

## HAEMATOLOGY OF FISH FROM TIBAGI RIVER. I. DIFFERENTIAL WHITE BLOOD CELL COUNTS IN *Pimelodus maculatus* FEMALES\*

[Hematologia de peixes do rio Tibagi. I. Contagem diferencial de leucócitos de fêmeas de *Pimelodus maculatus*]

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### ABSTRACT

The white blood cell picture of sixty-eight females of the "mandi-amarelo", *Pimelodus maculatus* Lacépède, 1803, from the Tibagi River, at Sertanópolis, Paraná State, Brazil, was analysed in relation to both the gonadal maturation and the water temperature at the times of specimen capture in each month. All classic leucocyte forms were found, i.e. lymphocytes, neutrophils, monocytes, basophils, eosinophils, special granulocytic cells and immature leucocytes. The lymphocytes were the most frequent leucocyte followed by the neutrophils. The other white cell types were scarce. A tendency to the lymphocytophilia and a significant neutropenia ( $P < 0.05$ ) were observed in the gonadal maturation. A different pattern was found when the mean percentages of the most abundant leucocytes were analysed monthly. A marked lymphocytopenia and neutrophilia was found during the highest water temperature period.

**Key words:** leucocytes, differential count, gonadal maturation, water temperature, *Pimelodus maculatus*

### RESUMO

O quadro leucocitário de sessenta e oito fêmeas do mandi-amarelo, *Pimelodus maculatus* Lacépède, 1803, capturadas no rio Tibagi, localidade de Sertanópolis, Estado do Paraná, Brasil, foi analisado frente à maturação gonadal e à temperatura da água no momento de captura dos peixes em cada mês. Foram verificados todos os leucócitos descritos comumente no sangue periférico de peixes, isto é, linfócitos, neutrófilos, monócitos, basófilos, eosinófilos, células granulocíticas especiais e leucócitos imaturos. Os linfócitos foram os leucócitos de maior frequência, seguidos pelos neutrófilos. As demais células apresentaram baixas frequências. Observou-se tendência à linfocitofilia e significativa neutropenia ( $P < 0.05$ ) em relação à maturação gonadal. Um padrão diferente foi encontrado quando as porcentagens médias dos leucócitos mais frequentes foram analisadas mensalmente. Marcadas linfocitopenia e neutrofilia foram verificadas nos períodos de temperaturas da água mais elevadas.

**Palavras-chave:** leucócitos, contagem diferencial, maturação gonadal, temperatura da água, *Pimelodus maculatus*

### Introduction

Some authors have found that haematological variations appear to be correlated to the reproductive cycle in several fish species (COLGROVE, 1966, EINZPORN-ORECKA, 1970, EZZAT; SHABANA; FARGHALY, 1974, CAVICCHIOLI and ZAVARINI, 1977, MCARTHUR; THOMSON; FLETCHER, 1985, PICKERING, 1986, SUZUKI, 1986) and seasonal variations (BENNETT and NEVILLE, 1975, JENEY and JENEY, 1982).

Changes in the blood cell composition during the spawning season of Brazilian fish species were studied by KAVAMOTO; RANZANI-PAIVA; TOKUMARU (1983) in *Rhamdia hilarii*, KAVAMOTO *et al.* (1985) in *Plecostomus albopunctatus*, RANZANI-PAIVA and GODINHO (1983) in *Prochilodus scrofa* and RANZANI-PAIVA (1995) in *Mugil platanus*. RIBEIRO (1978) analysed the morphology and also the differential leucocyte counts of *Pimelodus maculatus*, but refers neither to the gonadal maturation nor to the environ-

mental variations.

The aim of the present study was to examine the circulating white blood cell composition of females of the "mandi-amarelo", *Pimelodus maculatus* Lacépède, 1803, in relation to both the gonadal maturation and water temperature.

## Material and Methods

Sixty-eight females of the "mandi-amarelo", *Pimelodus maculatus* (17.0-34.7 cm total length and 40.2-474.1 g) were net fished monthly from July 1992 to July 1993 (except February) from the Tibagi river at Sertanópolis, Paraná State, Brazil. Water temperature was measured using an appropriate mercury thermometer at the exact time and locality of fish capture.

Sex and gonadal maturation stage (immature, initial maturation, maturing, mature and spent) were checked macroscopically. Fish with either parasites or lesions were discarded.

The blood samples were taken from unanaesthetised fish by caudal puncture, using heparin 2% as anticoagulant. The blood smears were made immediately after sampling, air-dried and stained with May-Grünwald and Giemsa. The white blood cells of each fish were identified according to the criteria used by several authors (BOYAR, 1962, CAVICCHIOLI and ZAVARINI, 1977, SARASQUETE, 1984, BURROWS and FLETCHER, 1987 and RANZANI-PAIVA, 1995). Percentage values of the different leucocytes were estimated by counting 200 cells under an immersion oil objective.

A statistical comparison ( $P < 0.05$ ) for the blood characteristics among the fish at different gonadal maturation stages was performed with a nonparametric test, the Mann-Whitney "U" Test.

## Results

The blood smears examined under the light microscope present a general blood picture for *Pimelodus maculatus* females. The red blood cells series in the peripheral blood contain erythrocytes and erythroblasts. Thrombocytes are found more often in groups than isolated. They are round rather than spindle shaped.

The white blood cells series show all the classic forms, i.e., lymphocytes, neutrophils, monocytes, basophils, eosinophils and immature leucocytes. Other type of granulocytic cell, described and called by RIBEIRO (1978) as special granulocytic cell, is also seen.

The distinguishing features of the leucocytes are the following:

- Lymphocytes: small cells with very little cytoplasm, circular or somewhat elliptical as a thin rim, pale blue. Protuberances can be observed on the thin layer of cytoplasm. The thick purple nucleus filling nearly the whole cell is circular.
- Neutrophils: circular cytoplasm, clear to very pale blue, with extremely fine granules dispersed. Elliptical or oval nucleus may be bilobed, eccentric, purple.
- Monocytes: big cells. Circular cytoplasm with irregular rim, dark blue to dark purple, often containing one or more vacuoles. The large and generally elliptical nucleus is mainly in the centre, dark red or purple and fills most of the cell.
- Basophils: they are round cells. The circular cytoplasm is pale blue coated with fine purple granules which give the cytoplasm the appearance of being dark-purple. Large nucleus, circular, blue or light purple, eccentric.
- Eosinophils: cell shape is mostly round. Circular cytoplasm, pale blue with large orange granules. Circular or elliptical nucleus, light purple, eccentric.
- Special Granulocytic Cells (SGC): similar to neutrophils. The circular cytoplasm is weakly stained with coarse granules uncoloured by usual stains which can be seen under a light microscope as a negative image. Eccentric nucleus, circular or elliptical, reddish-purple.
- Immature cells: very irregular shapes. Abundant cytoplasm from dark purple to dark blue and the large and eccentric nucleus has a reticulate dark purple structure.

### 1. Differential leucocyte counts

The differential leucocytes counts resulting of the total analysed specimens (68 females), are given in Table 1. Lymphocytes were the most common leucocyte followed by neutrophils. Monocytes, immature cells and special granulocytic cells constituted an average of 2.5%, 1.6 and 1.8% each one, respectively, of the white cells. The results of each leucocyte by gonadal maturation stage are indicated in Table 2 and the results of the Mann-Whitney "U" Test (P values) are reported in Table 3.

The variations of mean percentages of the most abundant leucocytes (lymphocytes and neutrophils) during the gonadal maturation can be better seen in Figure 1.

**Table 1.** Range (R), mean (m) and standard deviation (s) of the leucocyte percentages and number of females of *Pimelodus maculatus* analysed (N)

	Lymphocytes	Neutrophils	Monocytes	Basophils	Eosinophils	Immature Cells	Special Granulocytic Cells
R	18.0 - 98.5	0.5 - 80.5	0.0 - 16.0	0.0 - 4.0	0.0 - 1.5	0.0 - 9.5	0.0 - 12.0
m	71.2	22.3	2.5	0.5	0.1	1.6	1.8
s	23.7	21.4	2.9	0.9	0.3	1.7	2.8
N	68	68	68	68	68	68	68

**Table 2.** Leucocytes percentages in the peripheral blood of females of *Pimelodus maculatus*, at the different gonadal maturation stages (I=Immature, IM=Initial Maturation, Ma=Maturing, Mt=Mature, S=Spent, N=number of fish, m=mean, s=standard deviation and R=range)

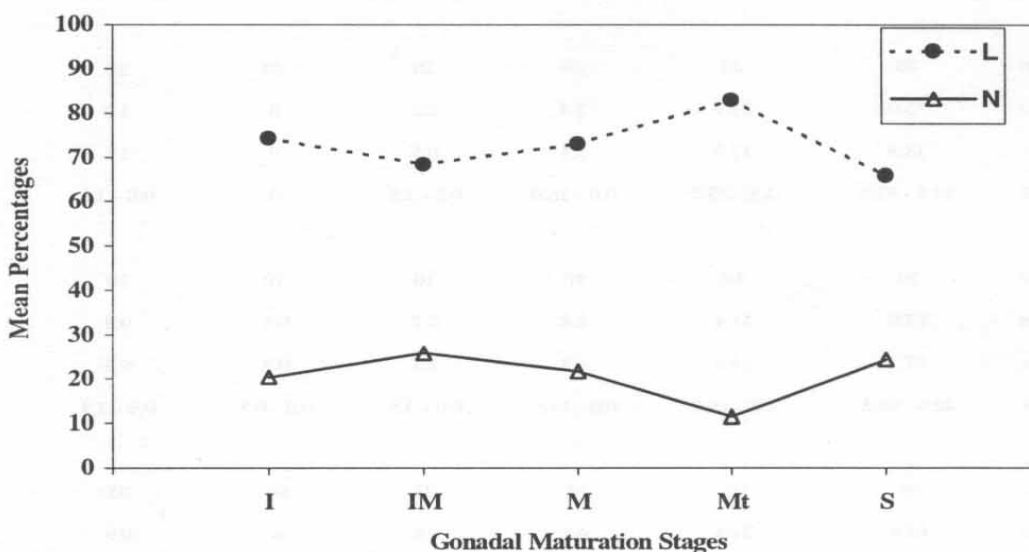
Stages		Lymphocytes	Neutrophils	Monocytes	Basophils	Eosinophils	Immature Cells	Special Granulocytic Cells
I	N	10	10	10	10	10	10	10
	m	74.3	20.4	2.3	0	0.3	2.3	0.4
	s	22.5	20.1	1.9	0	0.5	2.2	1.1
	R	29.5 - 94.5	1.0 - 58.5	0.0 - 5.5	0	0.0 - 1.5	0.0 - 6.5	0.0 - 3.0
IM	N	23	23	23	23	23	23	23
	m	68.4	25.8	2.1	0.4	0.1	1.8	1.4
	s	26.4	25.6	2.2	1.0	0.3	2.2	2.8
	R	18.0 - 97.0	1.5 - 80.5	0.0 - 7.0	0.0 - 4.0	0.0 - 1.0	0.0 - 9.5	0.0 - 10.5
Ma	N	20	20	20	20	20	20	20
	m	73.0	21.7	2.4	0.2	0	1.3	1.4
	s	18.9	17.5	3.5	0.5	0	1.1	2.8
	R	24.0 - 92.0	3.5 - 73.5	0.0 - 16.0	0.0 - 1.5	0	0.0 - 3.5	0.0 - 12.0
Mt	N	10	10	10	10	10	10	10
	m	82.9	11.4	2.8	0.7	0.1	0.8	1.3
	s	17.7	14.6	4.3	1.3	0.2	0.3	2.2
	R	42.0 - 98.5	0.5 - 45.5	0.0 - 11.5	0.0 - 3.5	0.0 - 0.5	0.0 - 2.5	0.0 - 5.0
S	N	05	05	05	05	05	05	05
	m	65.8	24.4	4.1	0.6	0	0.9	4.2
	s	22.8	23.8	2.4	0.7	0	1.0	4.4
	R	39.5 - 91.5	4.5 - 54.0	0.5 - 6.5	0.0 - 1.5	0	0.0 - 2.5	0.0 - 10.5

**Table 3.** Results of the Mann-Whitney “U” Test (P values) among fish at different gonadal maturation stage (I=immature, IM=initial maturation, Ma=maturing, Mt=mature and S=spent). (l=lymphocytes, n=neutrophils, mo=monocytes, b=basophils, e=eosinophils, ic=immature cells, sgc=special granulocytic cells). Significant differences are shown by underlined number (P<0.05)

	IM							Ma							Mt							S													
	l	n	mo	b	e	ic	sgc	l	n	mo	b	e	ic	sgc	l	n	mo	b	e	ic	sgc	l	n	mo	b	e	ic	sgc							
I	0.68							0.64							0.38								0.52												
n		0.83							0.64							0.38								0.94											
mo			0.68							0.64							0.57								0.17										
b				—							—							—								—									
e					0.65							—							0.65								—								
ic						0.34							0.30							0.11								0.22							
sgc							0.30							0.18							0.38								<u>0.04</u>						
IM								0.94						0.14								0.80													
l									0.67							0.12							0.92												
n										0.88							0.80							0.09											
mo											0.71							0.61							0.29										
b												—							0.80							—									
e													0.91							0.27							—							0.42	
ic														0.51							0.98							—							0.08
sgc															0.17							0.41						—							
Ma																	0.57						0.67					—							
l																		0.41						0.08				—							
n																			—						0.22			—							
mo																				0.22							—								
b																					0.75						—								
e																						0.09					—								
ic																							0.28				—								
sgc																								0.28			—								
Mt																									0.72		—								
l																											—								
n																											—								
mo																											—								
b																											—								
e																											—								
ic																											—								
sgc																											—								

The lymphocytes were also the dominant leucocytes in all the maturation stages followed by neutrophils. Basophils were not observed in the immature fish and eosinophils seem to be absent in

the peripheral blood of the maturing and spent specimens. The lymphocyte mean percentage showed an increase during the gonadal maturation but the observed change in the lymphocyte



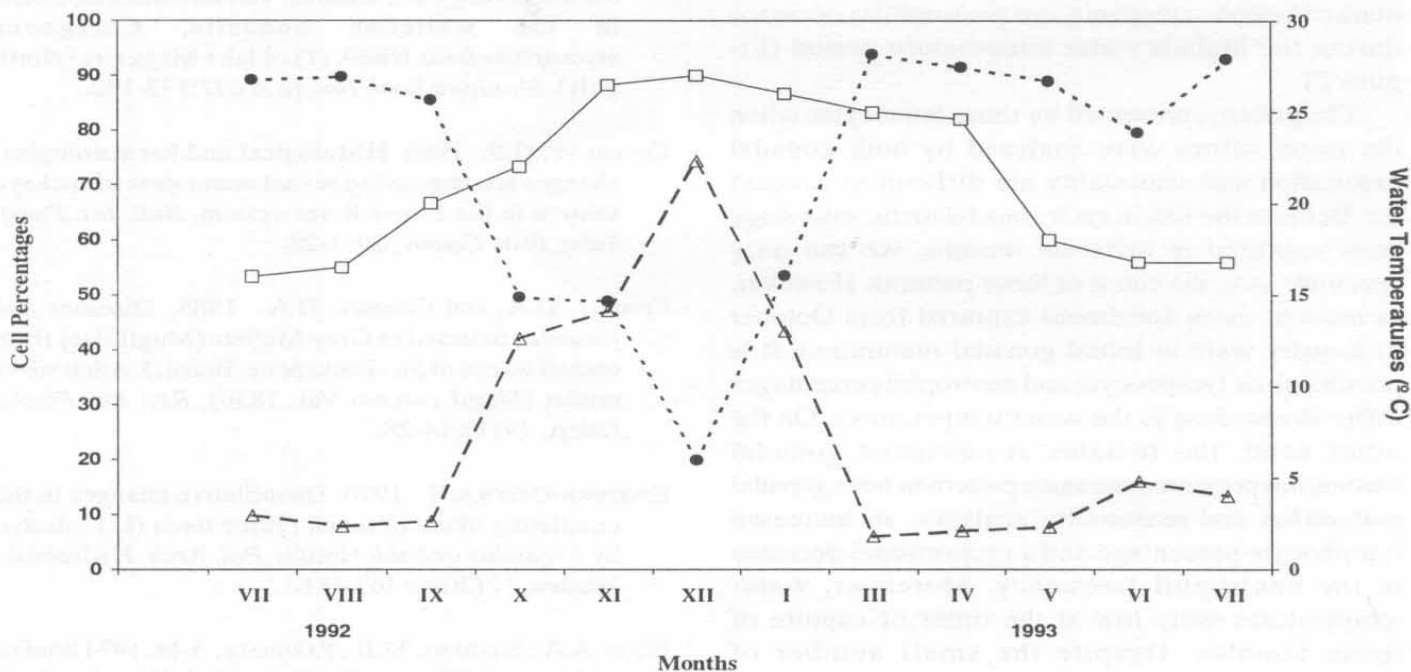
**Figure 1.** Mean percentage variations of lymphocytes (L) and neutrophils (N) in the different gonadal maturation stages (I=immature, IM=initial maturation, M=maturing, Mt=mature and S=spent)

percentages was not statistically significant. On the other hand, a significant decrease in the neutrophils percentage levels was evident in the blood of mature females when compared to the maturing specimens ( $P=0.04$ ). Significant differences were also found in the special granulocytic cell percentages between immature and spent females ( $P=0.04$ ). Non-significant variations were observed in the other peripheral blood leucocytes during the gonadal maturation of *Pimelodus maculatus* females. The mean percentages of lymphocytes and neutrophils for each month and the water temperature at the times of fish collection results are shown in Figure 2. The figures for the other types of leucocytes, monocytes, basophils, eosinophils, immature cells and special granulocytic cells have not been included, for they were very similar for all months. As one can see in Figure 2, the mean percentage of lymphocytes decrease significantly in female fish captured from

October to December, with a recovery by March. On the other hand, the neutrophils increase proportionally in those months. Water temperature was 22° C in October, 26,5° C in November, 27° C in December and 26° C in January. The lowest lymphocytes percentage and greatest frequency of neutrophils occurred in December, when the neutrophils were the most abundant leucocytes. Most of the females analysed in those months were at initial gonad maturation. The fish at advanced gonadal maturation stages (maturing, mature and spent) were collected in July and August, 1992 and from March, 1993 onwards. The sexually immature females were almost all captured in September, 1992.

### Discussion

Lymphocytes have usually been considered the most abundant of the leucocytes in the peripheral



**Figure 2.** Annual variation of water temperature (-□-) and mean percentages of lymphocytes (-•-) and neutrophils (-Δ-)

blood of healthy fish. On the other hand, neutrophils are scarce, basophils and eosinophils are only seen occasionally (ALEXANDER *et al.*, 1980, CONROY and CONROY, 1985, RANZANI-PAIVA, 1995). RIBEIRO (1978) found that the lymphocytes represented 58.5% of the white cells in the peripheral blood of *Pimelodus*

*maculatus*, neutrophils 24.6% and monocytic cells 7.2%. In our study, lymphocytes were specially more frequent (74.4%) than the neutrophils (19.6%) and monocytes (2.4%). Basophils and eosinophils were scarce in both studies.

COLGROVE (1966), RANZANI-PAIVA and GODINHO

(1983), KAVAMOTO *et al.* (1985) and PICKERING (1986) showed lymphocytopenia and neutrophilia related to the spawning period. In the present study, the opposite was found i.e. in *Pimelodus maculatus* females gonadal maturation appears to coincide with a tendency to lymphocytophilia and a significant neutropenia.

According to PICKFORD *et al.* (1971) and BENNET and NEVILLE (1975), lymphocytopenia and neutrophilia resulted of the stress caused by the water temperature. However, those studies were performed on fish exposed to a temperature of 1 or 2° C under experimental conditions. Evidences of the correlation between the fish leucocyte counts and the temperatures of their natural environments are scarce. MCLEAY (1973) *apud* BENNET and NEVILLE (1975) found that the lower the water temperature at the times of fish capture, the lower the lymphocyte percentages and the higher the neutrophil percentages. However, in our study, marked lymphocytopenia and neutrophilia occurred during the highest water temperature period (Figure 2).

The patterns presented by those leucocytes when the mean values were analysed by both gonadal maturation and seasonality are difficult to account for. Because the fish in each gonadal maturation stage were captured in different months, we can only speculate as to the cause of those patterns. However, as most of those specimens captured from October to January were at initial gonadal maturation, it is possible their lymphocyte and neutrophil percentages differ in response to the water temperatures. On the other hand, the females at advanced gonadal maturation presented the same pattern in both gonadal maturation and seasonality analyses, an increased lymphocyte percentage and a proportional decrease in the neutrophil frequency. Moreover, water temperatures were low at the times of capture of those females. Despite the small number of specimens, the results may suggest a main relation of the circulating white blood cells composition of the *Pimelodus maculatus* females to the environmental temperatures. This may explain the leucocyte percentage ranges found by gonadal maturation analyses.

Further studies on the differential leucocyte counts in *Pimelodus maculatus* sexual maturation and environmental temperature variations in following years are needed if these relationships are to be confirmed.

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