

## HEMATOLOGICAL PARAMETERS IN TWO NEOTROPICAL FRESHWATER TELEOST, *Leporinus macrocephalus* (Anostomidae) AND *Prochilodus lineatus* (Prochilodontidae)

### PARÂMETROS HEMATOLÓGICOS EM DOIS TELEÓSTEOS DE ÁGUA DOCE NEOTROPICAIS, *Leporinus macrocephalus* (Anostomidae) E *Prochilodus lineatus* (Prochilodontidae)

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**ABSTRACT:** *Leporinus macrocephalus* is a non-migratory omnivore fish which occurs in waters with a relatively high oxygen rate. *Prochilodus lineatus* is a migratory fish that inhabits deoxygenated hypolimnion and feeds on detritus material. Red blood cell, thrombocytes and white blood cell counts, hematocrit and hemoglobin, serum electrolytes (sodium, potassium, calcium, magnesium and chloride), metabolic products (total serum protein and plasma glucose) and blood cells major axis length for *L. macrocephalus* and *P. lineatus* were compared. White blood cell counts for both species indicated a similar activity of the immune functions. The concentration of total protein, sodium, calcium, magnesium and chloride, MCV, as well as red blood cells, monocytes, and thrombocytes size in *L. macrocephalus* were higher than in *P. lineatus*. However, *P. lineatus* had higher red blood cell counts, hematocrit and hemoglobin, which reflects a considerable adaptation to survive in an environment with low levels of oxygen.

**KEYWORDS:** Blood. Leukocytes. Metabolism. Freshwater fish. Hematology. *Leporinus macrocephalus*. *Prochilodus lineatus*.

## INTRODUCTION

Studies on fish blood parameters are important to known factors concerning their physiologic capacity (VILJOEN; VUREN, 1991; BALLARIN et al., 2004; WELLS et al., 2005), being also a helpful tool in the evaluation of their immune system (BALLARIN et al., 2004; TAVARES-DIAS; MORAES, 2004; TAVARES-DIAS; MORAES, 2007). Hematocrit, hemoglobin concentration and red blood cells size indicate their oxygen-carrying capacity (GRAHAM et al., 1985; TAVARES-DIAS; MORAES, 2004; WELLS et al., 2005). These primary red blood cells indices may be highly variable and dependent on the fish species. The hemoglobin rate needed to reversibly bind oxygen reflects the ability to extract oxygen from water and also the need for delivering oxygen to the tissue. Consequently, fish differ considerably in their activity patterns accordingly to their habits (GRAHAM et al., 1985; WELLS et al., 2005). Therefore, besides serving as an index of oxygen transport capability; the hematological parameters may also supply additional information about the health of a fish population and its immune system.

The purpose of this paper was to compare several blood parameters for healthy water breathing

*Leporinus macrocephalus* (GARAVELLO; BRITSKI, 1988) and *Prochilodus lineatus* (VALENCIENNES, 1836) bred on fish farms in Brazil. The *L. macrocephalus* is a non-migratory, omnivore fish that feeds on a wide variety of plant and animal materials (CASTAGNOLLI, 1992). The *P. lineatus* is a migratory fish that inhabits the deepness of rivers and lagoons and feeds on detritus material (CASTAGNOLLI, 1992; RIVAROLI et al., 2006).

## MATERIAL AND METHODS

### Fish and culture conditions

Young specimens of *Leporinus macrocephalus* (180 days old) weighting from 103.5 to 712.0 g and measuring from 20.5 to 36.5 cm and of *Prochilodus lineatus* (172 days old) weighting from 85.5 to 375.0 g and measuring from 17.0 to 32.0 cm were captured with nets in a fish farm (21° 07'45"S, 48° 03'57"W). They were transported to the Laboratory of Ichthyopathology of the Animal Health Research Center, at CPPAR/UNESP (Jaboticabal, São Paulo, Brazil) and acclimated for 15 days in tanks filled with tap water before the blood samples were collected. At the fish farm, the both species lived in similar water conditions and

were stocked in 1000 m<sup>2</sup> ponds (1fish/m<sup>3</sup>). This fact prevented major differences in the maintenance of the fish.

The fish were feed with extruded meal with 28% of crude protein. During this period, the water temperature varied from 24.5 to 30.2°C; the pH from 6.7 to 6.9; the dissolved oxygen from 5.4 to 6.2 mg/L and the electric conductivity from 115.6 to 129.8µS/cm.

### Hematological measurements

The fish were anesthetized with benzocaine (1 g/10 L) and two blood samples were collected by puncture of the caudal vessel. The first one was collected with syringe containing 0.01mg/mL of EDTA (10%) and divided into two aliquots. The second was taken without any anticoagulant. These procedures took about 1.0-1.2 minutes. One blood aliquot from the first blood sample taken with anticoagulant was centrifuged at 750G for determination of plasma glucose by the glucose oxidase method. The second blood aliquot was used for hematology. Red blood cells count (RBC) was done by automatic blood cell counter (Celm, Model CC510); hematocrit (Hct) by microhematocrit method and hemoglobin concentration [Hb] by cyanomethaemoglobin technique. From these primary indices resulted the secondary Wintrobe indices, such as the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Blood samples were used to prepare the blood smears for morphological observation, which were stained with a combination of May Grünwald-Giemsa-Wright (TAVARES-DIAS; MORAES, 2003). The blood smears were also used for white blood cell (WBC), total thrombocytes counts (TAVARES-DIAS; MORAES, 2007), and differential WBC counts. Other blood smears were stained with blue toluidine 0.5% pH 9.0 (TAVARES-DIAS, 2006a) for the determination of the length of the major axis of thrombocytes.

The second blood sample, taken without the use of anticoagulants, was let for 20 minutes at room temperature and then centrifuged for 10 minutes at 750G to obtain serum, which was collected and frozen at -80°C until the analyses were done. Total serum protein concentration was determined by biuret reaction. Serum ions concentrations were determined by flame photometer (Zeiss M4Q2) through emission (sodium and potassium) and through atomic absorption (calcium and magnesium). Serum chloride concentration was determined by the mercury thiocyanate method using a commercial kit (Sigma 461).

### Image analysis

To measure the major axis length (µm) of blood cells, erythrocytes, thrombocytes and WBC major axis lengths were determined using a Leica DMLB microscope equipped with an Image Pro-Lite image analysis system. The erythrocytes and WBC were analyzed in blood smears stained with MGGW, but the thrombocytes diameter were measured using only fusiform cells of blood smears stained with alkaline blue toluidine 0.5% stain (2006a). This solution, in pH 9.0, was chosen due to its better staining of thrombocytes in cytoplasm.

### Statistical analysis

The mean values of parameters for both species were statistically analyzed by *t* test, and the differences were considered significant at *p*<0.05.

## RESULTS AND DISCUSSION

Studies on fish electrolytes deserve great attention because they regulate several cellular processes, hence biochemical assessments can be used for a variety of purposes. It has been suggested that plasma glucose concentration is related to the activity and feeding habits, thus it is higher in active fish species (CARNEIRO; AMARAL, 1979; BALDISSEROTO, 2002). Contrastingly, for *L. macrocephalus*, a bottom feeder fish, and *P. lineatus*, an omnivore fish; two unlike taxonomic and ecological species, the concentration of plasma glucose and serum potassium are similar. However, sodium, calcium, magnesium, total protein and chloride concentration are higher for *L. macrocephalus* (Table 1). It is not surprising that these two fish species demonstrated differences for serum electrolytes concentrations, since they have different metabolic patterns, which may be associated to their particular way of life. Therefore, this indicates that the fish have specific needs for these electrolytes in the maintenance of their osmotic equilibrium (VILJOEN; VUREN, 1991; TAVARES-DIAS, 2004).

Studies on fish red blood cell, hematocrit and hemoglobin, besides being an important tool for health assessment (TAVARES DIAS; MORAES, 2004; TAVARES-DIAS; MORAES, 2006), can be used for finding out about factors concerning their oxygen-carrying capacity (GRAHAM et al., 1985; TAVARES DIAS; MORAES, 2004; WELLS et al., 2005). Red blood cell, hematocrit and hemoglobin levels for *L. macrocephalus* were lower than for *P. lineatus* (Table 1), suggesting a higher oxygen-carrying capacity in *L. macrocephalus*. In contrast, *P. lineatus* higher red blood cell indices presumably reflect a greater adaptation to survive in environments with low levels of oxygen, as

well as a higher metabolic activity. Because *P. lineatus* inhabits hypoxia regions of rivers and lagoons and is also an active swimmer (RIVAROLI et al., 2006), it

presumably improves its performance by increasing the number of erythrocytes and the hemoglobin concentration in peripheral blood.

**Table 1.** Biochemical and hematological parameters for *L. macrocephalus* and *P. lineatus* intensively farmed. Different letters indicate significant differences ( $p < 0.05$ ). Values are expressed as mean  $\pm$  SD

Parameters	<i>L. macrocephalus</i> (N=40)	<i>P. lineatus</i> (N=40)
Glucose (mg/dL)	57.7 $\pm$ 10.9 <sup>a</sup>	51.3 $\pm$ 22.9 <sup>a</sup>
Total protein (g/dL)	4.6 $\pm$ 0.6 <sup>a</sup>	3.6 $\pm$ 0.5 <sup>b</sup>
Sodium (mmol/L)	162.9 $\pm$ 5.7 <sup>a</sup>	144.0 $\pm$ 10.7 <sup>b</sup>
Potassium (mmol/L)	2.6 $\pm$ 1.1 <sup>a</sup>	2.8 $\pm$ 0.9 <sup>a</sup>
Calcium (mmol/L)	2.9 $\pm$ 0.4 <sup>a</sup>	2.6 $\pm$ 0.2 <sup>b</sup>
Magnesium (mmol/L)	1.5 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.2 <sup>b</sup>
Chloride (mmol/L)	124.8 $\pm$ 13.2 <sup>a</sup>	113.7 $\pm$ 18.0 <sup>b</sup>
RBC (x 10 <sup>6</sup> /μL)	2.407 $\pm$ 0.76 <sup>b</sup>	3.172 $\pm$ 0.55 <sup>a</sup>
Hemoglobin (mg/dL)	10.8 $\pm$ 1.2 <sup>b</sup>	13.3 $\pm$ 2.3 <sup>a</sup>
Hematocrit (%)	36.0 $\pm$ 4.9 <sup>b</sup>	39.4 $\pm$ 6.6 <sup>a</sup>
MCV (fL)	173.3 $\pm$ 85.3 <sup>a</sup>	128.9 $\pm$ 35.1 <sup>b</sup>
MCHC (mg/dL)	30.5 $\pm$ 5.7 <sup>a</sup>	34.6 $\pm$ 8.3 <sup>a</sup>
Thrombocytes (μL)	47,875 $\pm$ 20,962 <sup>a</sup>	21,409 $\pm$ 13,397 <sup>b</sup>
WBC (μL)	19,771 $\pm$ 18,866 <sup>a</sup>	24,689 $\pm$ 19,079 <sup>a</sup>
Lymphocytes (μL)	13,726 $\pm$ 20,736 <sup>a</sup>	8,825 $\pm$ 11,539 <sup>a</sup>
Neutrophils (μL)	4,376 $\pm$ 3,795 <sup>a</sup>	13,356 $\pm$ 8,374 <sup>a</sup>
Monocytes(μL)	745.0 $\pm$ 1,311 <sup>a</sup>	1,783 $\pm$ 1,922 <sup>a</sup>
Eosinophils (μL)	0.0 $\pm$ 0.0 <sup>b</sup>	725 $\pm$ 645 <sup>a</sup>
Basophils (μL)	969 $\pm$ 841 <sup>a</sup>	0.0 $\pm$ 0.0 <sup>b</sup>

Under extreme hypoxia, *P. lineatus* shows an elevated opercula movement rate, regulating its oxygen levels (FERNANDES et al., 1995). The fish that tolerate hypoxia possess coronary circulation and high myoglobin levels; hence, the heart muscle receives blood directly from the gills for its appropriate functioning (BALDISSEROTO, 2002). Interestingly, *L. macrocephalus* and *P. lineatus* have primary red blood cells parameters higher than water-breathing catfish *Arius leptaspis* and the facultative air-breathing tarpon *Megalops cyprinoids*, both of which have also a high oxygen carrying capability in environments with low levels of oxygen (WELLS et al., 2005). In *L. macrocephalus*, a higher MCV when compared to *P. lineatus*, is associated with a lower surface/volume ratio of red blood cells, which along with lower hematocrit and hemoglobin, might have a marked effect on its physiology, since the total area available for oxygen assimilation is reduced (GRAHAM et al., 1985; LAY; BALDWIN, 1999; BALLARIN et al., 2004).

Large cell size indicates a lower surface/volume ratio that influences all the processes involving plasma membrane, such as diffusion and transport, as well as other exchanges (BALLARIN et al., 2004). LAY; BALDWIN (1999) reported an inverse relationship between the size of fish's erythrocytes and its aerobic swimming ability. Functional basis for this relationship is found in larger surface area/volume ratios and in shorter diffusion distances, which allows faster oxygenation and deoxygenation of hemoglobin as the volume of erythrocytes decreases. The diameter of *P. lineatus* erythrocytes was larger than the diameter of *L. macrocephalus* erythrocytes (Table 2). Therefore, in *P. lineatus*, larger erythrocytes, a higher number of erythrocytes containing great hemoglobin concentration and a low cell volume (MCV) seem to be adaptations for the storage of oxygen. These adaptations have enabled the *P. lineatus* to live in the deoxygenated hypolimnion of rivers and lagoons, which is its natural habitat, indicating that fish differ considerably in their activity patterns according to their habits.

**Table 2.** Major axis length ( $\mu\text{m}$ ) of the blood cells for *L. macrocephalus* and *P. lineatus* intensively farmed. Different letters indicate significant differences ( $p < 0.05$ ). Values are expressed as mean  $\pm$  SD

Parameters	<i>L. macrocephalus</i>	<i>P. lineatus</i>
Erythrocytes (n=200)	11.46 $\pm$ 0.91 <sup>b</sup>	11.84 $\pm$ 1.00 <sup>a</sup>
Thrombocytes (n=100)	9.02 $\pm$ 1.24 <sup>a</sup>	7.75 $\pm$ 1.12 <sup>b</sup>
Lymphocytes(n=50)	7.11 $\pm$ 0.89 <sup>a</sup>	6.22 $\pm$ 0.97 <sup>b</sup>
Neutrophils (n=75)	10.00 $\pm$ 0.88 <sup>a</sup>	9.66 $\pm$ 0.92 <sup>a</sup>
Monocytes(n=75)	8.32 $\pm$ 0.87 <sup>a</sup>	7.92 $\pm$ 0.96 <sup>b</sup>
Eosinophils (n=50)	-	8.11 $\pm$ 0.85
Basophils (n=50)	9.06 $\pm$ 0.83	-

Fish leukocytes are the major cells component involved in immune responses (TAVARES-DIAS; MORAES, 2007), being the phagocytes directly involved in cell-mediated immune responses (BALLARIN et al., 2004; TAVARES-DIAS; MORAES, 2004; TAVARES-DIAS, 2006a). In *L. macrocephalus* and *P. lineatus*, the number of leukocytes, lymphocytes and monocytes were similar. However, the number of total thrombocytes and neutrophils was higher in *P. lineatus* than in *L. macrocephalus*. *P. lineatus* has eosinophils while *L. macrocephalus* has basophils (Table 1). Hence, this indicates a similar functioning of the immune functions for both species. It has been reported that few fish have basophils or eosinophils, and very rarely both basophils and eosinophils (TAVARES-DIAS; MORAES, 2004; TAVARES-DIAS, 2006a,b; TAVARES-DIAS; MORAES, 2007). Since environmental pressure during evolution induced to adaptations in the tissue distribution of the cells involved in the immune defence of host fish (REITE; EVENSEN, 2006), hence granulocytes similarly may have evolved (TAVARES-DIAS, 2006a).

Many events mediated by the plasma membrane occur as a follow up to the recognition of foreign particles by immunocytes; therefore lower surface/volume ratio can influence some of the leukocyte functions. For example, the interaction of phagocytes with foreign particles usually leads to the activation of plasma membrane NADPH oxidase

(BALLARIN et al., 2004). In *P. lineatus*, the size of thrombocytes, lymphocytes and monocytes are smaller than in *L. macrocephalus* (Table 2). However, fish lymphocytes are usually cells with small or large size, and this size may vary among species. Srivastava (1969) reported that the thrombocytes of sluggish fish are larger than those of fish with an active way of life. However, our findings do not corroborate these results, because *P. lineatus* is a migratory species and had smaller thrombocytes than *L. macrocephalus*, a species with a moderate way of life.

Blood evaluation may be an important tool in the assessment of the health status of fish. Therefore, baseline values were established for two important species in Brazilian aquaculture. These parameters can be used to interpret hematological data obtained for these species bred in intensive culture, as well as in other environmental conditions. Furthermore, they suggest that the blood oxygen affinity between species may represent an adaptation to their particular oxygen requirements, and the immune system functions may be similar when fish are in similar environmental conditions.

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**RESUMO:** O *Leporinus macrocephalus* é um peixe onívoro não migrador que ocorre em águas com concentrações de oxigênio relativamente altas. O *Prochilodus lineatus* é peixe migrador que habita o hipolímnio desoxigenado e alimenta-se de detritos. Foram comparados em *L. macrocephalus* e *P. lineatus* as contagens de eritrócitos, leucócitos e trombócitos, hematócrito, hemoglobina, eletrólitos séricos (sódio, potássio, cálcio, magnésio e cloreto), produtos metabólicos (proteína sérica total e glicose plasmática) e maior diâmetro das células sanguíneas. As contagens de leucócitos para ambas as espécies indicaram uma atividade similar das funções imunológicas. As concentrações de proteína total, sódio, cálcio, magnésio, cloreto, VCM e diâmetro de eritrócitos, monócitos e trombócitos em *L. macrocephalus* foram maiores que em *P. lineatus*. Porém, *P. lineatus* mostrou maior contagem de eritrócitos, hematócrito e concentração de hemoglobina, o que reflete uma considerável adaptação para sobreviver em ambientes com baixos níveis de oxigênio.

**PALAVRAS-CHAVE:** Hematologia. Leucócitos. Metabolismo. Peixes de água doce. *Leporinus macrocephalus*. *Prochilodus lineatus*.

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