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HEMATOLOGICAL CHARACTERISTICS OF THE CURIMBATÁ, *Prochilodus scrofa* STEINDACHNER, 1881 (OSTHEICHTHYES, CHARACIFORMES, PROCHILODONTIDAE), STOCKED IN EXPERIMENTAL CONDITIONS.

Características hematológicas do Curimbatá, *Prochilodus scrofa* Steindachner, 1881 (Osteichthyes, Characiformes, Prochilodontidae) cultivado em condições experimentais.

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RESUMO

O presente trabalho foi desenvolvido com o objetivo de identificar as características hematológicas do curimbatá, *Prochilodus scrofa*, uma espécie brasileira de água doce. Os exemplares foram obtidos através de reprodução induzida (usando-se Pregnyl – HCG) e mantidos em tanques na densidade de 2 peixes/m^2. As amostras foram feitas quando os indivíduos tinham a idade de 3 meses, 1 ano e 4 meses, 2 anos e 2 anos e 3 meses. O sangue foi retirado através de punção da artéria caudal e utilizado para as análises de: taxa de hemoglobina (g/100ml), contagem do número de eritrócitos (10^6/mm^3), hematócrito (%) e contagem diferencial dos leucócitos. Foram, também, calculados os índices hematimétricos: volume corpuscular médio (VCM), hemoglobina corpuscular média (HCM) e concentração de hemoglobina corpuscular média (CHCM).

ABSTRACT

The purpose of this study was to investigate the hematological characteristics of the Brazilian freshwater fish curimbatá, *Prochilodus scrofa*. The animals were obtained by hormone induced reproduction (using Pregnyl – HCG) and bred in stocking tanks with two animals per square meter. Samples were taken when the specimens reached the ages of: 3 months; 1 year and 4 months; 2 years and 2 years and 3 months. Blood colected from the caudal artery was used to determine: hemoglobin concentration (g/100ml), number of erytrocytes (10^6/mm^3), hematocrit (%), and differential leukocyte count. The following indices were calculated: MCV (mean corpuscular volume) MCH (mean corpuscular hemoglobin) and CMHC (concentration of mean corpuscular hemoglobin).

1. INTRODUCTION

The curimbatá, *Prochilodus scrofa*, belongs to the family Prochilodontidae and is widely distributed throughout the Southwest of Brazil and in Paraguay (FOWLER, 1951). It accounts for as much as 60% of fish production in the Mogi-Guaçu River (21°58'S - 47°26'W). It is a highly appreciated species because of the quality of its meat and the size it attains and hence shows great potential for aquiculture (LEITE et alii, 1984 a and b).

The hematological characteristics of this species were first studied by RANZANI-PAIVA (1981) with the aim of investigating the curimbatá's blood makeup in its natural habitat (river).

This paper sets out to certify possible changes in the blood composition of specimens of *Prochilodus scrofa* in differente stages of gonadal maturity, obtained by induced reproduction and bred in experimental stocking tanks, in order to compare them with obtained datas for the same species, in their natural environment.

2. MATERIALS AND METHODS

Specimens of *Prochilodus scrofa* were obtained by hormoneinduced reproduction (using Pregnyl-HCG) (GODINHO et alii, 1984) conducted at "Laboratório de Biologia de Peixes Fluviais" in Pirassununga, Brazil. Fifty 3-month-old individuals, measuring 15.7 cm on average and weighing 45,59 g on average, were stocked in tanks

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at a density of 2 animals per square meter. Natural food in the form of plankton was supplemented with a ration containing 28% raw protein. The food was administered daily at a proportion of 4% of the total live weight of the specimens. Water temperature were recorded three times a day (8, 12 and 16 hours).

Samples amounting to 20% of the original batch were taken at the start of the experiment, when individuals were three months old (March 1982); later samples were taken at 1 year and 4 months (April 1983), two years (December 1983) and 2 year and 3 months (March 1984).

For blood collection purposes, the fish were kept in aquaria for three days. After anesthetizing them with chlorobutanol, blood was collected from the caudal artery using heparinized disposable syringes.

The following analyses were performed:

– Determination of hemoglobin concentration (Hb) by the cyanmethemoglobin method, according to COLLIER (1944) and with the use of a Spectronic 20 spectrophotometer (Bausch Lomb);

– red cell count or count of total number of erythrocytes (Er), with blood diluted at 1:200 in Hayem fluid in a red-cell pipette and using an "improved" Neubauer hemocytometer, counting cells in both reticules and calculating the average;

– packed cell volume (hematocrit – Ht) was estimated using the microhematocrit technique (GOLDENFARB et alii., 1971): analyses were performed in duplicate and the average calculated;

– differential count of leuckocytes and erythroblasts in smears stained by the method given in ROSENFELD (1947).

Results for hemoglobin concentration, number of erythrocytes and hematocrit were used to estimate the following absolute hematimetric indices: MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration), in accordance with formulas used by WINTROBE (1934).

After blood collection, the animals were sacrificed and biometric analyse were performed. Total and standard length was noted in centimeters, and total weight in grams. Laparotomy was conducted to determine sex and gonadal maturation status using scales provided by NIKOLSKY (1963), GODINHO (1972) and GODINHO et alii (1974). The gonad was removed and weighed (in grams) in order to calculate the gonado-somatic index (GSI).

All results were analyzed by calculating means, standard deviations and variation coefficients for each sample and for each of the items studied.

3. RESULTS

A total of 39 individuals of the species curimbatá, *Prochilodus scrofa*, was used for this study between March 1982 and March 1984.

During the period, the fish presented a total length range of 13.9 to 35.5 centimeters and a weight range of 27.66 to 534.25 grams. At the start of the experiment the specimens had gonads in young stage, while later samplings showed later stages of gonad development, i.e. Resting (I and II) and Maturation. No individuals were found to have gonads in the Mature and Spent stages.

Analysis of peripheral blood in *Prochilodus scrofa* under conditions of culture showed that the cells found were the same as those described for the species under natural conditions (RANZANI-PAIVA & GODINHO, 1983).

In almost all analysis (FIGURE 1, TABLE 1) there was a drop in values calculated for the second and fourth samples, i.e. for individuals in gonadal Resting, except for the MCHC which has ascending values until the third sample, i.e. for Young individuals and individuals in Resting I and Maturation, but a slight decline in the fourth sample, when fish had gonads entering the Resting II stage of the second reproductive cycle. The same figure also permits the observation of variation in mean GSI values. It is worth highlighting the peak value in the third sample, i.e. in individuals with gonads in Maturation.

FIGURE 1 – Mean value per sample of hemoglobin rates, nº of erythrocytes, hematocrit, MCV, MCHC and GSI in *Prochilodus scrofa*.

TABLE 1

Mean values and standard deviations for red series, total length, total weight and GSI of *Prochilodus scrofa* bred in captivity

Sample	Lt (cm)	Wt (g)	GSI	Hb	Er	Ht	MCV	MCH	MCHC
Young									
Ax	13.9 – 17.5	27.66 – 60.68	0.0049 – 0.011	9.3 – 13.2	1.585 – 2.620	39.5 – 46.0	164.12 – 383.91	40.08 – 65.44	21.11 – 27.11
$\bar{x} \pm s$			0.012 ± 0.01	10.56 ± 0.95	2.147 ± 0.35	44.05 ± 1.98	210.96 ± 41.03	50.31 ± 8.83	23.98 ± 1.91
v.c.			83.33	8.71	16.50	4.49	19.45	17.55	7.96
Resting I									
Ax	23.5 – 29.4	151.88 – 301.78	0.0067 – 0.331	5.6 – 11.1	1.615 – 2.220	27.0 – 38.0	139.53 – 206.52	34.67 – 50.00	20.74 – 34.69
$\bar{x} \pm s$			0.152 ± 0.14	8.44 ± 2.06	1.977 ± 0.24	32.42 ± 3.9	165.40 ± 23.85	41.65 ± 5.74	26.79 ± 5.24
v.c.			92.10	24.41	12.14	12.03	14.42	13.78	19.51
Maturation									
Ax	27.4 – 35.5	211.65 – 534.24	0.473 – 17.349	8.9 – 13.4	1.735 – 2.650	33.0 – 45.0	143.40 – 202.70	43.84 – 58.96	26.28 – 33.95
$\bar{x} \pm s$			6.27 ± 6.65	11.29 ± 1.53	2.180 ± 0.27	38.6 ± 3.48	178.66 ± 19.39	51.85 ± 4.05	29.23 ± 2.92
v.c.			106.06	13.55	12.38	9.01	10.85	7.81	9.99
Resting II									
Ax	26.2 – 30.7	188.57 – 337.11	0.009 – 0.455	7.3 – 10.6	1.715 – 2.545	27.0 – 37.0	140.62 – 183.06	38.02 – 56.28	25.15 – 30.75
$\bar{x} \pm s$			0.12 ± 0.17	9.46 ± 1.10	2.074 ± 0.24	33.55 ± 2.98	162.29 ± 14.22	45.86 ± 5.26	28.16 ± 1.60
v.c.			141.67	11.63	11.57	8.88	8.46	11.47	5.68

Ax = range of variable
$\bar{x} \pm s$ = mean ± standard deviation
v.c. = variation coefficient
Lt = total length
Wt = total weight
GSI = gonado somatic Index
Hb = hemoglobin (g/100ml)
Er = nº of erythrocytes (10^6/mm³)
Ht = hematocrit (%)
MCV = mean corpuscular volume (μ^3)
MCH = mean corpuscular hemoglobin ($\mu\mu g$)
MCHC = mean corpuscular hemoglobin concentration (%)

TABLE 2
Mean values and standard deviations for differential counts of leukocytes and erythroblasts in *Prochilodus scrofa* bred in captivity

Sample	Lymphocyte	Monocyte	Neutrophil	Basophil	Erythroblast
Young					
Ax	19.93 – 87.83	0.99 – 18.50	0.49 – 45.91	0.0 – 0.0	1.66 – 85.33
$\bar{x} \pm s$	49.25 ± 28.49	5.26 ± 6.18	18.57 ± 18.07	0.0 ± 0.0	26.92 ± 27.47
v.c.	57.85	117.49	97.31	0.0	102.04
Resting I					
Ax	10.50 – 85.93	11.11 – 82.05	2.95 – 12.25	0.0 – 0.0	0.0 – 9.25
$\bar{x} \pm s$	32.63 ± 36.01	6.12 ± 4.38	58.76 ± 32.29	0.0 ± 0.0	2.48 ± 4.52
v.c.	110.36	71.57	54.95	0.0	182.25
Maturation					
Ax	8.46 – 59.30	6.10 – 20.53	29.65 – 77.11	0.0 – 0.49	0.0 – 4.39
$\bar{x} \pm s$	25.14 ± 16.21	12.79 ± 5.94	59.69 ± 17.85	0.12 ± 0.22	1.73 ± 1.55
v.c.	64.48	46.44	29.90	183.33	89.59
Resting II					
Ax	7.81 – 64.68	1.50 – 38.85	15.92 – 87.00	0.0 – 0.0	0.0 – 17.00
$\bar{x} \pm s$	35.49 ± 18.94	5.73 ± 3.84	53.54 ± 24.57	0.0 ± 0.0	5.24 ± 5.17
v.c.	53.37	62.02	45.89	0.0	98.66

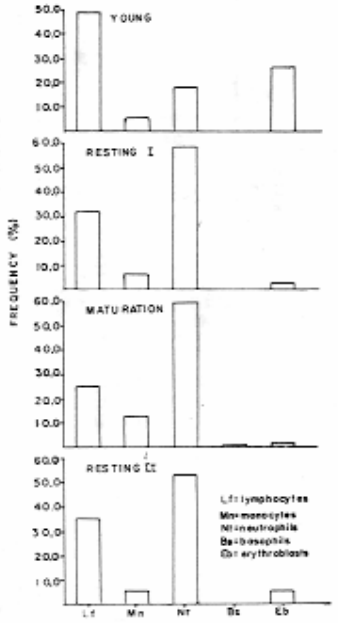

FIGURE 2 – Frequency distribution of leucocytes, by gonadal maturation stage in *Prochilodus scrofa*.

In the histogram there is an evident prevalence of lymphocytes and neutrophiles in almost all individuals, except for those in the Young stage, where a large percentage of erythroblasts can be observed. (FIGURE 2, TABLE 2).

Mean temperature was 24.7°C during the months when the first and second collections were made. It was 26.9°C during the third, and 27.3°C during the fourth.

4. DISCUSSION

Early work on the hematology of *Prochilodus scrofa* was conducted with individuals captured in their natural habitat, i.e. in rivers (RANZANI-PAIVA & GODINHO, 1983; RANZANI-PAIVA & GODINHO, 1985), considering time of year, sex and stage of gonad maturation. According to these studies, river fish presented no significant differences in terms of the mean values for blood characteristics between males and females, although means were slightly higher for males. No such differences were detected in fish bred in captivity. CLARK et alii (1979) also failed to find significant differences between the sexes for *Micropterus salmoides*. EZZAT et alii

(1974) found higher numbers of erythrocytes for males in *Tilapia zilli* and higher values still for the reproduction period.

If mean values for the hematological characteristics considered are compared, and considering the gonad maturation stage of fish captured in rivers and those bred in captivity, some differences can be found: river specimens almost always had higher values than bred specimens. This may be due to the fact that bred specimens were smaller and younger than river specimens, as well as to the fact that they were fed with artificial feed. It is also true, however, that these fish were found to be in excellent zootechnical condition.

The hemoglobin rates for *Prochilodus scrofa* was lower than for *Plecostomus albopunctatus* and *Rhamdia hilarii*, studied by KAVAMOTO et alii (1983a and b) while the hematocrit rate for *Prochilodus scrofa* was higer than both species.

Differential leukocyte counts are very important for determining the hematological characteristics of fish, as they are in human and veterinary medicine. Owing to the highly varied cell shapes and the relative differences in leukocyte counts, however, it is impossible to reach a definit conclusion even for a single species, as variation from one individual to another is enormous.

RHYZOVA & KUPCHINSKIY (1984) compared *Abramis brama orientalis*, Cyprinidae fish from two environments, lakes and reservoirs. They failed to find significant differences in leukocyte composition. During the spawning period they found that these counts changed but there was no relation with any pathological problems.

The only fact observed with regard to *Prochilodus scrofa* in this respect was a higher percentage of lymphocytes in bred fish.

For some other species, lymphocytes are also more frequent, as found by PITOMBEIRA et alii (1968) for *Opisthonema oglinum*, by PITOMBEIRA (1972) for *Astronotus ocellatus*, Mac CARTHY et alii (1973) for trout, *Salmo gairdneri*, BLAXHALL & DAISLEY (1973) for *Salmo trutta*, RIBEIRO (1978) for *Pimelodus maculatus* and KAVAMOTO et alii (1983a) for *Rhamdia hilarii*.

For *Prochilodus scrofa*, however, it is important to note that a high percentage of neutrophiles was found, specially in adults, both in river specimens and specimens bred in captivity.

The basophile is a rare cell, found in few fish species. It has been found however by Mc KNIGHT (1966) in *Prosopium williamsoni*, PITOMBEIRA et alii (1968) in *Opisthonema oglinum*, PITOMBEIRA (1972) in *Astronotus ocellatus*, EZZAT et alii (1974) in *Tilapia zilli*, KAVAMOTO et alii (1983a) in *Rhamdia hilarii*, and RANZANI-PAIVA & GODINHO (1983) in *Prochilodus scrofa* from rivers (but only in very small percentages in bred individuals of this species).

In both bred and river specimens, a large number of erythroblasts were found and counted among leukocytes. In individuals bred in captivity, the highest frequency of this cell type was noted in individuals in Maturation.

5. CONCLUSIONS

1 — No significative differences between males and females were observed.

2 — Individuals kept in captivity showed values of blood analisys lower than individuals from natural environment.

3 — Lymphocit was the most frequent cell in blood smears.

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