparing the analyzed seasons (spring/summer vs. autumn/winter), statistical analysis showed significant differences ($p<0,02$) in the mean values of hemoglobinometry, MCV, CHCM, relative lymphocyte count and absolute and relative monocyte count, with higher values in the colder period (autumn/winter); and in the mean values of hematimetry, global leukometry, absolute neutrophil count, absolute and relative heterophil count and absolute and relative eosinophil count, with higher values in the warmer period (spring/summer).

Table 2 shows the mean values, standard deviation and 95% confidence intervals for the hematological parameters observed in the spring/summer period.

Table 2. Mean values and standard deviation and confidence intervals of hematological parameters observed in juveniles of *Prochilodus lineatus*, in the spring/summer period, at average water temperature of 26°C.

Parameters	Mean ± sd	CI 95% min	CI 95% max
Ht (%)	43.67 ± 6.18	42.25	45.08
He ($x10^6$/uL)	2.24 ± 0.56	2.11	2.37
Hb (g/dL)	10.62 ± 2.22	10.11	11.13
MCV (fL)	207,5 ± 63.14	193.07	221.93
MCH (pg)	50.4 ± 15.32	46.9	53.9
MCHC (%)	24.74 ± 4.74	23.6	25.83
TPP (g/dL)	5.06 ± 1.12	4.8	5.31
Tr (/uL)	40,045 ± 7,500.64	38,331.37	41,759.31
LG (/uL)	10,369.78 ± 3.403,22	9,592.12	11,147.45
L abs (/uL)	6,763.21 ± 3.419,21	5,981.89	7,544.54
L rel (%)	64.06 ± 21.41	59.17	68.95
N abs (/uL)	2,377.41 ± 1,944.85	1,932.99	2,821.83
N rel (%)	25.39 ± 21.31	20.52	30.26
M abs (/uL)	169.63 ± 203.30	123.17	216.08
M rel (%)	1.53 ± 1.71	1.14	1.93
H abs (/uL)	792.51 ± 1,245.24	507.96	1,077.06
H rel (%)	6.60 ± 9.58	441	8.79
E abs (/uL)	204.6 ± 296.14	136.93	272.27
E rel (%)	1.8 ± 2.25	1.28	2.31
PAS+ abs (/uL)	16.28 ± 55.65	3.56	28.99
PAS+ rel (%)	0.11 ± 0.39	0.02	0.2

Ht = hematocrit; He = hematimetry; Hb = hemoglobinometry; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobine; MCHC = mean corpuscular hemoglobine concentration; TPP = total plasma proteins; Tr = number of thrombocytes; LG =global leukometry; L abs = absolute number of lymphocytes; L rel = relative number of lymphocytes; N abs = absolute number of neutrophils; N rel = relative number of neutrophils; M abs = absolute number of monocytes; M rel = relative number of monocytes; H abs = absolute number of heterophils; H rel = relative number of heterophils; E abs = absolute number of eosinophils; E rel = relative number of eosinophils; PAS+ abs = absolute number of granulocytes PAS+; PAS+ rel = relative number of granulocytes PAS+.

Table 3 shows the mean values, standard deviation and 95% confidence intervals for the hematological parameters observed in the autumn/winter period.

Table 3. Mean values and standard deviation and confidence intervals of hematological parameters observed in juveniles of *Prochilodus lineatus*, in the autumn/winter period, at average water temperature of 21.5°C.

Parameters	Mean ± sd	CI 95% mín	CI 95% max
Ht (%)	45,7 ± 6,7	43,95	47,44
He (x10^6/uL)	1,96 ± 0,61	1,8	2,11
Hb (g/dL)	11,74 ± 1,99	11,19	12,22
MCV (fL)	266,44 ± 125,61	233,99	298,89
MCH (pg)	67,5 ± 31,7	59,31	75,69
MCHC (%)	26,05 ± 5,34	24,67	27,43
TPP (g/dL)	5,37 ± 1,15	5,07	5,67
Tr (/uL)	38.705 ± 12.615,29	35.446,12	41.963,87
LG (/uL)	8.932,16 ± 4.185,2	7.851,01	10.013,31
L abs (/uL)	6.937,61 ± 3.879,84	5.935,33	7.939,88
L rel (%)	75,05 ± 15,22	71,11	78,98
N abs (/uL)	1.423 ± 1.460,39	1.045,74	1.800,26
N rel (%)	24,77 ± 66,14	7,68	41,86
M abs (/uL)	494,80 ± 478,92	371,08	618,52
M rel (%)	4,85 ± 3,8	3,86	5,83
H abs (/uL)	231,82 ± 341,23	143,67	319,97
H rel (%)	2,71 ± 3,74	1,74	3,68
E abs (/uL)	75,75 ± 137,5	40,22	111,27
E rel (%)	0,93 ± 1,56	0,53	1,33
PAS+ abs (/uL)	34,71 ± 82,96	13,27	56,14
PAS+ rel (%)	0,4 ± 0,96	0,15	0,64

Ht = hematocrit; He = hematimetry; Hb = hemoglobinometry; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobine; MCHC = mean corpuscular hemoglobine concentration; TPP = total plasma proteins; Tr = number of thrombocytes; LG =global leukometry; L abs = absolute number of lymphocytes; L rel = relative number of lymphocytes; N abs = absolute number of neutrophils; N rel = relative number of neutrophils; M abs = absolute number of monocytes; M rel = relative number of monocytes; H abs = absolute number of heterophils; H rel = relative number of heterophils; E abs = absolute number of eosinophils; E rel = relative number of eosinophils; PAS+ abs = absolute number of granulocytes PAS+; PAS+ rel = relative number of granulocytes PAS+.

Table 4 show the mean values for the hematological parameters observed in both periods.

Table 4. Mean values and standard deviation of hematological parameters observed in juveniles of *Prochilodus lineatus*, in the spring/summer period and in the autumn/winter period, at average water temperature of 26°C and 21.5°C, respectively.

	SPRING / SUMMER	AUTUMN / WINTER
Parameters	**Mean ± sd**	**Mean ± sd**
Ht (%)	43,67 ± 6,18	45,7 ± 6,7
He (x10^6/uL)	2,24 ± 0,56	1,96 ± 0,61
Hb (g/dL)	10,62 ± 2,22	11,74 ± 1,99
MCV (fL)	207,5 ± 63,14	266,44 ± 125,61
MCH (pg)	50,4 ± 15,32	67,5 ± 31,7
MCHC (%)	24,74 ± 4,74	26,05 ± 5,34
TPP (g/dL)	5,06 ± 1,12	5,37 ± 1,15
Tr (/uL)	40.045 ± 7.500,64	38.705 ± 12.615,29
LG (/uL)	10.369,78 ± 3.403,22	8.932,16 ± 4.185,2
L abs (/uL)	6.763,21 ± 3.419,21	6.937,61 ± 3.879,84
L rel (%)	64,06 ± 21,41	75,05 ± 15,22
N abs (/uL)	2.377,41 ± 1.944,85	1.423 ± 1.460,39
N rel (%)	25,39 ± 21,31	24,77 ± 66,14
M abs (/uL)	169,63 ± 203,30	494,80 ± 478,92
M rel (%)	1,53 ± 1,71	4,85 ± 3,8
H abs (/uL)	792,51 ± 1.245,24	231,82 ± 341,23
H rel (%)	6,60 ± 9,58	2,71 ± 3,74
E abs (/uL)	204,6 ± 296,14	75,75 ± 137,5
E rel (%)	1,8 ± 2,25	0,93 ± 1,56
PAS+ abs (/uL)	16,28 ± 55,65	34,71 ± 82,96
PAS+ rel (%)	0,11 ± 0,39	0,4 ± 0,96

Ht = hematocrit; He = hematimetry; Hb = hemoglobinometry; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobine; MCHC = mean corpuscular hemoglobine concentration; TPP = total plasma proteins; Tr = number of thrombocytes; LG =global leukometry; L abs = absolute number of lymphocytes; L rel = relative number of lymphocytes; N abs = absolute number of neutrophils; N rel = relative number of neutrophils; M abs = absolute number of monocytes; M rel = relative number of monocytes; H abs = absolute number of heterophils; H rel = relative number of heterophils; E abs = absolute number of eosinophils; E rel = relative number of eosinophils; PAS+ abs = absolute number of granulocytes PAS+; PAS+ rel = relative number of granulocytes PAS+.

4. Discussion

The leukocytes found in the present study were lymphocytes, neutrophils, monocytes, eosinophils, heterophils and PAS-positive granulocytes. Other studies that addressed hematological aspects of *Prochilodus lineatus* reported the presence of lymphocytes, neutrophils, monocytes and eosinophils (Ranzani Paiva et al. 1997, Tavares Dias et al. 2008, Cazenave et al. 2009, Belo et al. 2013, Tavares Dias, Tavares Dias, 2015) and the presence of all these cells and also the PAS-positive granulocyte (Ranzani Paiva et al. 1999). Cells classified as heterophils in this study had small, rod-shaped granules, some of them eosinophilic, others basophilic. As for cell morphology, it is similar to heterophils of birds and reptiles (Clauss et al. 2008, Campbell, 2015). The simultaneous presence of neutrophils and heterophils, found in this study, has already been described in other species of the Characiformes order, such as *Brycon orbignyanus* (Tavares Dias & Moraes, 2006), *Salminus maxillosus* (Veiga et al. 2000) and *Salminus brasiliensis* (Pádua et al. al. 2009). PAS positive granulocytes are small cells, with slightly basophilic cytoplasm containing granules that do not stain with acidic or basic dyes, with an appearance of fine granulation (Tavares Dias et al. 1999b). These leukocytes have also been reported in other species of the Characiformes order, such as *Piaractus mesopotamicus* (Tavares Dias et al. 1999a) and *Colossoma macropomum* (Tavares Dias et al. 1999b) and Cypriniformes order, such as *Aristichthys nobilis* (Tavares Dias, 2006) and *Cyprinus carpio* (Tavares Dias et al. 2003, Tavares Dias et al. 2015).

There were no significant inclusions or morphological disorders (nuclear or cytoplasmic abnormalities) in the peripheral blood cells of the analyzed specimens, indicating absence of evidence of chronic stress, nutritional deficiencies, infectious diseases or environmental contaminants. Morphology of the peripheral blood cells of the specimens of *Prochilodus lineatus* analyzed in this study was consistent with the observations of Tavares Dias (2015) for the same species. However, the referred author did not report the presence of heterophils or PAS-positive granulocytes. According to other studies on hematological aspects of *Prochilodus lineatus* (Ranzani Paiva et al. 1997, Cazenave et al. 2009, Belo et al. 2013) lymphocyte is the prevalent leukocyte in healthy animals, followed by neutrophil, corroborating the findings of the present study. According to Clauss et al. (2008), in healthy teleosts, lymphocytes are the most prevalent leukocytes, followed by neutrophils, which are the most prevalent granulocytes in the peripheral blood of these fishes. In the present study, increase in the mean values of global leukometry was detected in the spring/summer period, and a decrease in these values in the colder period. This pattern is recognized in teleost species (Grant, 2015), and in the analyzed specimens of *Prochilodus lineatus*, increase in global leukometry in warmer periods can be explained by the greater presence of granulocytes (neutrophils, heterophils and eosinophils), while in colder periods, there was a significant increase in the mean values of monocytes, with a decrease in the presence of granulocytes. In tropical fish species, cooler temperatures tend to have a negative impact on both specific immune responses (mediated by the T helper lymphocyte) and nonspecific immune responses. One possible explanation for this is the change in cell membrane components, such as proteins and carbohydrates, involved in the cell recognition process, and phagocytes tend to be more resistant to these changes compared to lymphocytes. Thus, there would be a predominance of non-specific immunity in colder periods (Le Morvan et al. 1998). Moreover, fish immunity is based more on innate responses than on specific acquired functions, and phagocytes show better recognition of antigens at lower temperatures, compensating for lower humoral immunity (more efficient in warmer periods). Thus, during colder seasons, innate (nonspecific) functions predominate, and in warmer periods, acquired (specific) functions represent the main line of immune defense of teleosts (Marnila & Lilius, 2015). Therefore, monocytes are more prevalent in the autumn/winter period in the specimens analyzed in this study, as these cells are phagocytes involved in nonspecific responses (Clauss, 2008), predominant in the referred seasons. The higher prevalence of granulocytes (mostly heterophils and eosinophils) in the peripheral blood of the specimens investigated during spring/summer may be due to the more efficient specific immune function - eosinophils have functions related to antigenic stimulation, while the function of heterophils is presumably similar to that of neutrophils, participating in inflammatory processes in interaction with specific immune mechanisms (Campbell, 2015). Monocytes and eosinophils are less prevalent in the peripheral blood of healthy teleosts - eosinophils make up between 0 and 3% of leukocytes in peripheral blood, while monocytes correspond to at most 5% of the global leukometry (Campbell, 2015), which is consistent with the observations of the present study.

Mean hematocrit values did not vary significantly between seasons in this study. However, the lowest average values were observed in the spring/summer period, and MCV was significantly lower in this period, which is due to both the lower hematocrit values and the significantly higher hematimetric indices. At higher temperatures, erythrocytes were smaller in the individuals analyzed in this study, which may be an adaptation mechanism to seasonal variation in the concentration of dissolved oxygen in water - at higher temperatures, concentration of dissolved oxygen in water tends to decrease (Pascoli et al. 2011, Kavadias et al. 2013, Witeska, 2013), with the need for a more efficient gas capture system. Lay and Baldwin (1999) established the existence of an inverse correlation between erythrocyte size and aerobic activity in teleosts – smaller erythrocytes have a larger surface area for gas exchange and a smaller distance for gas diffusion, allowing faster oxygen flow from the blood to the tissues, compensating for the lower concentration of oxygen in the environment. Thus, in more active species, or during periods when the increase in water temperature increases metabolism, erythrocytes tend to be smaller. Hematimetry is also influenced by seasonal variations, and in this study, it was significantly higher in the spring/summer period, which reflects the trend observed in teleosts (Seriani et al. 2011, Grant, 2015). Such increase may be a response to greater metabolic activity (and consequently,

greater oxygen demand) and the tendency of decrease in the concentration of diluted oxygen in water when temperatures are higher (Satheeshkumar et al. 2011, Campbell, 2015, Islam et al. 2020). Even when temperatures do not vary significantly over seasons, most teleost species have higher mean hematimetric values in warmer seasons, suggesting photoperiod is also a key factor for this variation (Zapata et al. 1992). Higher hematimetric indices in the summer are essential to meet the greater metabolic demand of *Prochilodus lineatus* specimens, due to reproductive and migratory period of the species in its natural habitat, situated between October and January (Agostinho et al. 2003). This trend was maintained even in farming conditions when migration is not possible.

Regarding hemoglobinometry, the higher mean values observed in autumn/winter, as well as the higher means of MCH values, indicate that in these seasons red blood cells have higher hemoglobin levels, possibly as a response to the lower hematimetric indices observed in these periods.

Comparing the present study with other research on reference intervals for farmed streaked *Prochilodus lineatus* and at higher temperatures (between 24 and 30°C) (Tavares Dias et al. 2008, Tavares Dias, 2015), there was a predominance of neutrophils compared to lymphocytes in these previous studies. However, the presence of heterophils or PAS-positive granulocytes was not considered. Regarding red blood cells, these studies showed lower mean values for MCV and hematocrit parameters, and higher mean values for hemoglobinometry and hematimetry, which led to differences also in hematimetric indices (lower mean values of MCV and higher MCHC values). These experiments used larger specimens compared to those in the present study (17 to 32 cm), and it is known that age and size influence the hematological profile of teleosts. Adult fish, which is older and larger than juveniles, usually have blood parameters with higher values, possibly due to more efficient hematopoiesis and adaptation to non-environmental factors, such as diet, metabolic adaptations and activity level (Ahmed et al. 2020).

In the present study, juveniles of *Prochilodus lineatus* showed tolerance to the wide temperature variation during the experiment (minimum of 15°C and maximum of 28°C). This observation is consistent with the study conducted by Barrionuevo & Fernandes (1995), which reports that young individuals of *Prochilodus lineatus* have a high tolerance to low temperatures and wide temperature variations (variations of 29°C on average), which can be attributed to the seasonal variations of its natural environment, that usually has significant temperature variations during the day, especially in winter.

5. Conclusions

Seasonality has a significant influence on the hematological parameters of farmed streaked juvenile individuals of *Prochilodus lineatus*, leading to changes in the size of red blood cells, hematimetic indices, hemoglobinometry, global leukometry and prevalence of white blood cell types. These findings demonstrate the importance of establishing reference intervals for farmed fish species, considering the factors responsible for physiological changes, especially seasonal features. The authors emphasize the importance of environmental and physiologic factors in the assessment of the hematological parameters of teleost fishes, and suggest that future researches in this regard take such factors highly into consideration, especially in species recently introduced into aquaculture.

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