HEMATOLOGICAL PARAMETERS OF "CACHARA", *Pseudoplatystoma fasciatum* LINNAEUS, 1766 (OSTEICHTHYES, PIMELODIDAE), REARED IN CAPTIVITY

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ABSTRACT

This study was carried out with *Pseudoplatystoma fasciatum* ("cachara") reared at PDSA - APTA, Pariquera-Açu, São Paulo, Brazil, stocked in eight 200 m² ponds, from January 1999 to February 2001, totalizing 190 fishes (71 males, 99 females and 20 indeterminated). The total weight (138.5 to 2430.0 g) and total length (29.5 to 79.0cm) were recorded. The sex and the gonadal stages (Juvenile, Resting, Maturation, Final Maturation, Regression) were also identified. Blood was collected to determine the hematocrit (mean value: 28.0±0.6%), hemoglobin level (mean value: 6.4±0.2 g/100 mL) and red blood cell (mean value: 224.0±6.0 10^4/mm³). Mean corpuscular volume (MCV) (mean value: 131.0±2.3 µm³), mean corpuscular hemoglobin (MCH) (mean value: 26.6±1.0 ppg) and mean corpuscular hemoglobin concentration (MCHC) (mean value: 19.8±0.7%) were calculated. There was difference between males and females, and a slight increase in the mean values of the hematological variables with gonadal maturation was registered. Differences among fish according to the several classes of total length were also verified.

Key words: *Pseudoplatystoma fasciatum*; "cachara"; fish; hematology; reproductive cycle

PARÂMETROS HEMATOLÓGICOS DE CACHARA, *Pseudoplatystoma fasciatum* LINNAEUS, 1766 (OSTEICHTHYES, PIMELODIDAE), CRIADO EM CATIVEIRO

RESUMO

Foram analisados 190 exemplares jovens e adultos de cachara, *Pseudoplatystoma fasciatum*, em Pariquera-Açu, SP, entre janeiro de 1999 e fevereiro de 2001, estocados em viveiros de 200 m² cada um. De cada exemplar foram anotados dados de peso total (g) e comprimento total (cm) e identificados os sexos e os estádios de maturação gonadal (Jovem, Repouso, Maturação, Maturação Final e Regressão), sendo 71 machos, 99 fêmeas e 20 indeterminados. O peso e o comprimento total variaram de 138,5 a 2430,0 g e de 29,5 a 79 cm, respectivamente. Amostras de sangue foram coletadas e utilizadas na determinação de hematócrito (média: 28,0±0,6%), taxa de hemoglobina (média: 6,4±0,2 g/100 mL) e contagem total de células (média: 224,0±6,0 10^4/mm³). Foram calculados: volume corpuscular médio (VCM) (média: 131,0±2,3 µm³), hemoglobina corpuscular média (HCM) (média: 26,6 ±1,0 ppg) e concentração de hemoglobina corpuscular média (CHCM) (média: 19,8±0,7%). Verificaram-se diferenças entre os valores hematológicos determinados para os sexos e pequena elevação dos mesmos com o avanço da maturação gonadal. Foi também encontrada diferença entre peixes de diferentes classes de comprimento total.

Palavras-chave: *Pseudoplatystoma fasciatum*; cachara; peixe; hematologia; ciclo reprodutivo

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INTRODUCTION

In Brazil, aquaculture has been developed over the last few years at a rate of around 30% a year (FAO, 1998). One of the native species that has an excellent potential for culture is the "cachara", *Pseudoplatystoma fasciatum*, belonging to the neotropical Siluriformes (IBGE, 1988; MIRANDA, 1997). This wild catfish performs long reproductive migrations, exhibiting total spawning (RESENDE *et al.*, 1995) when in captivity. It presents attractive zootechnical and market characteristics (ROMAGOSA *et al.*, 2003a).

On the other hand, the intensification of fish farming represents a challenge for the health of the animals. The possibility of illness during the rearing process affects the growth and survival of the fish (PAVANELLI *et al.*, 1998). Some physical signs, such as loss of appetite, behavioral changes, hemorrhage, abnormal body coloration and decrease of motility, can be indicators of illness in the animals. One of the tools to evaluate the health condition of the fish is the use of hematological exams (HENDRICKS, 1952; HESSER, 1960; SRIVASTAVA, 1968; RANZANI-PAIVA and GODINHO, 1985; RANZANI-PAIVA, 1995) in both wild and captive specimens.

Considering that the hematological parameters are susceptible to alterations according to changes of the aquatic environment, they can help to understand the process of adaptation of the animals to their environment (RUUD, 1954) and to obtain some reference that would allow to identify any modification in the health of the individuals. These aspects could help in the intensive and semi-intensive fish culture (RANZANI-PAIVA *et al.*, 1997).

The objective of this paper was to identify the hematological status of "cachara", *Pseudoplatystoma fasciatum*, reared in captivity, and to relate it to sex, gonadal maturation and total length. The correlation among the hematological and biological parameters was also verified. Assuming that these parameters are considered "normal" for the species, they can be used as reference in a future study with parasitized fish.

MATERIAL AND METHODS

Four hundred specimens of "cachara", *Pseudoplatystoma fasciatum*, were acquired from a private fish farm and kept at the Fishery Department of "Pólo de Desenvolvimento Sustentável dos Agronegócios (PDSA) - APTA", in Pariquera-Açu City, São Paulo State, Brazil (24º43' S and 47º53' W). The animals were stocked in eight 200 m² earthen ponds (50 fish in each one) and were fed with 40% crude protein ration, daily and twice a day. Table 1 shows the number of fish collected during the total period of samplings. The total weight of the fish ranged from 138.5 to 2430.0 g and the total length, from 29.5 to 79.0 centimeters.

Table 1. Months of sampling and number of specimens of "cachara", *Pseudoplatystoma. fasciatum*, captured for the hematological analysis

Month	*Number of fish*
January/1999	20
March/1999	20
October/1999	20
December/1999	20
February/2000	20
April/2000	20
July/2000 *	10
September/2000	20
November/2000	20
February/2001	20
Total	**190**

* *Water temperature below 21 ^{0}C*

The maximum and minimum water temperatures were daily measured.

The fish were captured 24 hours before sampling was done and kept in the laboratory in 500 L tanks, in order to minimize the stress. They were then anesthetized (2 g benzocaine, 150 mL alcohol 96 °GL) and 2 mL of blood were withdrawn by caudal puncture, using disposable heparinized syringes and needles. The samples were used to determine: hematocrit (Ht), by the microhematocrit method (GOLDENFARB *et al.*, 1971); hemoglobin level (Hb), by the cyanometahemoglobin method (COLLIER, 1944); total cell count, performed in a Neubauer chamber, using Hayem's diluent; hematimetric indexes, according to WINTROBE (1934): mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

After the blood was collected, the fish were measured (Lt=total length, in centimeters) and weighed (Wt=total weight, in grams), and then, decapitated in order to have their gonads removed for macroscopic inspection. Sex and stage of gonad maturation (Juvenile, Resting, Maturation, Final Maturation and Regression) were identified, following the method used by ROMAGOSA *et al.* (2003a, b).

The mean values, standard deviations and confidence intervals for each hematological evaluation were calculated considering the sex, stage of gonad maturation and class of total length. The results obtained were studied through graphic interpretation (RANZANI-PAIVA, 1995) and the differences were established using the analysis of variation (ANOVA). The relationship between the hematological parameters and the biological variables was tested in pairs, estimating the Pearson coefficient for linear correlation (r) by the least-squares method. The confidence intervals were calculated considering $P \leq 0.05$.

RESULTS AND DISCUSSION

A total of 71 males, 99 females and 20 indeterminate specimens of "cachara", *Pseudoplatystoma fasciatum,* were analyzed. In this species, the macroscopical differentiation between sexes is not easily perceptible in individuals with total length between 29.5 and 39.4 centimeters.

ROBERTS (1981) verified that lordosis, scoliosis, fin damage, corporal lesion, hemorrhagy, organs and tissues with abnormal coloration and even mortality may be caused by nutritional deficiency or parasites. However, in the present study, no signal of disease was observed.

During the study, the water temperature values ranged from 17.0 to 32.8 °C.

Comparing the hematological data obtained in this study (Table 2) with those registered in other siluriforms, it was verified that they were similar to the values obtained by VAL *et al.* (1985) with *Hypostomus regani* and by KAVAMOTO *et al.* (1983) with *Rhamdia hilarii*, both fish from the wild, and by BEELEN *et al.* (1998) with *Pseudoplatystoma coruscans* in captivity. It has been demonstrated that the range of different results for the hematological parameters of fish may be associated with individual or environmental factors (RANZANI-PAIVA, 1995).

The mean values of Ht, Hb, RBC and MCHC were significantly different between the sexes (males exhibiting higher values than females and indeterminates). The Ht was higher in males, but MCV was lower (Table 2 and Figure 1). These results corroborate those described for *Salvelinus fontinalis* (CHRISTENSEN *et al.*, 1978), *Prochilodus scrofa* (RANZANI-PAIVA and GODINHO, 1985), *Brycon* sp (PAIVA, 1991) and *Mugil platanus* (RANZANI-PAIVA, 1995).

GRAY (1946), KLAWE *et al.* (1963), EISLER (1965), GLAZOVA (1976) and RANZANI-PAIVA (1995) noticed a positive relationship between hemoglobin level and the activity of the fish in its natural environment (Hb is higher in animals with greater muscular activity, as that which occurs in the migration for instance). In this study, the average value of Hb for *P. fasciatum* was relatively low (6.4 g/100 mL). However, since the specimens used in the present study had been reared in captivity, where the energy expense is probably lower than that in the wild, it would be acceptable to consider the low value of hemoglobin level as a normal one.

VAL *et al.* (1985) verified that there was no significative difference between the Hb mean levels for *Hypostomus regani* caught in both lotic and lentic environments. Those authors postulated that the differences were, probably, due more to abiotic factors, such as pH, dissolved CO_2 and O_2, than to the condition of the fish itself, when kept in confinement. On the other hand, LUSKOVÁ (1997) affirmed that the Hb content is linked with the different proportions of erythrocytes showing several degrees of ripeness. Imature erythrocytes contain less hemoglobin than the older and ripe ones. Once in the blood circulation, the erythrocytes should start synthesizing hemoglobin, which complicates the definition of the normal condition, and, probably, even the photoperiod may influence this process.

In figure 1, a slight raise in the mean values of Hb, RBC and MCHC is observed, in relation to the reproductive cycle, for males and females. Similar results were found for *Prochilodus scrofa* and *Brycon* sp, by RANZANI-PAIVA and GODINHO (1985) and PAIVA (1991), respectively, who observed a small raise in the hematological variables in relation to the reproductive cycle. However, in this study significant lower values of RBC were registered in females (Table 2).

Among the biological manifestations, spawning appears to be the most important event changing the animal homeostasis, which is reflected in the hematological parameters (LUSKOVÁ, 1997). In this study, a more detailed analysis of the hematimetric parameters along the reproductive cycle was harmed by the low number of males at final maturation and regression and also by the total absence of females at the stage of regression (Figure 1). Therefore, the confidence intervals included the entire variation range of the hematological parameters.

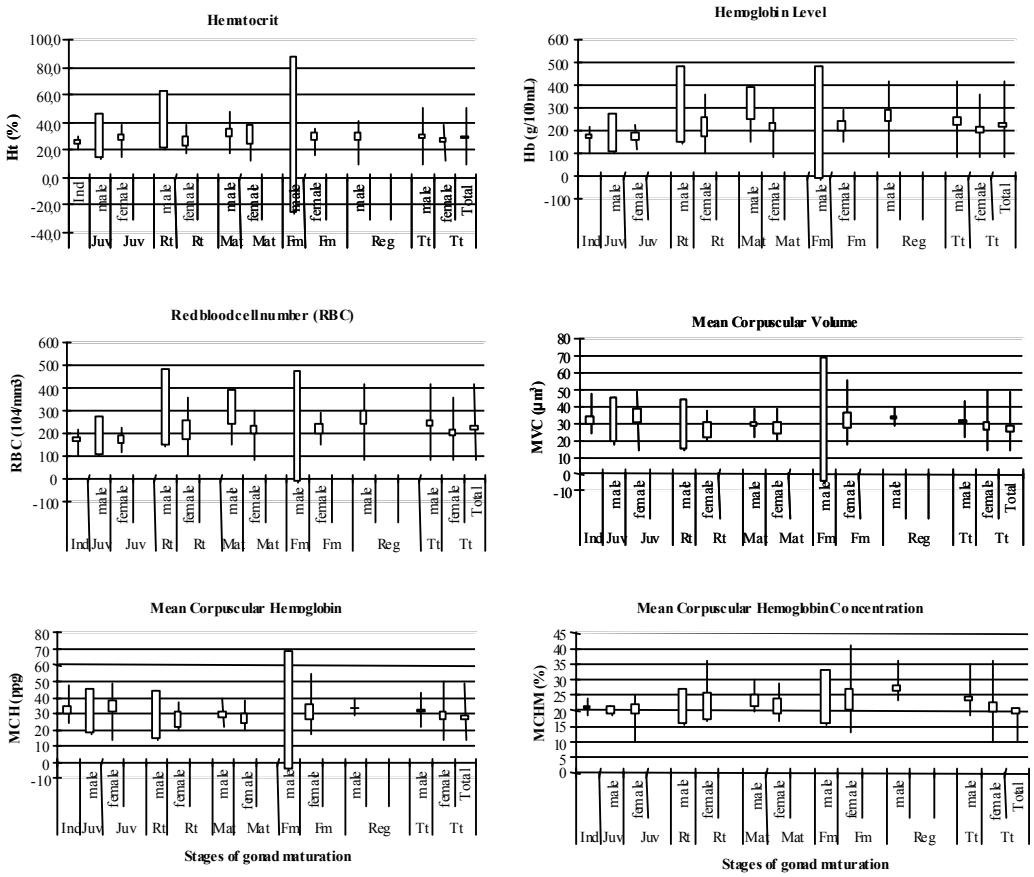

Figure 1. Variation range, means, and confidence intervals of the hematological parameters of "cachara", *Pseudoplatystoma fasciatum,* reared in capitivity (Ind=indeterminated; Juv=juvenile; Rt=resting; Mat=maturation; Fm=final maturation; Reg=regression)

The absolute hematimetric indexes are used to assess the size of the red cells and the content of hemoglobin inside them. Through those indexes it is possible to evaluate and classify morphologically the blood of the animals, characterizing some anemia that may occur. Examining such indexes in *P. fasciatum*, the lowest mean values of MCV were observed as gonad maturation progressed, with overlap of all the confidence intervals. The same was observed in *Brycon* sp (PAIVA, 1991), through the decrease of MCV at the time of reproduction.

In order to check if the differences between the means of the hematological parameters of indeterminate sex individuals and of the adults were due to sexual differentiation or to ontogenetic development, analyses according to class of total length were carried out. In table 3 it is possible to see that all the hematological parameters showed significantly different values among the classes of total length. As the length raised, Ht value increased, probably due to the raise of RBC and MCV. The largest specimens (class 70.0 - 79.9 cm) presented the lowest values of Hb, MCV, MCH and MCHC. RANZANI-PAIVA (1995) demonstrated increase of the hematological parameters related to the raise in total length in mullet, *Mugil platanus*. According to Joshi and Tandon (1977), *apud* AMADIO (1985), the hematological variables tend to augment as the fish grows, to fill the increasing metabolic needs, stabilizing when the animal becomes older, larger and less active, with lower respiratory demand.

Since erythrocyte count and hemoglobin level cannot be quickly determined in the field or at the fish facilities, a lot of effort has been made to establish relationships between such parameters and Ht, which is simpler, faster and more feasible in the field. Those relationships are shown in table 4, where a high correlation between the pairs Wt-Lt, Ht-Hb, Ht-RBC and Hb-RBC can be verified. Relationships between those variables were also found by KAVAMOTO *et al.* (1985), with *Plecostomus albopunctatus*, RANZANI-PAIVA and GODINHO (1985), with *Prochilodus scrofa*, TAVARES-DIAS *et al.* (1998) and TAVARES-DIAS and SANDRIM (1998), with *Colossoma macropomum*, and TAVARES-DIAS *et al.* (1999), with *Piaractus mesopotamicus*.

Table 2. Variation range (Aẋ), means values (ẋ), standard deviation of the means (Sẋ) and confidence intervals (Iẋ) of the values of the hematological parameters in "cachara", *Pseudoplatystoma fasciatum*, according to the sex

PARAMETER		SEX			Total	P
		Indeterminate	Male	Female		
		n=20	n=71	n=99	n=190	
Ht (%)	Aẋ	20.0 – 30.5	9.0 – 50.0	12.0 – 38.5	9.0 – 50.0	< 0.0001 *
	ẋ	25.0	29.4	26.3	28.0	
	Sẋ	0.7	1.1	0.8	0.6	
	Iẋ	1.5	2.2	1.6	1.2	
Hb (g/100 mL)	Aẋ	4.2 - 6.1	2.6 – 13.2	2.8 - 10.0	2.6 - 13.2	< 0.0001 *
	ẋ	5.2	6.9	6.0	6.4	
	Sẋ	0.1	0.3	0.2	0.2	
	Iẋ	0.2	0.6	0.4	0.3	
RBC ($10^4/mm^3$)	Aẋ	103.0 - 215.5	81.0 – 415.5	86.5 - 353.5	81.0 - 415.5	< 0.0001*
	ẋ	169.7	239.2	200.7	224.0	
	Sẋ	7.3	9.6	7.7	6.0	
	Iẋ	15.3	19.2	15.4	11.9	
MCV (μm^3)	Aẋ	11.7 - 237.9	73.0 – 250.0	82.7 - 221.7	73.0 -250.0	< 0.0001 *
	ẋ	151.7	127.3	135.8	131.0	
	Sẋ	6.4	3.4	3.6	2.3	
	Iẋ	13.4	6.8	7.2	4.6	
MCH (ppg)	Aẋ	24.3 - 47.6	21.7 – 42.7	13.9 - 48.7	13.9 - 48.7	0.5886 NS
	ẋ	31.6	30.9	28.3	26.6	
	Sẋ	1.3	0.6	1.3	1.0	
	Iẋ	2.7	1.2	2.6	1.9	
MCHC (%)	Aẋ	18.5 - 23.9	18.5 – 35.1	9.8 - 36.4	9.8 - 36.4	0.0019 *
	ẋ	20.9	23.4	20.9	19.8	
	Sẋ	0.4	0.4	0.9	0.7	
	Iẋ	0.8	0.9	1.8	1.4	

Ht=hematocrit; Hb=hemoglobin level; RBC=erythrocyte count; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; n=number of specimens
NS=not significant **=significant: P ≤ 0.05*

Table 3. Mean values (ẋ) and standart error (SEM) of the hematological parameters of "cachara", *Psedoplatystoma fasciatum*, studied according to class of total length

Class of total length (cm) (n)		***Ht*** (%)	***Hb*** (g/100 mL)	***RBC*** ($10^4/mm^3$)	***MCV*** (μm^3)	***MCH*** (ppg)	***MCHC*** (%)
20.0 - 29.9 (1)		24.3	5.4	206.0	117.7	26.0	22.0
30.0 - 39.9 (22)	ẋ	24.9	5.1	167.0	152.2	31.5	20.7
	SEM	0.8	0.2	6.7	6.2	1.3	0.4
40.0 - 49.9 (32)	ẋ	30.1	6.5	211.4	146.9	32.3	21.7
	SEM	1.4	0.3	11.1	5.9	1.2	0.6
50.0 – 59.9 (83)	ẋ	28.1	7.0	240.9	120.0	30.5	24.7
	SEM	0.9	0.2	8.6	2.2	0.5	0.4
60.0 - 69.9 (30)	ẋ	26.6	6.0	215.5	125.4	29.1	23.1
	SEM	1.0	0.2	10.2	3.4	1.1	0.7
70.0 - 79.9 (2)	ẋ	26.5	4.9	244.8	107.5	20.1	18.9
	SEM	4.5	0.2	17.3	10.8	0.6	2.5
P		**0.094 NS**	**0.0007 ***	**0.0003 ***	**0.004 ***	**0.0133 ***	**< 0.0001 ***

* *significant* *NS=not significant* *numbers in brackets=n*

Table 4. Pearson coefficient correlation (r) values utilized to evaluate the hematological and biological parameters of "cachara", *Pseudoplatystoma fasciatum*

	Wt	*Lt*	*Ht*	*Hb*	*RBC*	*MCV*	*MCH*	*MCHC*
Wt	1							
Lt	**0.93514**	1						
Ht	- 0.05899	- 0.02196	1					
Hb	0.04628	0.11190	**0.84724**	1				
RBC	0.12699	0.20329	**0.76326**	**0.81306**	1			
MCV	- 0.19174	- 0.28259	- 0.00576	- 0.27116	- 0.28294	1		
MCH	- 0.21591	- 0.21967	- 0.01483	0.17737	- 0.39170	0.15904	1	
MCHC	0.21696	0.24519	- 0.22992	0.26396	0.02287	- 0.41258	0.35094	1

Wt=total weight (g); Lt=total length (cm); Ht=hematocrit (%); Hb=hemoglobin level (g/100 mL); RBC=erythrocyte count (10^4/mm^3); MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration
Bold values are significant: P ≤ 0.05

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