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(Research Article)

First hematologic and serum biochemistry record of black drum *Pogonias cromis* (Linnaeus, 1766) in a Neotropical hypersaline Lagoon

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Abstract

This study is a pioneer in describing the first hematological parameters and serum biochemistry record of black drum, *Pogonias cromis* (Linnaeus, 1766) in a Neotropical hypersaline Lagoon. We collected a total of 32 individuals of *P. cromis*, with a total length (TL) ranging between 160 and 710mm, and total weight (TW) ranging between 24 and 39.8g. Blood samples were taken and the presence of granulocytes, lymphocytes, and monocytes indicated that *P. cromis* had developed an immune system. Increased levels of thrombocytes with decreased levels of the lymphocytes, may be due to an increased frequency of lymphocytes among the leukocytes, which is also seen in the trend of higher levels of lymphocytes associated with an increased leukocyte count. The mean count of red blood cells (1.97 ± 0.07 106 mm^3) was relatively low, whilst the hematocrit (37.4 ± 0.9%) and mean corpuscular volume (195.8 ± 1.9 fL) were higher when compared with those of other species of marine fish. High total plasma protein values (6.3 ± 0.1 g/dL) suggest an osmotic imbalance, which could be due to the stress of the environment. The relatively high values of ALT and AST (90 ± 0.7 and 229 ± 4.5 U/L, respectively) and urea (24.7 ± 0.6 mg/dL), and the low levels of creatinine (0.10 ± 0.02 mg/dL), suggest possible liver dysfunction in this species in this ecosystem. This work indicates the use of hematological analysis of *P. cromis* could be used as an effective biomarker for environmental risk assessments.

Keywords: Scianidae; Araruama Lagoon; Generalized additive models (GAMs)

1. Introduction

The human population growth experienced in recent decades, coupled with increased technological advances and an increase in industrial processes (including the manufacture of chemicals such as fertilizers, insecticides and herbicides), has led to an expansion in the level of xenobiotic compounds in aquatic ecosystems. This has contributed to the reduction in the quality of different habitats, as well as compromising the health of the living organisms that inhabit these ecosystems [1].

Constant exposure of aquatic biota to toxic substances released into the environment can lead to multiple changes in the living organisms, which can have serious consequences on populations, communities, or ecosystems, depending on the degree of contamination and length of exposure [2].

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Environmental monitoring programmers have recommended the use of biomarkers to detect the damage caused by contaminants, making it possible to observe the early effects on organisms, before irreversible damage has occurred [3]. In this context, hematological studies of different fish species are of ecological and physiological interest, since they improve our understanding of the relationship between blood characteristics, phylogeny, physical activity, fish habitat, and adaptability in the environment [4].

Studies on the hematology of fish in Brazil began in the 1930s, became focused on marine fish in the 1960s [5], and more recently have targeted commercially important species in river systems or in captivity [6].

However, despite the great importance of hematology, there is still a paucity of information on the hematology of teleosts in hypersaline ecosystems.

The Araruama Lagoon is a 220 km^2 Neotropical hypersaline Lagoon located on the north coast of the state of Rio de Janeiro (RJ), with salinity generally greater than 35 and a poorly studied fish fauna [7].

The black drum, *Pogonias cromis*, a native demersal Scianidae fish that lives in shoals, weighs up to 15 kg, and is one of the most popular edible fish in the region. However, the degradation and pollution of this ecosystem, together with overfishing has reduced the stocks of this fish [8]. The aim of this study was describe the first hematological parameters and serum biochemistry record of black drum, *Pogonias cromis* (Linnaeus, 1766) in a Neotropical hypersaline Lagoon.

2. Material and methods

2.1. Study area

The Araruama Lagoon consists of seven bays delimited by sandy tips formed by the destructive and constructive action of marine currents. It covers 220 km^2, with a shoreline of 190 km, an average depth of 3 m and a volume of 636 million m^3. Its maximum width and length is 14 km and 33 km, respectively. Its connection to the sea is through the 5.5 km long Itajuru channel, with a width rangingfrom 100 to 300 m, being limited by a coastal resting on the ocean side [9].

A unique characteristic of this ecosystem is its hypersalinity, ranging from 56-77 in the main body and 35-43 in the Itajuru channel [9]. The reason for the high salinity is the high evaporation rate, reduced rainfall and intense northeast wind (NE), with this lattersignificantly influencing the circulation of water in the Lagoon, causing the emergence of large vortices that rotate clockwise in southern portions of the Lagoon, and anticlockwise in northern portions [10]. The high salinity can also be linked to the reduced drainage basin, with only two perennial rivers flowing into its western portion, namely the Moças and Mataruna rivers. Water renewal is slow, every 84 days 50% of the volume of the Lagoons exchanged [11].

Figure 1 Map of Araruama Lagoon - RJ, Brazil. Sampled site (*)

2.2. Sampling and data collection

Pogonias cromis was collected from Araruama Lagoon in January 2013, with purse-seine net, at four sampling sites near the town of Iguaba Grande in zone 3 (22°51′19.3′′S 42°11′12.7′′W), where evaporation rates are higher and more typical of the interior regions of the Lagoon (more saline area) (Fig. 1).

All *P. cromis* were weighed and measured. Condition factor (K) was determined by the following equation: $K = TW/TL^b$[12].

Blood samples were taken to describe the hematological parameters and serum biochemistry of this specie. Finally, all specimens were released back into the Araruama Lagoon.

The blood samples were obtained via flow venipuncture using 1-mL syringes and an appropriately sized needle (13 × 4.5; 25 × 6; 20 × 5.5 gauges) according to the size of the fish, previously rinsed with the anticoagulant ethylenediaminetetraacetic acid (EDTA) [13]. After the puncture, the samples were placed in 1.5-mL Eppendorf tubes and stored refrigerated until arrival at the Laboratory for Clinical and Molecular Research, Faculty of Veterinary Medicine, Fluminense Federal University.

The red blood cells count (H) and the global leukometry (GL) were performed according [14] in which all cell types are present in a Neubauer chamber, by diluting 10 mL of blood with 1 mL saline. All erythrocytes, leukocytes, and thrombocytes contained in 25 squares within the larger central square were counted. The result was multiplied by the correction factor (5.000) and the response expressed as cells/mm^3.

The specific leukometry was performed by visual identification of leukocyte types using optical microscopy with an immersion lens (×1.000) and counting the frequency of each leukocyte type in a total of 100 leukocytes to give a percentage value. This percentage was used to obtain the total number of each leukocyte type per mm^3 of blood. Four leukocyte cell types were identified: neutrophils, eosinophils, lymphocytes and monocytes. Basophils were not observed. Hematoscopy and morphological observations of thrombocytes were also performed.

The packed cell volume or hematocrit (Ht) was obtained using the microhematocrit technique with centrifugation at 11,000 rpm/5 min. The mean corpuscular volume (MCV) was calculated by applying the formula Ht × 10/ H. The mean corpuscular hemoglobin concentration (MCHC) was calculated using the formula Hb × 100/Ht. Total plasma protein (TPP) was obtained by measuring the separated plasma in the capillary tube used for the analysis of cell volume, using a manual clinical refractometer. The results were expressed in terms of g/dL.

Serum biochemistry was performed using specific commercial Labtest® kits for each analysis: ALT (alanine aminotransferase, kit number 74), AST (aspartate aminotransferase, kit number 75), creatinine (kit number 96) and urea (kit number 104). All measurements were performed in plasma using a spectrophotometer (BioPlus 200).

For data analysis, a generalized additive models (GAMs) were also applied to identify possible relationships between hematological parameters. GAMs are an extension of generalized linear models, allowing some covariates to have a nonlinear effect on the response associated predictor variable, through a link function [15]. The complexity of the GAM model was selected by the stepwise procedure using the Akaike information criterion (AIC), available in the CANOCO 4.5 program.

3. Results

A total of 32 were analyzed, with a total length (TL) ranging between 160 and 710 mm, and total weight (TW) ranging between 24 and 39.8g.

Of the hematological parameters are shown in the table 1. The GAM identified significant relationships between the values of hemoglobin, thrombocyte, leukometry, lymphocytes, and neutrophils (Fig. 2). There was a positive nonlinear trend between hemoglobin, TL ($F = 5.68$, $p = 0.008$), and TW ($F = 6.39$, $p = 0.005$), where lower concentrations of hemoglobin were observed at shorter lengths and weights (Fig. 2).

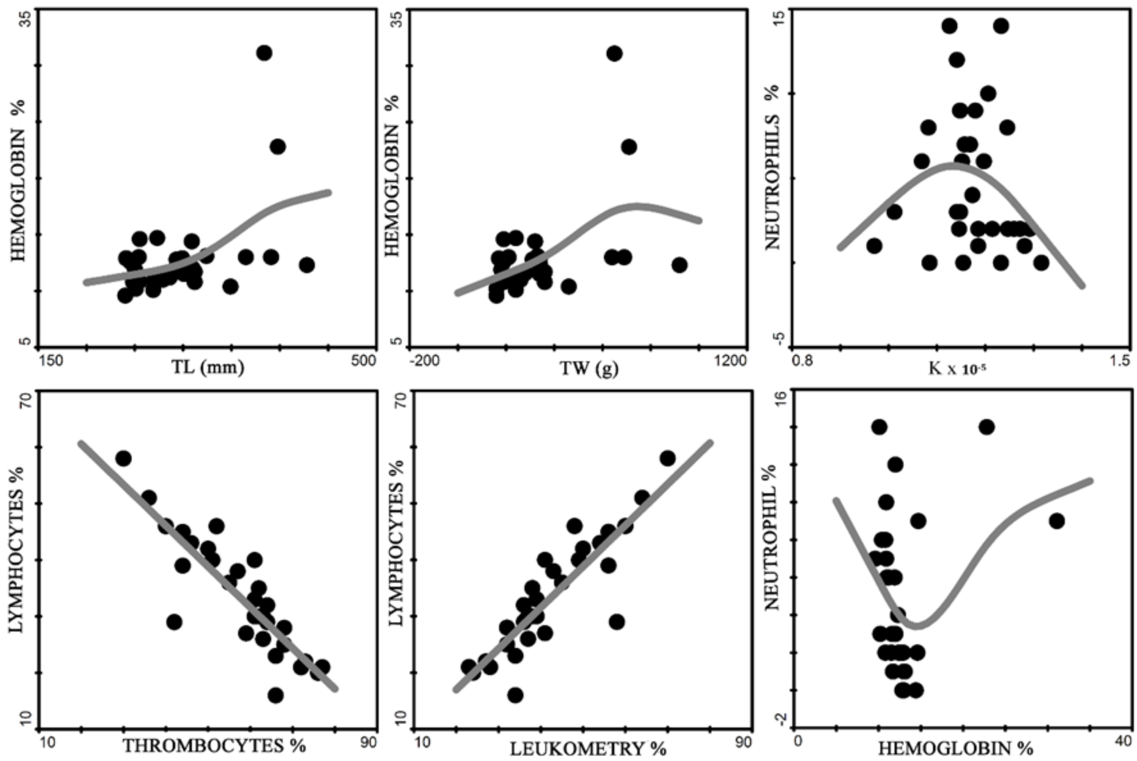

Figure 2 Relationships between hemoglobin and (%) total length (mm) and total weight (g) and between neutrophils (%) and the condition factor (K); Relationship between lymphocytes and thrombocytes, and leucocytes and lymphocytes; Relationship between neutrophils and hemoglobin of *Pogonias cromis* in Araruama Lagoon, RJ, Brazil. The lines represent the generalized additive models (GAMs), selected by the Akaike information criterion**.**

Table 1 Hematological parameters of black drum, *Pogonias cromis* in Araruama Lagoon, RJ, Brazil. MCV= mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; TPP= Total plasma protein; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Hematological parameters	**Mean±SE**
Hematocrit (%)	37.4±0.9
Red blood cells (10^6 /mm^3)	1.97±0.07
Hemoglobin (mg/L)	13.0±0.8
MCV (fL)	195.8±1.9
MCHC (g/dL)	35.2±2.3
Global leukometry (10^3 /mm^3)	339.2±28.1
Thrombocytes (%)	57.3±2.2
Lymphocytes (%)	33.6±1.8
Neutrophils (%)	4.7±0.7
Monocytes (%)	3.3±0.4
Eosinophils (%)	0.3±0.1
TPP (g/dL)	6.3±0.1
ALT (U/L)	90±0.7
AST (U/L)	229±4.5
Creatinine (mg/dL)	0.10±0.02
Urea (mg/dL)	24.7±0.6

Neutrophils showed a nonlinear trend with K (F = 4.24, p = 0.05), being the highest values found between the extreme values of K (Fig. 2). The lymphocytes showed a negative linear trend in relation to thrombocytes (F = 89.55, p < 0.01), with higher counts of thrombocytes being associated with lower lymphocyte counting. Moreover, lymphocytes showed a positive linear trend with leukometry (F = 93.63, p <0.01), where higher leukometry values were associated with an increase in the number of lymphocytes (Figure 2 and 3).

Neutrophils showed a nonlinear trend with hemoglobin (F = 9.21, p <0.01), with high percentages of neutrophils being related to low concentrations of hemoglobin.

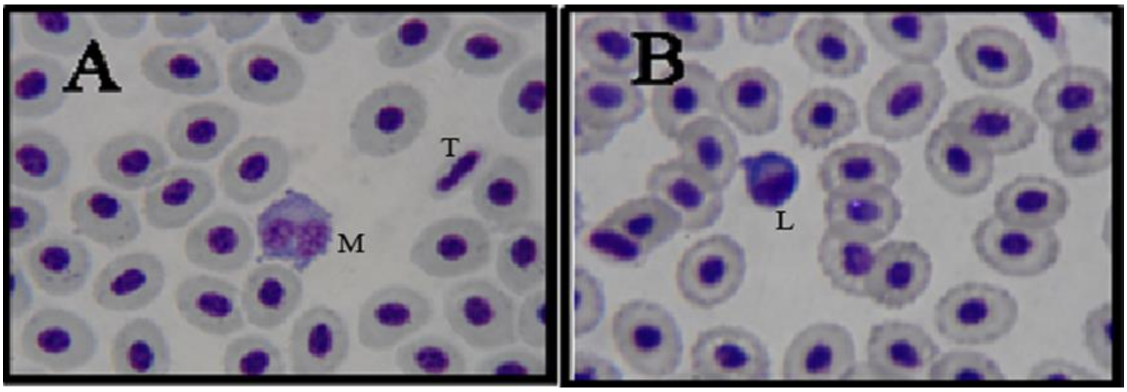

Figure 3 A. Monocytes (M) and thrombocytes (T); B. Lymphocytes (L) of *Pogonias cromis* in Araruama Lagoon, RJ, Brazil.

4. Discussion

In the present study, *P. cromis* with lower weight and length had lower hemoglobin concentrations. Similar results were obtained by Pandey and Pandey [16], Clarke [17] and Ranzani-Paiva and Godinho [18], who also observed a positive correlation between hemoglobin concentration and the length and age of *Micropterus salmoides*, *Cyprinus carpio*, *Prochilodus lineatus*, and *Rita rita,* respectively. Moreover, Ranzani-Paiva et al [19] reported that, in *Piaractus mesopotamicus,*hemoglobin concentration is higher in older individuals. According to Tavares-Dias and Moraes [20] the young fish are smaller in size and have the same amount of hemoglobin per erythrocyte than adult fish, what conducts to higher values in juveniles. Juveniles also have a greater number of erythrocytes and erythropoietic activity than adults. Probably because individuals of different sizes release energy in different amounts according to their body size and this can thus interfere with the hematological framework.

Similar to length, weight gain can also be influenced by environmental and nutritional factors. The quantity and quality of food, for example, can have significant effects on the physiology of fish, and thus interfere with the hematologic variables [20]. Furthermore, hematologic values may be influenced not only by growth but also by the ecophysiological conditions [4]. Tavares-dias et al [21, 22] found a positive correlation between TW and the concentration of hemoglobin in *Piaractus mesopotamicus* and *Oreochromis mossambicus*. However, Gaviria and Pérez [23] did failed to observe a correlation for these variables in *Mugil curema*.

The fact that basophils were not observed in the examined specimens is consistent with the study by Campbell [13], who noted that these cells are rare in the blood of fish.

The presence of granulocytes (neutrophils and eosinophils), lymphocytes, and monocytes, indicate that *P. cromis* has a developed immune system [24] and could corroborate the future use of hematology for the health monitoring of this Lagoon system.

The nonlinear trend of neutrophils in relation to the condition factor (K), where the highest values of neutrophils lie between the extremes of K, can be explained by the strong relationship between body condition of the individual and the full operation of its immune system [24].

The nonlinear positive correlation of neutrophils with hemoglobin can be explained by the leukocyte function of neutrophils. These comprise the first line of defense in acute inflammatory processes and are produced under conditions such as reduced bioavailability of iron, a major component of hemoglobin [14].

Thrombocytes are rarely observed in samples where there is no response to the antigenic stimulation; in addition, there are reports that they have other functions such as phagocytosis and reduce the likelihood of infections [21; 20]. The increased number of thrombocytes and decreased levels of lymphocytes, may be due to an increased frequency of lymphocytes compared to leukocytes, as seen in the trend of higher levels of lymphocytes associated with increased leukocyte count. These relationships are evident from the calculations of specific leukocyte counts.

The mean values of *P. cromis* erythrocytes were smaller than for the marine fish found off the southern coast of Brazil by Filho et al [25]. According to Tavares-Dias and Moraes [20] lower hematocrit values occur in benthic fish, which explains the lower values observed in this study for the black drum, since it is characterized by demersal and benthic habitats.

The red blood cell count indices can be used to control diseases and stress in an animal, whatever the cause, and further demonstrate the physiological state of the animal (26, 27). The low erythrocyte counting and high hematocrit can suggest that black drum has changed its erythrocyte volume due to environmental stress. Compared to Rodrigues [28] and Seriani et al [29], specimens of black drum in Araruama Lagoon, showed lower values of red blood cell count than the values described for another fish species from the same taxonomic family (Scianidae), *Micropogonias furnieri* collected in Guanabara and Ribeira bays, Rio de Janeiro State, Brazil, and in the estuary of Itanagar, São Paulo, Brazil. At the same way, *P. cromis* showed higher hematocrit and mean corpuscular volume than *M. furnieri* (Table 2). Since there was no access to previous published data that described *P. cromis* hematology, the comparison with a species of the same taxonomic family is the closest evaluation that could be done to understand how hematological parameters were expected to range for this fish species.

Table 2 Mean and standard error of hematological values obtained in different coastal areas in southeastern Brazil for *Micropogonias furnieri*. Values in parentheses = number of samples.

	Guanabara Bay, RJ, Rodrigues et al. (28)	**Ribeira Bay, RJ, Rodrigues et al. (28)**	**Estuary of Itanhaém, SP, Seriani et al. (29)**	**Estuary of Santos, SP, Seriani et al. (29)**
H $10^6/mm^3$	2.65±0.6 (2)	2.57±0.2 (5)	-	-
Ht %	18.0±2.8 (2)	26.5±7.1 (6)	32.6±3.8 (13)	39.1±2.3 (11)
MCV fL	68.5±5.0 (2)	100.8±24.7 (5)	113.0±51.7 (13)	110.7±47.6 (11)
TPP g/dL	4.0±0.6 (2)	4.4±0.5 (6)	-	-
L $10^3/mm^3$	375.0±190.9 (2)	236.0±166.2 (5)	-	-

H=blood cells; Ht= hematocrit; MCV= mean corpuscular volume; TPP= Total plasma protein; L= Global leukometry

The red blood cells carry oxygen and carbon dioxide by hemoglobin and hematocrit, hemoglobin concentration and the total erythrocyte count can be used as indicators of the ability to transport oxygen, suggesting a proxy for the available oxygen concentration in the original habitat of the animal. The solubility of oxygen in water decreases as salinity increases; therefore, to meet its demand for oxygen, the fish in the Araruama Lagoon need high hemoglobin levels to survive in a hypersaline ecosystem. High values for the total plasmatic protein (TPP) (6.3 ± 0.1 g/dL) of the black drum collected in Araruama Lagoon suggest an osmotic imbalance, which could be due to the stress of the environmental. However, it should be noted that there are no normal values established for the species. Furthermore, similar values described in this study were found for other species, as for specimens of catfish *Genidens genidens* of Ribeira Bay (5.3 ± 1.2 g/dL) and Guanabara Bay (6.0 ± 1, 0 g / dL) [30]. The total leukometry was on average lower (339.2 ± 28.1 .$10^3/mm^3$) than that found for *M. furnieri* in Guanabara and Ribeira bays (Table 2), with a prevalence of lymphocytes, indicating the absence of inflammatory processes or infection in specimens evaluated.

The serum activities of ALT and AST enzymes are used as indicators of liver dysfunction. The activities of these enzymes may be increased in severe hepatocellular diseases [13]. A black drum in Araruama Lagoon had relatively high ALT and AST values of 90 ± 0.7 and 229 ± 4.5 U/L, respectively, when compared with data from *Herichthys steindachneri* (55.3 ± 37.4 and 105.6 ± 56.1, respectively) in Ribeira Bay, Rio de Janeiro [28], which suggests possible liver dysfunction in this species in this ecosystem, or even a natural difference between species metabolism.

Regarding creatinine, black drum showed lower values than those found in *G. genidens* (0.5 ± 0.9 mg/dL) and *Hypostomus* sp. (0.3 ± 0.2 mg/dL) in Guanabara Bay, Rio de Janeiro [30]. The measurement of creatinine in blood plasma of fish can help diagnose kidney-related diseases, since creatinine is formed from creatine, which is secreted by the kidneys. Approximately 50% of the waste nitrogen excreted through the kidneys is in the form of creatinine [13]. Taking into consideration that the values of creatinine should not be too high for healthy fish, the values found showed no kidney problems.

In general, the values obtained for urea in black drum in Araruama Lagoon were high (24.7 ± 0.6 mg/dL), when compared to data for urea reported for *G. genidens* (13.6 ± 6.3 mg/dL) and *Hypostomus* sp. (20.8 ± 9.2 mg/dL) in Guanabara Bay, Rio de Janeiro [30]. An increase in urea concentration above 5 mg/dL may indicate fish gill epithelial disease, since fish gills appear to important in the excretion of urea from the kidneys [13].

5. Conclusion

Although there are few references concerning about standards or normality values for hematological parameters and for serum biochemistry of *P. cromis* and other tropical fish species, the results found in the present work suggested a normal range of the species in the Lagoon. However, it's important to continue this work in order to investigate if the values described here reflect a normality scenario for the species. Additionally it's important to point out that hematological researches are extremely necessary to provide data for establish a reference standard of this and other fish species. This way fish species and their hematology could be a useful tool for evaluation of environmental quality and of the fish health. Therefore fishes could be used as bioindicators health for environmental risk assessments.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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