

**Some haematological parameters
in *Prochilodus lineatus*
(Pisces, Curimatidae) (1)**

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ABSTRACT

Values of haematological indices were measured in 56 adult "sábalo" (*Prochilodus lineatus*) captured in the floodplain of the Salado River (Argentina). The blood was sampled from the caudal vessels. The values of the haematological parameters were measured using standard methods used for humans. The data obtained were then used to establish normal values for this species.

The following mean values were established for the red blood cells : RBC = $1,580 \cdot 10^3/\text{mm}^3$ (870 - $2,610 \cdot 10^3/\text{mm}^3$); Ht = 32.85 % (20.80 - 47.20 %) and Hb = 8.3 g/100 ml (5.2 - 11.9 g/100 ml). No significant differences in any blood parameters were found between sexes or maturation stages, but some significant correlations between haematological variables were found.

KEYWORDS: Haematological parameters — Red cells — *Prochilodus lineatus* — South America.

RÉSUMÉ

CARACTÉRISTIQUES HÉMATOLOGIQUES DE *PROCHILODUS LINEATUS* (PISCES, CURIMATIDAE).

Les valeurs des indices hématologiques ont été mesurées chez 56 adultes de *Prochilodus lineatus* ("sábalo") capturés dans les plaines d'inondation du Salado (Argentine). Le sang a été collecté dans les vaisseaux de la partie caudale des poissons. Les valeurs des paramètres hématologiques ont été mesurées en utilisant les méthodes classiquement utilisées en hématologie humaine. Les résultats obtenus ont ensuite été normalisés pour servir de standard à cette espèce.

En ce qui concerne les globules rouges les valeurs moyennes suivantes ont été obtenues : nombre de globules rouges = $1580 \cdot 10^3/\text{mm}^3$ (870-2610); hématocrite = 32,85 % (20,80-47,20); concentration en hémoglobine = 8,3 g/100 ml (5,2-11,9). Le sexe ou le stade de maturation ne paraissent pas affecter les paramètres sanguins, toutefois il semble exister des corrélations significatives entre les différents indices hématologiques.

MOTS CLÉS : Paramètres hématologiques — Globules rouges — *Prochilodus lineatus* — Amérique du Sud.

(1) This work was financed through a grant from the National Council of Technical and Scientific Research of the Argentine Republic (PID No. 3-093800/88).

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INTRODUCTION

Studies of the haematology and blood biochemistry in different species of fish are of comparative physiological interest. They contribute to a greater understanding of habitat, food selection and mode of life. In general, these studies have been developed from two view points. The first, with the object of establishing a possible relationship between blood characteristics with regard to such factors as phylogenetic position, ecological blood characteristics and environmental changes (SOIVIO and OIKARI, 1976; LOCHMILLER *et al.* 1989; PETERSON, 1990; MARINSKY *et al.*, 1990). The second relating haematological parameters to variables such as food, sex, growth, reproduction and migratory patterns (KAVAMOTO *et al.*, 1983; RANZANI-PAIVA and GODINHO, 1985; PICKERING, 1986; RANZANI-PAIVA, 1991). In all cases, haematological analyses in fish are difficult to interpret due to intra and interspecific variations attributable to many causes that involve, among others, mainly blood sampling and laboratory procedures (HESSER, 1960; WILHELM FILHO *et al.*, 1992).

Prochilodus lineatus is a widely distributed neotropical fish, and one of the most important species of the Parano-Platense ichthyofauna (BONETTO *et al.*, 1969; BONETTO *et al.*, 1970; BONETTO *et al.*, 1971; OLDANI and OLIVEROS, 1984). The adults are detritivores and in the Rio Paraná comprise 60 % of the total ichthyomass (BONETTO, 1970). The catch is strongly dependent on the demand for industrial processing or export (QUIRÓS and CUCH, 1989) and fish abundance has been related to concentrations of organic matter in the water column in many places in the basin (QUIRÓS and BAIGÚN, 1985). The significance of this species in South America has been repeatedly emphasized by several authors studying fish communities (BONETTO, *et al.*, 1969; WELLCOMME, 1985; LOWE-Mc CONNELL, 1987; CORDI-VIOLA DE YUAN, 1992, among others).

The present paper deals with some haematological characteristics of *Prochilodus lineatus*. Besides increasing our fundamental knowledge of comparative blood physiology, the data provides the first information on the natural composition of blood in this species, in relation to weight, sex and maturation stages during a spring-summer season.

MATERIAL AND METHODS

Fish were sampled in the inundated floodplain of the Salado River (31° 39'36" S - 60° 45'58" W) during the period November 1992-March 1993 in the spring and summer season. Specimens were caught by

"artesanal" fishery, and were immediately transferred to individual 850 l tanks equipped with filters and aerators. They were allowed to rest for >24 h without external stimuli, to reduce stressful activity, prior to the taking of blood samples. The temperatures in the tanks ranged between 21 °C to 27 °C and the pH between 8.0-8.4. The O₂ supply was maintained above 4.0 ppm.

The analyses were carried out on 56 adult fish ranging in weight from 1,059-3,640 g and in length from 33-48 cm, respectively. Individual blood samples were drawn from the caudal vessels of unanaesthetized specimens by means of heparinized syringes and the blood collection was completed within 3 minutes to reduce effects of handling on blood constituents. All individuals were killed between 08.00 and 09.00 h.

The haematocrit (Ht) in percentage (%) was determined by the micromethod using capillary tubes and centrifugated at 12,000 r.p.m. for 5 minutes. Hemoglobin (Hb) concentration (g/100 ml) was determined by spectrophotometry by the cyanomethemoglobin procedure. The red blood cell counts (RBC) per mm³, were performed with Neubauer chambers, using Hayem diluting solution.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined according to RANZANI-PAIVA (1991) :

$$\text{MCV} = \text{Ht}(\%) \cdot 10/\text{RBC} (10^6/\text{mm}^3) = \mu\text{m}^3$$

$$\text{MCH} = \text{Hb} (\text{g}/100 \text{ ml}) \cdot 10/\text{RBC} (10^6/\text{mm}^3) = \mu\text{g}$$

$$\text{MCHC} = \text{Hb} (\text{g}/100 \text{ ml}) \cdot 100/\text{Ht} (\%) = \%$$

After the blood samples were taken, each fish was weighted, killed by a blow to the head and the state of maturation of the gonads assessed by internal examination. The different stages recorded were : resting, maturation, mature and spent.

Regression analyses were employed and statistical comparisons were made using Student's t-test (SOKAL and ROHLF, 1979).

RESULTS AND DISCUSSION

The number of erythrocytes (RBC) in the blood of *Prochilodus lineatus* ranged from 870 to 2,610 10³/mm³ with an overall mean of 1,580 10³/mm³ (Table I). The mean for males was 1,598 10³/mm³ and for females 1,574 10³/mm³. No significant differences were found between the sexes ($t = 0.24$; $P = 0.05$) but males had higher concentrations of cells than the females.

The statistical comparison between the sexes at different maturation stages showed no significant

TABLE I

Red cell haematological data from *Prochilodus lineatus*. All values are means. Numbers in parentheses are standard deviations. *Données sur les globules rouges de Prochilodus lineatus. Toutes les données sont des moyennes, avec l'écart-type entre parenthèses.*

SEX	RBC ($\times 10^3 \text{ mm}^3$)	Hb (g/100 ml)	Ht (%)	MCV (μm^3)	MCH (μg)	MCHC (%)
MALE	1,598 (234)	8.58 (1.29)	34.31 (4.68)	217.18 (31.21)	54.31 (10.11)	25.00 (2.48)
FEMALE	1,574 (400)	8.20 (1.68)	32.27 (6.69)	209.42 (31.38)	53.25 (7.58)	25.57 (2.15)
TOTAL	1,580 (359)	8.30 (1.57)	32.85 (6.21)	211.63 (31.24)	53.55 (8.30)	25.38 (2.23)

differences ($P < 0.05$) (Tables II and III). The higher red cell numbers were registered in resting and spent males and in resting and mature females; red cell numbers were lower in the maturation stage of both sexes.

Regression analysis revealed a poor correlation between the weight of specimens and erythrocyte counts ($r = 0.39$; $P < 0.05$), and also between the condition factor (K) and the number of erythrocytes ($r = 0.37$; $P < 0.05$).

Erythrocyte counts have proved to be highly variable among fishes, with values between $60 \times 10^3/\text{mm}^3$ for *Squalus acanthias* (WINTROBE, 1933) and $6,130 \times 10^3/\text{mm}^3$ for *Scomberomorus maculatus* (ENGEL and DAVIS, 1964, in AMADIO, 1985). The values reported for *P. lineatus* are within the range found for other neotropical fish species.

RANZANI-PAIVA and GODINHO (1985) found red blood cell concentrations to range from $2,000 \times 10^3/\text{mm}^3$ for male to $1,900 \times 10^3/\text{mm}^3$ for female specimens of *Prochilodus scrofa*, captured in the Mogi-Guaçu River (Sao Paulo, Brazil), values somewhat higher than those found in our studies of *P. lineatus*. RANZANI-PAIVA (1991) mentioned values between $2,078$ and $1,780 \times 10^3/\text{mm}^3$ for *Brycon* sp. Other representative values include a mean value of $1,580 \times 10^3/\text{mm}^3$ for *Rhamdia hilarii* (KAVAMOTO *et al.*, 1983) and $2,540 \times 10^3/\text{mm}^3$ for *Pimelodus maculatus* (RIBEIRO, 1978). Several workers have shown variations in erythrocyte counts as fish advanced towards maturity (SNIESZKO, 1961; YADAV *et al.*, 1978; LANE, 1979; PICKERING, 1986, among others). However, it can be seen from the present investigations that no difference was evident in relation to maturation stages.

TABLE II

Red cell haematological data from male of *Prochilodus lineatus*. All values are means. Numbers in parentheses are standard deviations.

Données sur les globules rouges des males de Prochilodus lineatus. Toutes les données sont des moyennes, avec l'écart-type entre parenthèses.

STAGE	RBC ($\times 10^3 \text{ mm}^3$)	Hb (g/100 ml)	Ht (%)	MCV (μm^3)	MCH (μg)	MCHC (%)
RESTING	1,830 (99)	8.75 (1.48)	34.24 (3.30)	186.90 (7.91)	47.70 (5.51)	25.46 (1.87)
MATURATION	1,423 (100)	7.97 (1.43)	30.81 (4.47)	218.37 (45.72)	56.43 (13.24)	25.77 (1.31)
MATURE	1,533 (228)	8.95 (1.18)	36.77 (5.35)	241.00 (22.58)	59.48 (11.81)	24.66 (3.84)
SPENT	1,688 (258)	8.34 (1.41)	33.49 (3.81)	200.00 (16.60)	49.50 (4.44)	24.77 (1.48)

TABLE III

Red cell haematological data from female of *Prochilodus lineatus*. All values are means. Numbers in parentheses are standard deviations.

Données sur les globules rouges des femelles de Prochilodus lineatus. Toutes les données sont des moyennes, avec l'écart-type entre parenthèses.

STAGE	RBC ($\times 10^3 \text{ mm}^3$)	Hb (g/100 ml)	Ht (%)	MCV (μm^3)	MCH (μg)	MCHC (%)
RESTING	1,601 (311)	8.39 (1.30)	33.77 (6.00)	214.06 (31.93)	53.43 (8.32)	24.98 (1.50)
MATURATION	1,389 (348)	7.68 (1.88)	30.78 (6.95)	224.59 (30.31)	55.83 (7.27)	24.95 (2.17)
MATURE	1,673 (369)	8.63 (1.84)	32.72 (7.19)	196.27 (20.19)	51.89 (5.75)	26.46 (1.95)
SPENT	1,493 (502)	8.04 (1.99)	30.82 (7.73)	195.37 (36.66)	50.94 (8.91)	26.28 (3.05)

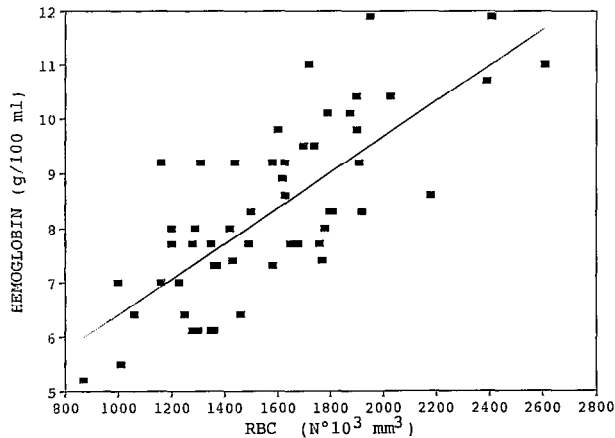


FIG. 1. — Correlation between red cell numbers (RBC) and haemoglobin content.

Corrélation entre le nombre de globules rouges (RBC) et la concentration en hémoglobine.

The haemoglobin concentration (Hb) ranged between 5.2 and 11.9 (g/100 ml) with a mean of 8.3 g/100 ml (Table I) for the combined totals and a mean of 8.6 and 8.2 for males and females, respectively. Haemoglobin values do not appear to be related either to sex of the fish ($t = 0.72$; $P = 0.05$) or to maturation stages ($P = 0.05$) (Table II and III), but higher values were registered in males. The highest haemoglobin concentrations were found in both sexes at the mature stage and minimum concentrations during maturation. RANZANI-PAIVA and GODINHO (1985) reported high haemoglobin values in *Prochilodus scrofa* during the breeding season and COBURN and FISCHER (1973) stated that fish haemoglobin values usually vary from 2.5 to 61 g/100 ml and that 41 % of these are in the range 5 to 10 g/100 ml.

Our data suggests no significant correlation of haemoglobin with weight ($r = 0.22$) and a poor correlation with condition factor ($r = 0.29$; $P = 0.05$); although there was a positive correlation between red cell number and haemoglobin content ($r = 0.75$; $P < 0.05$) (Fig. 1). Several workers have shown that the haemoglobin concentration in the blood of fish increases in response to enhanced activity (RANZANI-PAIVA and GODINHO, 1985) and that pelagic forms showed higher values than sedentary or less active species. The mean haemoglobin values given here are smaller than those of other migratory freshwater fishes of the same family, such as *Prochilodus scrofa* and *Semaprochilodus* sp. (RANZANI-PAIVA and GODINHO, 1985; AMADIO, 1985) in similar natural conditions. Lower values have been reported in *Astronotus ocellatus* (PITOMBEIRA, 1972) and higher

ones in *Pimelodus maculatus* (RIBEIRO, 1978) and *Rhamdia hilarii* (KAVAMOTO *et al.*, 1983). Haemoglobin values are amongst the most thoroughly documented haematological indices for evaluating fish health; anaemia; toxic metals and certain physiological stresses (CLARK *et al.*, 1979).

The mean haematocrit (Ht) value was 32.85 % (range 20.80-47.20 %) (Table I), with higher values in males (mean of 34.31 %) than in females (mean of 32.27 %). Haematocrit values, like haemoglobin and erythrocyte counts were not statistically different for sex ($t = 1.17$; $P = 0.05$) or for sex grouped by maturation stages of the gonads ($P = 0.05$)

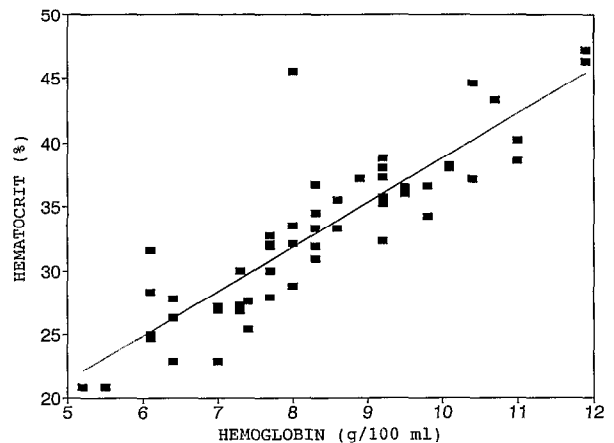


FIG. 2. — Linear relationship between haematocrit values and haemoglobin concentration.

Relation linéaire entre le pourcentage d'hématocrite et la concentration en hémoglobine.

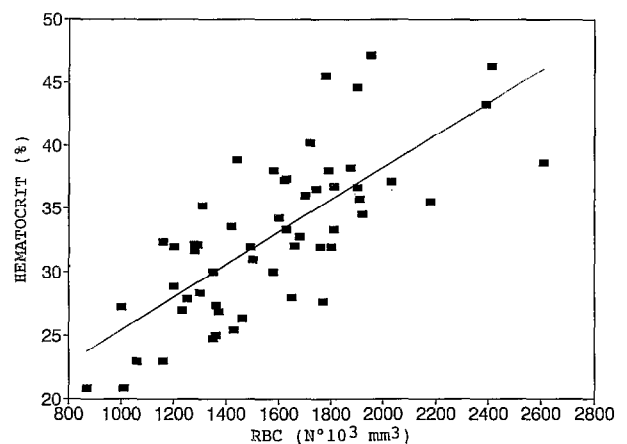


FIG. 3. — Regression analysis related haematocrit values and erythrocyte counts (RBC).

Régression entre le pourcentage d'hématocrite et le nombre de globules rouges (RBC).

(Tables II and III). Males had higher haematocrit values than the females. The highest haematocrit values were registered in mature males and in resting females and the minimum in both sexes at maturation stage.

We found a positive linear relationship between haematocrit values and haemoglobin concentration ($r = 0.88$; $P < 0.05$) (Fig. 2), a feature frequently reported for fish (WINTROBE, 1933 and WILHELM FILHO *et al.*, 1992) and also between haematocrit values and RBC counts ($r = 0.74$; $P < 0.05$) (Fig. 3). However, no significant correlation was found for the relationships with condition factor ($r = 0.32$; $P = 0.05$) or weight of the fish ($r = 0.13$).

With respect to haematological indices, the values for MCV were between $278.10 \mu\text{m}^3$ and $147.90 \mu\text{m}^3$ with a mean of $211.63 \mu\text{m}^3$; for MCH between $39.50 \mu\text{g}$ and $79.30 \mu\text{g}$ with a mean of $53.55 \mu\text{g}$ and for MCHC between 17.60 and 30.50% with a mean value of 25.38% (Table I). The data on the mean values of MCV, MCH and MCHC (Tables II and III) revealed no significant sex differences ($t = 0.80$; 0.43 and 0.78 , respectively; $P = 0.05$), but the mean values of MCV and MCH were slightly higher for males and MCHC for females.

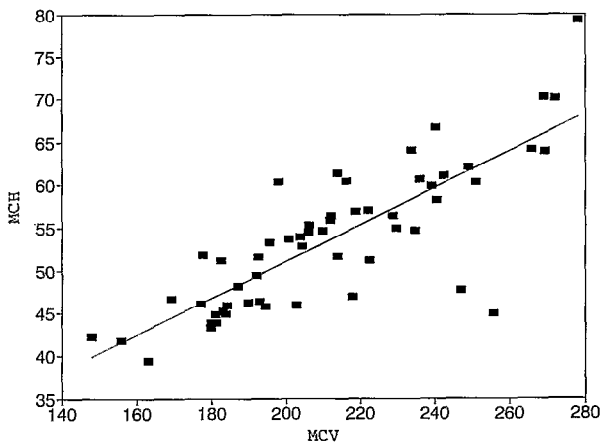


FIG. 4. — Correlation between mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH).
Corrélation entre le volume moyen cellulaire (MCV) et l'hémoglobine moyenne cellulaire (MCH).

Regression analysis revealed a high correlation between MCV and MCH ($r = 0.81$; $P < 0.05$) (Fig. 4) and between the erythrocyte counts vs. MCH ($r = 0.54$) (Fig. 5) and RBC vs. MCV ($r = 0.54$) (Fig. 6).

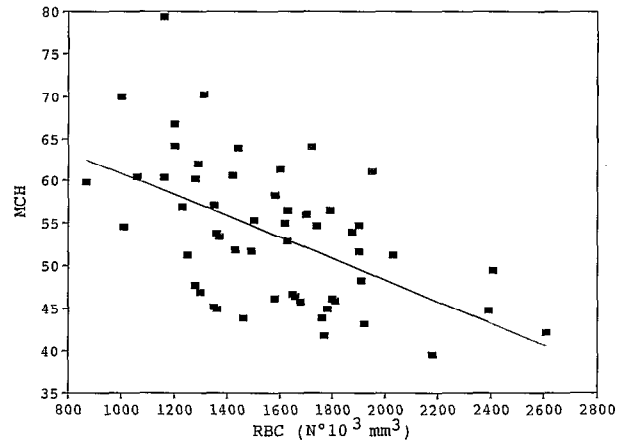


FIG. 5. — Linear relationship related erythrocyte counts (RBC) and mean corpuscular haemoglobin (MCH).
Relation entre le nombre d'érythrocytes (RBC) et l'hémoglobine moyenne cellulaire (MCH).

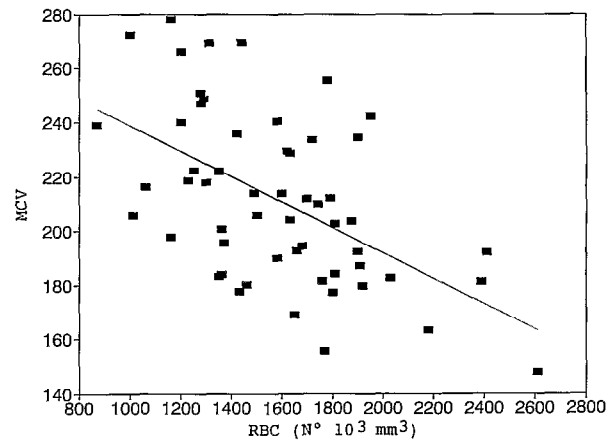


FIG. 6. — Regression analysis between erythrocyte counts (RBC) and mean corpuscular volume (MCV).
Régression entre le nombre d'érythrocytes (RBC) et le volume cellulaire moyen (MCV).

CONCLUSIONS

The importance of haematology in the diagnosis of fish diseases, for the assessment of the effects of pollution and knowledge of environmental conditions has been widely accepted. However, to interpret blood analyses a thorough understanding of the normal range of the blood parameters is needed. The great individual variability of haematological indices is the result of biological variability and sensitivity

to the influences of the external environment. Factors such as sex, age, size, reproduction, water temperature and quality of the environment, influence the values of haematological parameters in fishes (LANE, 1979; PICKERING, 1986; SANDSTROM, 1988).

Nevertheless, the mean values found in *Prochilodus lineatus* are considered to fall within a "normal" range and are within the range found for other fish species. In general, males showed higher blood values than females, but no significant differences in any blood parameters were found between sex or maturation stages. Some significant correlations bet-

ween haematological variables were found. The difference in similar blood indicators of *Prochilodus scrofa* may be connected with the differences in their environmental conditions.

ACKNOWLEDGEMENTS

To Stella G. de Paira and Mirta Campana (INALI-CONICET) for laboratory assistance. To Dr. Stephen Bowen (Michigan Technological University, Michigan, USA) for critical review of the manuscript.

Manuscript accepté par le Comité de rédaction le 27 juillet 1995

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