

# Association between the hematological characteristics and the biology of the “dourado” *Salminus maxillosus* Valenciennes, 1840 from Mogi-Guaçu River, state of São Paulo, Brazil

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**ABSTRACT.** In the present research 293 specimens of “dourado”, *Salminus maxillosus* Valenciennes, 1840, captured every month from August 1996 to December 1997, in Mogi-Guaçu River, downstream and upstream the dam of the Emas Falls (21°58' S - 47°26' W, 560 meters above sea level) were analyzed. The specimens were weighted (Wt - total weight, in grams) and measured (Lt - total length, in cm). Blood samples were taken to determine hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte count (Er) and hematimetric indexes: MCV, MCH and MCHC. Sex and development stage were identified through macroscopic inspection of the gonads (immature, in maturation, mature, spent and resting). Mean values were calculated in relation to sex, gonadal maturation stage and class of total length. Results demonstrate that mean of hematological analyses did not differ significantly when sex, gonadal maturation stage and class of total length were evaluated. Correlation coefficients between hematological variables and between these and biological variables were also calculated, although not much correlation has been found in these analyses.

**Key words:** hematology, biology, *Salminus maxillosus*, dourado, Mogi-Guaçu river.

**RESUMO.** Associação entre as características hematológicas e a biologia do dourado, *Salminus maxillosus* Valenciennes, 1840 do rio Mogi-Guaçu, Estado de São Paulo, Brasil. Foram analisados 293 espécimes de dourado, *Salminus maxillosus* Valenciennes, 1840, capturados mensalmente, no período de agosto de 1996 a dezembro de 1997, no rio Mogi-Guaçu, cachoeira das Emas (21°58' S - 47°26' W, altitude 560 metros). Cada indivíduo foi pesado (Wt - peso total, em gramas) e medido (Lt - comprimento total, em cm). Amostras de sangue foram utilizadas para as determinações de: hematócrito (Ht), taxa de hemoglobina (Hb), contagem de eritrócitos (Er) e calculados os índices hematimétricos absolutos: VCM, HCM e CHCM. Calcularam-se as médias das análises por sexo, por estágio de maturação gonadal e por classe de comprimento total. Os resultados encontrados demonstraram que as médias das análises hematológicas não diferem significativamente quando analisadas por sexo, estágio de maturação gonadal e classe de comprimento total. Calcularam-se as correlações entre as variáveis hematológicas entre si e entre estas e as biológicas, observando-se pouca correlação entre elas.

**Palavras-chave:** hematologia, biologia, *Salminus maxillosus*, dourado, rio Mogi-Guaçu.

Hematology is the study of blood or the sum of all our knowledge about it. Most data consist of measurements of hematological variables in normal and abnormal conditions. Hematological studies in animal research and in human diseases are well accepted and considered to be routine procedure in diagnoses.

Information on the condition of organisms may be obtained when blood is studied through physiological, biochemical or other methods. In

hematological analyses not only the determination of indicators in the liquid part of the blood (plasma and serum) but also the study of the morphological composition of the blood are important (Blaxhall, 1972; Beelen *et al.*, 1998).

Hendricks (1952) and Hesser (1960) were the first researchers who tried to adapt techniques used in human hematology to the study of fish, as a powerful tool for the diagnosis of diseases in these animals.

The objective of this paper is to study the possible variations of hematological variables in *Salminus maxillosus* in its natural environment, so that these variables could be related to specimens with pathological conditions or even to those kept in captivity.

*Salminus maxillosus*, popularly called dourado, belongs to the Characidae family and is one of the most commercially and ecologically important species in the regions where it is found. Geographically the dourado is found in Brazil, or rather, in the Amazon, high Amazon, Paraguai river and in the basin of the high, medium and low Paraná river (Ringuelet *et al.*, 1967), and in Bolivia, Colombia, Peru and Uruguay (Fowler, 1951). It exhibits a rheophilic behavior (popularly known as "piracema") and has high economic value since it is extremely appreciated by professional and amateur fishermen (Moraes Filho and Schubart, 1955; Britski, 1972; Godoy, 1975).

### Material and methods

Twenty specimens of dourado, *Salminus maxillosus*, were captured monthly from August 1996 to December 1997, in the Mogi-Guaçu river, at the Emas Falls (21°58'S - 47°26'W, 560 meters above sea level), in the town of Pirassununga SP Brazil.

After capture the fish were removed to the "Biology Lab of River Fish Dr. Pedro de Azevedo", belonging to the "Instituto de Pesca", where they were kept in 1,000L water tanks made of asbestos cement, for a maximum of 2 hours. Water was continuously aerated by means of a compressor. Blood samples were collected by caudal puncture, with the use of syringes and needles with heparin. The following analyses were undertaken: total cell count, using Natt and Herrick (1952) solution as a dilutant; hematocrit, using the microhematocrit method (Goldenfarb *et al.*, 1971); hemoglobin concentrations, using the cyanomethemoglobin method (Collier, 1944). Hematimetric indexes calculated were MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration), according to Wintrobe (1934).

After blood collection, specimens were anesthetized using benzocaine and then killed by brain concussion. They were weighed (Wt - total weight, in grams) and total length was measured (Lt, in cm). A ventral cranium-caudal incision was performed in order to expose the internal organs. During the macroscopic inspection of the gonads, sex and development stages were determined and classified as Immature (I); in Maturation (II),

Mature (III); Spent (IV) and Resting (V), according to Nikolsky (1963), modified by Barbieri *et al.* (1999).

Mean value and standard deviation for each hematological analysis were determined according to sex, gonad maturation stage and class of total length. Differences were analyzed using the ANOVA. The correlation between hematological variables and biological data in *S. maxillosus* was analyzed by Pearson's coefficient for linear correlation (*r*) (Costa Neto, 1977).

Employing Lt (total length) and Wt (total weight) for each individual, the trendline for the equation  $Wt = a.Lt^b$  was adjusted and values for the coefficients "a" and "b" were estimated. They were considered to be the total number of specimens studied. The coefficients "a" and "b" were used in the estimation of the theoretically expected values for body weight (We), using the expression  $We = a.Lt^b$ . In order to complement this analysis, the relative factor of condition (Kn) was calculated. This factor is the quotient between the observed weight and the theoretically expected weight, for a given length, that is,  $Kn = Wt / We$  (Le Cren, 1951). Means and standard deviations related to the groups were then estimated by sex. Mean values were then compared to the standard value  $Kn = 1,0$  by Student's "t" test.

### Results and discussion

In our research 293 specimens were analyzed, 113 were male (total length 37.6 to 73.5 cm and total weight, 540.0 to 4,400.0 g) and 180 females (total length 36.6 to 87.0 cm and total weight, 480.0 to 7,030.0 g).

Some authors state that the management of fish may produce alteration in their hematological values. However, Pickering *et al.* (1982) verified in *Salmo trutta* that there was no alteration in the number of erythrocytes after the fish had been managed. In this study, collection of samples was performed as soon as the specimens were placed in the water tank to prevent interference of stress factors.

Means and standard deviations for the hematological variables and hematimetric indexes are found in Table 1, in relation to sex, considered separately, and to the total of specimens. Table 1 shows that there were no significant differences for the mean values found in males and females in all the analyses.

Although these differences were not significant, it may be observed that the mean values for Ht, Hb and Er found for males are higher than those of

females. This fact has already been noted in other teleosteous species of fish (Ranzani-Paiva and Godinho, 1985; Silva, 1987; Ranzani-Paiva, 1995).

**Table 1.** Variation range (Ax), means ( $\bar{X}$ ) and standard deviations ( $\bar{Sx}$ ) for values of hematocrit (Ht), hemoglobin concentration (Hb), number of erythrocytes (Er), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), from *Salminus maxillosus* specimens, captured in Mogi-Guaçu River, at Emas Falls, Pirassununga, SP, analyzed according to sex

	Sex	Ax	$\bar{X}$	$\bar{Sx}$	N
Ht (%)	♂	24.0 - 59.0	43.18 a	0.64	113
	♀	26.5 - 66.5	41.80 a	0.48	180
	Total	24.0 - 66.5	43.60 a	0.40	293
Hb(g/100 ml)	♂	5.9 - 12.5	8.91 a	0.14	113
	♀	4.2 - 13.8	8.60 a	0.14	180
	Total	4.2 - 13.8	8.98 a	0.09	293
Er (10 <sup>6</sup> /mm <sup>3</sup> )	♂	107.0 - 320.5	218.95 a	3.44	113
	♀	123.5 - 353.5	212.38 a	2.92	180
	Total	107.0 - 353.5	214.90 a	2.24	293
MCV (μm <sup>3</sup> )	♂	126.9 - 295.3	199.61 a	2.87	113
	♀	90.5 - 384.6	206.59 a	2.85	180
	Total	90.5 - 384.6	207.00 a	2.10	293
MCH (pg)	♂	26.0 - 56.1	41.45 a	0.70	113
	♀	21.5 - 85.9	43.67 a	0.65	180
	Total	21.5 - 85.9	42.63 a	0.49	293
MCHC (%)	♂	13.6 - 37.1	20.88 a	0.38	113
	♀	10.7 - 39.9	20.86 a	0.26	180
	Total	10.7 - 39.9	20.87 a	0.21	293

Means followed by the same letter are not significantly different ( $P < 0.01$ )

It may be also noted that mean values for hematological analyses are within the normal range established for other species of teleosteous fish (Ranzani-Paiva and Godinho, 1985; Facchini, 1987; Ranzani-Paiva, 1995; Ranzani-Paiva and Ishikawa, 1996; Ueda *et al.*, 1997; Ranzani-Paiva *et al.*, 1998, 2000). However, since *S. maxillosus* is a species with reproductive migration ("piracema"), these values could be higher owing to an increase in energy expenses produced by this kind of behavior (Ranzani-Paiva and Godinho, 1985). This may be noted especially in relation to hemoglobin concentrations. According to Hall and Gray (1929) and Ranzani-Paiva and Godinho (1985), there seems to be a relationship between hemoglobin concentrations and fish activity. Pelagic and migrating species apparently present higher values for this parameter than benthonic species.

Table 2 presents values for the relative factor of condition (Kn), for male and female *S. maxillosus*. When comparing mean Kn to 1.0 by the Student's "t" test there was no significant difference for males. This means that the weight of the individuals was equal to that theoretically expected. Females had a

Kn different from 1.0 ( $1.02 > 1.0$ ), indicating that they were above the expected weight. This demonstrates that females, in general, have a better physical condition, even when going through the reproduction period when they probably display a higher consumption of body reserves.

**Table 2.** Means, standard deviations ( $\bar{Sx}$ ), range of variation and Student's "t" test for the values of the relative factor of condition (Kn) for total weight (Wt) and total length (Lt) of *Salminus maxillosus* specimens analyzed according to sex

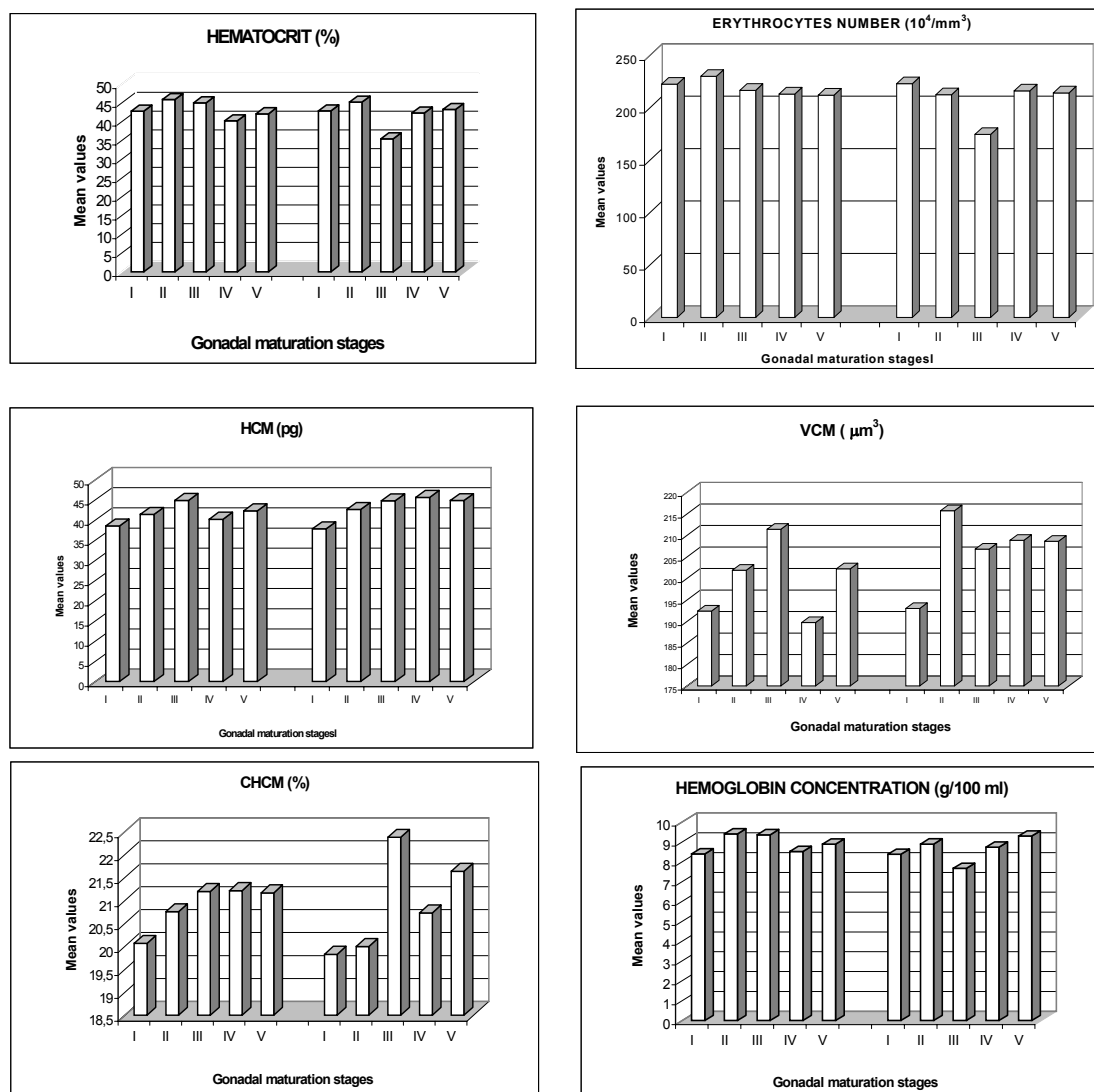
Statistics analysis	Males	Females
mean Kn $\pm$ Sx	0.97 $\pm$ 0.1203	1.02 $\pm$ 0.0089
Range of variation	(0.76-0.95)	(0.61-1.92)
n	69	97
"t" test. Ho: Kn = 1.0	t = 0.506	t = 2.2361
P	P = 0.6137	P = 0.0266 *

n = number of specimens; \* significant difference,  $p < 0.05$ )

No significant differences were found with ANOVA when the hematological variables for different sex, maturation stages and interactions sex-stage were compared. It was however verified that Ht presented a significant difference ( $P < 0.01$ ) for the gonad maturation stages. This fact demonstrates a distinct behavior for each sex when considered within a given stage.

Analysis of Figure 1 shows that females tend towards lower average values of Ht, Hb and Er in stage III. However, these differences were not significant. Although variations found for males are not so clear, they tended to decrease in the spent stage. With regard to hematimetric indexes, it was verified that, although differences between mean values were not significant neither according to sex, nor according to stage or interactions, values found for MCV and MCHC in females were lower in stage I. MCV present a peak in stage II, and then returns to the previous values. A discreet oscillation may be observed until the end of the reproductive period. McCarthy *et al.* (1973), Rhyzova and Tugarina (1979), Alexander *et al.* (1980) and Pickering (1986) also reported variation in blood components during the reproductive cycle of the species studied.

Lack of significance for the different mean values of the hematological variables of *S. maxillosus* was not expected, for there is a decrease in the food intake during the spawning season and fish are submitted to a great physical effort during the "piracema" period. According to Alexander *et al.* (1980), the relationship between the reproductive phase and the number of erythrocytes is suggestive. Individuals in pre-reproductive period present lower values for this parameter than when they are in the post-reproduction phase. Figure 1 shows this in a discreet manner.



**Figure 1.** Mean values for blood variables in *Salminus maxillosus*, captured in Mogi-Guaçu River, at Emas Falls, Pirassununga, SP, according to gonad maturation stage (I - immature; II - in maturation; III - mature; IV - spent; V - resting)

**Table 3.** Means ( $\bar{X}$ ), standard deviations ( $\bar{S}_X$ ) and number of specimens (n) for values of blood analyses and hematimetric indexes in *Salminus maxillosus* captured in Mogi-Guaçu River, at Emas Falls, Pirassununga, SP, analyzed according to the class of total length

Lt (cm) class	30 40 — (n=5)	40 50 — (n=47)	50 60 — (n=132)	60 70 — (n=92)	>70 (n=17)
	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$
Ht (%)	46.20 ± 4.00	42.05 ± 0.90	43.42 ± 0.55	44.91 ± 0.69	41.59 ± 1.82
Hb (g/100 ml)	7.93 ± 0.17	8.69 ± 0.22	8.92 ± 0.13	9.51 ± 0.23	8.78 ± 0.30
Er ( $10^3/\text{mm}^3$ )	230.70 ± 9.02	216.35 ± 4.47	210.04 ± 3.22	224.62 ± 4.64	193.03 ± 4.93
MCV ( $\mu\text{m}^3$ )	198.90 ± 11.46	195.85 ± 3.62	209.91 ± 2.98	207.73 ± 4.46	215.36 ± 7.79
MCH (pg)	34.57 ± 1.44	40.65 ± 0.97	42.84 ± 0.73	44.30 ± 1.28	45.55 ± 1.21
MCHC (%)	17.77 ± 1.78	20.85 ± 0.51	20.65 ± 0.36	21.29 ± 0.44	21.55 ± 0.89

Lt = total length; MCV = Mean Corpuscular Volume; Er = erythrocyte counts; MCH = Mean Corpuscular Hemoglobin; Ht = hematocrit; MCHC = Mean Corpuscular Hemoglobin Concentration; Hb = hemoglobin concentration

For the identification of a possible alteration in hematological variables in individuals of different sizes, some analyses of the mean values for hematological variables and hematimetric indexes were performed, according to the class of total length (Table 3).

When Ht and Er are analyzed, it may be observed that as individuals get larger, mean values for the variables tend to be lower. However, hemoglobin concentration does not present the same pattern. As for hematimetric indexes, the pattern is rather the opposite; that is, larger individuals present higher mean values for MCV, MCH and MCHC. On the other hand, Ranzani-Paiva (1995) in a study on *Mugil platanus* verified that mean values for Ht, Hb, Er, MCV, MCH and MCHC showed a slight increasing tendency as individuals got larger. Amadio (1985) stated that Er values increase as the individual grows, but these values stabilize as fish get older and reach body homeostasis.

These blood variables are not always related to total length of the specimens, as reported by Pitombeira (1972); van Vuren and Hattingh (1978) and Kori-Siakpere (1985). However, other authors also observed this increase trend in some hematological variables when compared to the total length of the individuals (Korzhuev *et al.*, 1982; Pastore, 1983; Amadio, 1985 and Facchini, 1987).

Biological variables of *Salminus maxillosus* as total length (Lt), total weight (Wt) and hematological variables are presented in Table 4. Correlation analyses were performed in all. It has been established that correlations are acceptable if they are either positive or negative and above 0.5. When all individuals are considered, a strong positive correlation between length and weight was found (0.95). In the comparisons between the hematological variables and the biological ones, few positive or negative correlations over 0.5 were found. Correlations tended to be weak or absent in these cases. Higher correlations were found for the hematimetric indexes, which indicates a link between them. In the case of MCV and Er, a negative correlation (-0.60) was found, indicating that as one variable increases, the other decreases. This was expected once MCV is calculated on the values of Ht and Er, where the latter is the denominator in the formula; thus, the higher the Er, the lower the MCV. The same pattern occurred in the comparison between MCH and Hb; theoretically, when the number of erythrocytes increased, so did the hemoglobin concentration. This influences the MCH value, in whose formula

Er is also the denominator. Since the correlations for MCH, MCHC and Hb were the highest, this indicates that the more hemoglobin is found in the blood, the higher its concentration and quantity inside the erythrocytes.

**Table 4.** Correlations found in the comparison of blood and biological variables in *Salminus maxillosus* captured in Mogi-Guaçu River, at Emas Falls, Pirassununga, SP

	Lt (cm)	Wt (g)	Ht (%)	Hb (g/100ml)	Er (10 <sup>6</sup> /mm <sup>3</sup> )	MCV (μm <sup>3</sup> )	MCH (pg)	MCHC (%)
Lt (cm)	1.00	0.95	0.08	0.15	-0.02	0.14	0.20	0.10
Wt (g)		1.00	0.05	0.11	-0.03	0.13	0.18	0.07
Ht (%)			1.00	0.46	0.48	0.37	-0.01	-0.37
Hb (g/100ml)				1.00	0.39	-0.01	0.59	0.63
Er (10 <sup>6</sup> /mm <sup>3</sup> )					1.00	-0.60	-0.49	-0.02
MCV (μm <sup>3</sup> )						1.00	0.54	-0.31
MCH (pg)							1.00	0.62
MCHC (%)								1.00

Lt = total length; Er = erythrocyte number; Wt = total weight; MCV = Mean Corpuscular Volume; Ht = hematocrit; MCH = Mean Corpuscular Hemoglobin; Hb = hemoglobin concentration; MCHC = Mean Corpuscular Hemoglobin Concentration

The establishment of relationships between blood variables and biological data of fish is important, principally when the aim is a rational breeding system. Generally, it is difficult to determine hematological variables in fish especially when study is performed in natural conditions. The determination of the correlations in results for the hematological variables has been shown to be an adequate tool in diagnosis procedures. Once correlations are established, values for Er and Hb may be estimated on hematocrit determination, for example. The latter is a simple and low-cost analysis. Some authors have found high correlations for these variables (Pandey *et al.*, 1976; Green, 1977; Everaarts, 1978; Clark *et al.*, 1979; Pastore, 1983; Ranzani-Paiva and Godinho, 1985; Facchini, 1987; Dheer, 1988; Ranzani-Paiva, 1995).

In a study on *Salmo gairdneri*, *Cyprinus carpio* and *Salvelinus fontinalis*, Houston and De Wilde (1968) suggest that hematocrit values should be used as a general indicator of "hematological status" in routine examinations. Based on the result, the number of erythrocytes and hemoglobin concentration may be estimated. However, correlation coefficient (r) for these variables was low, less than 0.5, according to some authors. This has been also demonstrated in *Scomberomorus maculatus*, by Pitombeira and Martins (1970); in *Astronotus ocellatus* by Pitombeira (1972); in *Tilapia zilli*, by Ezzat *et al.* (1974); in *Pimelodus maculatus* by Ribeiro (1978); in *Plecostomus albopunctatus*, by Kavamoto *et al.* (1985); in *Oncorhynchus mykiss*, by Wells and Weber (1991) and in *Oncorhynchus mykiss* and *Dicentrarchus labrax*, by Garcia *et al.* (1992).

Results for hematological analysis compared to biological data in “dourado” *Salminus maxillosus*, captured in Mogi-Guaçu River, at Emas Falls, in Pirassununga SP Brazil, enable one to reach the following conclusions: variations found for hematological values present no difference with regard to sex and gonad maturation stage; total length of the individuals affects some of the hematological variables, and smaller individuals present higher values for Ht, Hb and Er than larger ones. The opposite pattern occurs for hematimetric indexes (MCV, MCH, MCHC); the estimation of the relative factor of condition for males and females demonstrate that, as an average, females weigh more than it was theoretically expected.

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