

HAEMATOLOGICAL CHARACTERISTICS ASSOCIATED WITH PARASITISM IN MULLET, *MUGIL PLATANUS* GÜNTHER, FROM THE ESTUARINE REGION OF CANANÉIA, SÃO PAULO, BRAZIL

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ABSTRACT. This study involved 344 Mulletts, *Mugil platanus* Günther, 1880, captured in the estuarine region of Cananeia, São Paulo, Brazil (25°00'S, 47°55'W). The following data were recorded for each fish: total weight, total length, haemoglobin ratio (Hb), haematocrit (Ht), erythrocyte count (Er), differential leucocyte count (lymphocytes, monocytes, neutrophils, basophils and "other cells") and haematometric indices; MCV, MCH, and MCHC. The sex and stage of gonadal maturity (undifferentiated, juvenile, resting, maturing, mature, and spent) were determined by macroscopical inspection of the gonads. Parasitological inspections revealed the following infestations: Gills – Trichodinidae, Monogenea, Copepoda, and Hirudinea. Liver: cysts of Digenea. Intestine: Nematoda, Digenea and Acanthocephala. Blood: *Trypanosoma* sp. and *Haemogregarina* sp. Analysis of variance was done to evaluate how far blood and gill parasitism had any effect on total length, total weight, the biological condition factor or the measured blood parameters. Significant differences were found for the haematocrit (infested versus noninfested by Trichodinidae) and for MCHC (infested versus noninfested by Trichodinidae and Copepoda). No differences were found with regard to leucocytes.

KEY WORDS. *Mugil platanus*, hematology, parasitism, mullet, estuarine region

The simplicity of determining certain blood parameters is probably responsible for the rise in the use of haematology as a tool for testing of health problems in fish, even those not usually directly associated with the blood.

In medicine, haematological techniques are used for the following three objectives (listed in order of frequency as reported by DAVIDSON & HENRY 1969 *apud* BLAXHALL 1972): 1) Differentiating normal from abnormal blood, 2) Diagnosis of disease or abnormality, 3) Detailed studies in haematology.

Pathological conditions in fish are manifested in the form of changes in the behavior (symptoms) and/or in the integrity of the tissues (lesions), leading to a decrease in weight gain and often, death (ROBERTS 1981).

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According to TATCHER (1981), many parasites can live in a host, sometimes causing damage, sometimes not. Therefore, it is necessary to associate changes in haematological parameters with various parasitoses to establish a data base, as ROBERTS (1981) infers, in order to allow precise diagnoses which will reliably guide the implementations of treatment or preventive measures, which are indispensable in fish farming.

Despite of the potential and present value of mullet in brazilian aquaculture (SCORVO FILHO *et al.* 1992), information about diseases been confined to isolated studies concerned with occurrence of parasites (PAPERNA & LAHAV 1974; PAPERNA & OVERSTREET 1981; CONROY & CONROY 1984; FACCHINI 1987). A success in aquaculture is keeping health fishes, resistant, to diseases and handling. Fish health maintenance is arising in economic impotence. This requires diagnoses and fight against sickness, important step for good quality in research and to fish culture (KINKELIN *et al.* 1991).

The aim of the present study is to determine changes that occur in the blood in association with parasitism, allowing for more intensive production of this species on fish farms.

MATERIAL AND METHODS

Mullets, *Mugil platanus* Günther, 1880, were collected every month from July 1988 to June 1989 from the estuarine region of Cananea, São Paulo, Brazil (25°00'S, 47°55'W). Those with a total length of more than 30cm were captured in fixed fence-traps in the region between the delta and the outlying islands and those smaller individuals were captured with throw nets in lagoons of the Comprida and Cardoso islands, where salinity is lower than in the first region.

The fish were brought to the laboratory of the Oceanographic Institute of the University of São Paulo in Cananea, where they were kept in 1000-liter asbestos-reinforced concrete tanks, the water being continuously aerated by means of compressed air. The blood was sampled soon after the fish were put into the tanks.

The blood was withdrawn by heart puncture, with disposable 3-ml syringes with heparinized 8x25 needles, and used for the following analysis: haematocrit (Ht) by the microhaematocrit method (GOLDFARB *et al.* 1971); haemoglobin ratio (Hb) by the cyanometahaemoglobin method (COLLIER 1944); erythrocyte or total cell count (Er) done in a Neubauer chamber and using Haven's solution as the diluent; haematimetric indexes (MCV, MCH and MCHC) using the formulas of WINTROBE (1934); differential leucocyte count, using smears stained by Leshman's stain.

After the blood was drawn, each fish was weighed (Wt – total weight in grams), measured (Lt – total length in centimeters) and then killed by decerebration, introducing a knife in its brain.

A ventral-longitudinal cut was made from the anus to the heart cavity, to expose the organs. The sex was determined by macroscopic inspection of the gonads, that were cut out and weighed (Wg – gonad weight in grams). The results of Lt, Wt, and Wg were used to calculate the allometric condition factor (K) following ISSAC-NAUM & VAZZOLLER (1983).

Each fish used for haematological studies was also analysed parasitologically, being liver, gills and intestine removed. Liver fragments were squashed between slide and cover slide, and inspected in a light microscope. Scrapings were taken from the gills, and also inspected between slide and cover slide in a light microscope. The entire gills were removed, and inspected through a stereomicroscope, the parasites being looked for along each gill arch, separating the lamellas. The intestine was opened longitudinally and examined under a stereomicroscope.

The parasites were removed and fixed in AFA or 70% ethyl alcohol depending on the taxonomic group, and identified to the lowest possible taxonomic level.

The blood parasites were detected during the differential leucocyte counts on the blood smears.

Statistical analysis were made considering the degree of parasitic infestation in each organ studied. For Copepoda the degree of infestation was classified from 0 to 2. For all parasites a binary classification was used: either with (1) or without parasites(0), as that the number of infected fish was low, as was the number of parasites per fish.

To evaluate if the percentages of fish infested versus not infested bore any correlation to sex, the chi-square test was used following COSTA NETO (1977).

To establish if the type and degree of parasitic infestation bore any correlation to the haematological data, the computer program LSMLMW was used (HARVEY 1987), the analysis made by the least-squares method, due to the unequal number of fish in the subclasses.

RESULTS AND DISCUSSION

This study involved 344 mullets, *Mugil platanus* (144 males, 123 females, 1 hermaphrodite and 76 undifferentiated, this term being applied to those whose gonads were difficult to characterize macroscopically).

Parasites of the following taxonomic categories were found in the respective organs: Gills: Trichodinidae, Monogenea, Copepoda and Hirudinea. Liver: cysts of Digenea. Intestines: Nematoda and Acanthocephala. Blood: *Trypanosoma* sp. and *Haemogregarina* sp.

In about 20 fish, flagellate protozoans were found in the gills, identified as *Cryptobia* sp. The two mullets with the highest degree of infestation by this protozoan were captured in the same sample (June/1989) and didn't show anything special in terms of their blood characteristics, except for a readily apparent neutrophil (88.50 and 84.00%). However, this neutrophil couldn't be considered an indicator, in that there were other specimens that also showed a high percentage of neutrophils in the absence of this parasitosis.

For the test of correlation between the haematological and parasitic data, gill and blood parasites were considered, since according to TATCHER (1981), biological agents that attack the gills or simply attach themselves there can be considered pathogenic, even in the absence of any apparent pathogenic conditions. Any decrease in environmental dissolved oxygen will affect first those fish that have a

problem of the respiratory system, especially the gills, whether in the wild or in captivity.

The percentages of infested fish, by sex, are given in table I. In this table it can be noted by the chi-square test that only for the haemogregarines there is a significant sex-correlated difference in the percentages of infested versus not infested fish ($p \leq 0.01$). It can also be noted that even though the difference is not statistically significant, the undifferentiated (captured in coastal lagoons) and the males always show a higher infestation percentage than the females, with the one exception of infestation by leeches, in which the females show the higher infestation percentage. Studies should be developed to better establish whether the higher percentage of infestation is really due to the sex of the fish, or to the environment they live in. It should be remembered that in the coastal lagoons the fish are more closely confined, a factor which may favour the transmission of parasites, especially those whose life cycle involves just one host species, as is the case in the present study.

The degree of infestation was not found to vary in any way according to the seasons of the year. The same was found by EIRAS (1987b) in relation to the infestation of *Blennius pholis* Linnaeus, 1758 by *Haemogregarina bigemina* (Laveran & Mesnil, 1901). However, CARNEVIA *et al.* (1988) studying *Mugil liza* Valenciennes, 1836 found a higher percentage of fish infested by Trichodinidae during the winter, in a context of high percentages all through the year.

Table I. Percentages of males, females and undifferentiated specimens infested by gi blood parasites: *Mugil platanus* from the estuarine region of Cananéia, São Paulo, Brazil.

Parasites (%)	Infestation level	Indifferentiated		Male		Female		Total	
		%	n	%	n	%	n	%	n
Trypanosome	Infested	8.33*	6	6.25*	9	3.79*	5	5.75	20
	Not infested	91.67*	6	93.75*	135	96.21*	127	94.25	328
	Total		72		144		132		348
Haemogregarine	Infested	19.44	14	23.13	31	21.97	29	21.89	74
	Not infested	80.55	58	76.87	103	78.03	103	78.11	264
	Total		72		134		132		338
Trichodinidae	Infested	91.43	64	93.43	128	89.76	114	91.62	306
	Not infested	8.57	6	6.57	9	10.24	28	8.38	28
	Total		70		137		127		334
Monogenea	Infested	56.34	40	30.94	43	24.43	32	33.72	115
	Not infested	43.66	31	69.06	96	75.57	99	66.28	226
	Total		71		139		131		341
Copepoda	Infestation level 1	67.60	48	51.08	71	40.46	53	50.44	172
	Infestation level 2	26.76	19	30.22	42	2.48	33	27.57	94
	Not infested	5.63	4	18.71	26	34.35	45	21.99	75
	Total		71		139		131		341
Hirudinea	Infested	4.23	3	15.83	22	24.43	32	16.72	57
	Not infested	95.77	66	84.17	117	75.57	99	83.28	284
	Total		71		139		131		341

*. $p \leq 0.1$; (n) number of individuals.

In table II it can be noted that there was a significant correlation between infestation by *Monogenea* and total length, the infested fish being shorter. This significant difference, however, does not extend to total weight or (K). KHAN (1985), TORT *et al.* (1987), as well as PAVANELLI & SANTOS (1991) found significant differences for the allometric condition factor (K) for the fish they studied, in that the infested fish showed lower values for K than the noninfested ones.

Table II. Means (\bar{X}) and standard deviation ($S\bar{x}$) of the biological characteristics of *Mugil platanus* from the estuarine region of Cananéia, São Paulo, Brazil, according to the degree of the parasitic infestation.

Parasites (%)	Infestation level	Total length (cm)	Total weight (g)	Condition factor (K)
		$\bar{X} \pm S\bar{x}$	$\bar{X} \pm S\bar{x}$	$\bar{X} \pm S\bar{x}$
Trypanosome	Not infested	42.88 ± 1.07	871.00 ± 57.00	7.81 ± 0.21
	Infestation level 1	40.96 ± 1.83	685.00 ± 98.00	7.29 ± 0.35
Haemogregarine	Not infested	42.87 ± 1.29	849.00 ± 69.00	7.54 ± 0.25
	Infestation level 1	40.97 ± 1.53	708.00 ± 82.00	7.56 ± 0.30
Trichodinidae	Not infested	41.99 ± 1.33	754.00 ± 71.00	7.45 ± 0.26
	Infestation level 1	41.85 ± 1.41	801.00 ± 75.00	7.65 ± 0.27
Monogenea	Not infested	40.43 ± 1.31	726.00 ± 70.00	7.67 ± 0.25
	Infestation level 1	43.41 ± 1.42**	831.00 ± 76.00	7.43 ± 0.28
Copepoda	Not infested	43.33 ± 1.98	821.00 ± 106.00	7.48 ± 0.39
	Infestation level 1	41.15 ± 1.23	751.00 ± 66.00	7.45 ± 0.24
	Infestation level 2	41.28 ± 1.41	762.00 ± 75.00	7.72 ± 0.27
Hirudinea	Not infested	41.09 ± 1.23	718.00 ± 65.00	7.39 ± 0.23
	Infestation level 1	42.75 ± 1.54	839.00 ± 82.00	7.71 ± 0.30

*. $p \leq 0.1$.

In the haematologic variables, (Tabs III, IV), significant differences ($p \leq 0.01$) were found only for the following: Both Ht and MCHC in relation to infested or not infested by Trichodinidae; MCHC in relation to infestation by Copepoda. There were no changes in blood characteristics for *Mugil platanus* infested by trypanosomes. This result is similar to that found by FACCHINI (1987), for *Mugil liza* captured in the same estuarine region of Cananeaia.

The percentages of leucocytes showed no significant correlation with parasitic infestation (Tab. V). One should bear in mind that the high degree of variance in the data would make small actual correlations hard to detect. It should also be remembered that in this study neither the degree of infestation nor the length of time infested were substantially part of the data.

Studies of trypanosomes in mammals have clearly shown that some species are highly pathogenic while in other classes of vertebrates the infections appear benign except in a few isolated cases (KHAN 1985). In fish there are some reports of alteration in tissues, with emphasis on alterations in the blood (COTTRELL 1977; PULSFORD 1984).

Table III. Means (\bar{X}) and standard deviation (Sx) of the blood characteristics of *Mugil platanus* from the estuarine region of Cananéia, São Paulo, Brazil, according to the degree of the parasitic infestation.

Parasites (%)	Infestation level	Hematocrit (%)	Haemoglobin concentration (g/100ml)	Erythrocyte number ($10^6/\text{mm}^3$)
		$\bar{X} \pm Sx$	$\bar{X} \pm Sx$	$\bar{X} \pm Sx$
Trypanosome	Not infested	33.04 \pm 1.06	7.94 \pm 0.24	303.62 \pm 9.20
	Infestation level 1	34.02 \pm 1.82	7.86 \pm 0.35	314.64 \pm 16.52
Haemogregarine	Not infested	32.74 \pm 1.24	7.94 \pm 0.28	304.09 \pm 11.18
	Infestation level 1	43.31 \pm 1.54	7.86 \pm 0.35	314.17 \pm 13.83
Trichodinidae	Not infested	35.30 \pm 1.31*	8.02 \pm 0.30	313.74 \pm 11.91
	Infestation level 1	31.75 \pm 1.38*	7.78 \pm 0.31	304.51 \pm 12.43
Monogenea	Not infested	33.11 \pm 1.29	7.75 \pm 0.29	306.44 \pm 11.52
	Infestation level 1	33.94 \pm 1.39	8.05 \pm 0.32	311.82 \pm 12.59
Copepoda	Not infested	32.19 \pm 1.98	8.06 \pm 0.45	302.33 \pm 18.04
	Infestation level 1	33.85 \pm 1.22	7.57 \pm 0.28	307.64 \pm 10.90
	Infestation level 2	34.54 \pm 1.39	8.07 \pm 0.31	317.41 \pm 12.51
Hirudinea	Not infested	32.42 \pm 1.17	7.66 \pm 0.27	299.44 \pm 10.62
	Infestation level 1	34.64 \pm 1.57	8.14 \pm 0.36	318.82 \pm 13.62

*. $p \leq 0.1$.

Table IV. Means (\bar{X}) and standard deviation (Sx) of the haematimetric indexes of *Mugil platanus* from the estuarine region of Cananéia, São Paulo, Brazil, according to the degree of the parasitic infestation.

Parasites (%)	Infestation level	Mean corpuscular volume (μ^3)	Mean corpuscular haemoglobin ($\mu\mu\text{g}$)	Mean corpuscular haemoglobin concentration (%)
		$\bar{X} \pm Sx$	$\bar{X} \pm Sx$	$\bar{X} \pm Sx$
Trypanosome	Not infested	104.75 \pm 3.98	28.02 \pm 0.96	23.35 \pm 0.95
	Infestation level 1	101.14 \pm 6.80	26.09 \pm 1.64	23.15 \pm 0.70
Haemogregarine	Not infested	105.04 \pm 4.64	26.42 \pm 1.12	24.46 \pm 0.65
	Infestation level 1	98.42 \pm 5.76	27.69 \pm 1.39	23.70 \pm 0.80
Trichodinidae	Not infested	107.47 \pm 4.93	26.99 \pm 1.19	25.01 \pm 0.72*
	Infestation level 1	98.42 \pm 5.17	27.12 \pm 1.25	25.47 \pm 1.03*
Monogenea	Not infested	102.03 \pm 4.81	26.94 \pm 1.16	23.98 \pm 0.73
	Infestation level 1	103.86 \pm 5.22	27.18 \pm 1.26	24.34 \pm 0.90
Copepoda	Not infested	98.21 \pm 7.41	27.87 \pm 1.79	22.63 \pm 0.64*
	Infestation level 1	105.74 \pm 4.55	25.92 \pm 1.10	24.15 \pm 0.73*
	Infestation level 2	104.87 \pm 5.21	27.37 \pm 1.26	24.10 \pm 0.61*
Hirudinea	Not infested	103.59 \pm 4.38	26.60 \pm 1.06	24.10 \pm 0.61
	Infestation level 1	102.29 \pm 5.85	27.51 \pm 1.41	24.06 \pm 0.82

*. $p \leq 0.1$.

KHAN (1977) and KHAN *et al.* (1980) suggested that for *Gadus morhua* Linnaeus, 1758 and *Myxocephalus octodecemspinosus* Pinchuk, 1814, respectively, the anemia associated with trypanosome infestation is similar to the anemia that

trypanosomes provoke in humans (JENNINGS 1976 *apud* PULSFORD 1984). LETCH & BALL (1979) suggested that the anemia in fish is more the result of repeated feeding of the leech (the vector), than a direct effect of the protozoan infestation.

Table V. Means (\bar{X}) and standard deviation ($S\bar{x}$) of the leucocyte percentages of peripheral blood of *Mugil platanus* from the estuarine region of Cananéia, São Paulo, Brazil, according to the degree of the parasitic infestation.

Parasites (%)	Infestation level	Lymphocytes	Monocytes	Neutrophils	Basophils	Other cells
		$\bar{X} \pm S\bar{x}$	$\bar{X} \pm S\bar{x}$	$\bar{X} \pm S\bar{x}$	$\bar{X} \pm S\bar{x}$	$\bar{X} \pm S\bar{x}$
Trypanosome	Not infested	50.78 ± 3.94	3.71 ± 1.02	45.00 ± 3.87	0.67 ± 0.12	0.59 ± 0.27
	Infestation level 1	49.74 ± 7.08	5.07 ± 1.83	44.89 ± 6.95	0.80 ± 0.21	0.51 ± 0.49
Haemogregarine	Not infested	44.86 ± 4.67	4.54 ± 1.21	49.98 ± 4.59	0.56 ± 0.14	0.77 ± 0.32
	Infestation level 1	55.66 ± 6.03	4.24 ± 1.56	36.91 ± 5.92	0.91 ± 0.18	0.33 ± 0.41
Trichodinidae	Not infested	48.53 ± 5.02	4.77 ± 1.30	46.42 ± 4.93	0.73 ± 0.15	0.37 ± 0.34
	Infestation level 1	51.99 ± 5.31	4.01 ± 1.37	43.46 ± 5.22	0.74 ± 0.16	0.73 ± 0.36
Monogenea	Not infested	47.02 ± 5.36	5.10 ± 1.27	41.22 ± 4.80	0.77 ± 0.15	0.48 ± 0.34
	Infestation level 1	54.51 ± 6.56	3.68 ± 1.39	48.67 ± 5.27	0.70 ± 0.16	0.62 ± 0.37
Copepoda	Not infested	44.59 ± 7.43	4.33 ± 1.92	50.44 ± 7.29	0.92 ± 0.22	0.82 ± 0.51
	Infestation level 1	55.05 ± 4.69	3.91 ± 1.21	40.63 ± 4.61	0.72 ± 0.14	0.22 ± 0.32
	Infestation level 2	51.15 ± 5.36	4.93 ± 1.39	43.77 ± 5.26	0.57 ± 0.16	0.60 ± 0.37
Hirudinea	Not infested	51.75 ± 4.55	4.17 ± 1.18	42.81 ± 4.46	0.69 ± 0.14	0.69 ± 0.31
	Infestation level 1	48.77 ± 5.74	4.62 ± 1.49	47.08 ± 5.64	0.78 ± 0.17	0.78 ± 0.39

PULSFORD (1984), for *Scyliorhinus canicola* Linnaeus, 1758 infested by trypanosomes, found a reduction of Ht and Er, and an increase in the number of leucocytes. KHAN (1985) experimentally infested *Gadus morhua* with *Trypanosoma murmanensis* Nikitin, 1927, finding alterations in blood characteristics; the Ht and the Hb being considerably lower in infested fish. Anemia was the most common aspect, positively correlated with parasitism. Anemic fish are lethargic.

In *Mugil platanus* no haemogregarines were found outside the erythrocytes, nor were there found any infested leucocytes. SMIRNOVA (1971), studying *Cyprinus carpio* Linnaeus, 1758 infested by haemogregarines found various phases of destruction of the erythrocytes in the blood of the outlying tissues of the infested fish. On certain occasions in infested fish it occurs that a high percentage of erythrocytes are destroyed by haemogregarines, so the capacity of the blood to carry out its respiratory function falls off considerably, which could be associated with the periodic deaths of carp. That author also verified a large quantity of basophils similar to monocytes but bigger and with small pseudopods, associated with parasitism. Some authors believe that part of the life cycle of haemogregarines occurs in the leucocytes (BECKER 1970; KIRMSE 1980). Infested lymphocytes show a larger number of pseudopods, which indicates an increase in cell physiology. PAPERNA & OVERSTREET (1981) hold that *Haemogregarina mugili* Carini, 1932 is, at least in the stages observed in Mugilideos, an infester of erythrocytes. Infested fish show a large quantity of pathological forms of the leucocytes in the blood of outlying tissues.

According to EIRAS (1987a), there is circumstantial evidence of a relation between the degree of water pollution and the occurrence of haemogregarines in the blood of fish. Larger fish and thus older ones are also more infested. No change in any blood characteristic was found for fish infested by this parasite, as was the case of *M. platanus*.

The copepods found in the mullets attach themselves to the gills, depending on the species, in two ways: they hug a gill filament using their anterior antenna, or they have a proboscis which they insert into a filament. The infested gills get white in their extremities due to the attachment systems of the parasites. These parasites belong to one of two families: Ergasilidae and Lernaecidae.

According to EINSZPORN-ORECKA (1970), the copepod parasites of fish cause damage to the respiratory epithelia of the gill filaments of the fish, mainly with their swimming appendages, parts of their mouths and their second antenna. The damage caused by movements, feeding and attachment of the parasites results in the appearance of necrotic alterations.

Destruction of the capillaries or deeper arteries of the gills cause constant hyperemia, resulting in a decrease in the number of erythrocytes, chronic anemia, iron deficiency and alteration in the haemoglobin ratio. These alterations depend on the time length of infestation and the extent of damage to the gill filaments.

Prolonged action of the pathogenic factor results in an impairment to haematopoietic function, and an oxygen deficiency in the tissue which intensifies, resulting in subsequent metabolic disturbances, impairing the activity of the organism. Among the problems caused, there is a visible insufficiency in the development of the reproductive organs.

NATARAJAN & FELIX (1987) observed in *Mystus gulio* Merza, 1975 contaminated by various parasites, including *Ergasilus* sp., a drastic reduction of Er, Hb and Ht, with an elevation in MCV, MCH and MCHC as compared to the noninfested fish. In the beginning of the infestation there was an increase in the values of the blood parameters. In terms of both total count and relative ratios, more lymphocytes were found in infested fish and more granulocytes in noninfested ones.

The leeches, besides provoking lesions and anemia by their own feeding, are vectors of haemogregarines and trypanosomes (KHAN 1985; PULSFORD 1984). However, KIRMSE (1980) thinks that the leeches transmit trypanosomes and that the haemogregarines are transmitted by copepods, isopods or blood-feeding monogeneans.

Although in the present study leeches were not found on every *Mugil platanus* infested by trypanosomes or haemogregarines, they were found on 16.72% of the fish (Tab. I). Lesions at the edges of the fins suggest places of leech attachment. Attached leeches may have disattached themselves when the fish were captured.

The few studies of leeches that feed on mullets are cited by PAPERNA & OVERSTREET (1981) and none of these refer to alterations in the blood provoked by these parasites.

It is also necessary to keep in mind that the alterations in blood characteristics

could be due to endogenous conditions of the fish. In this case it becomes difficult to distinguish between the effects of other variables; age, size, maturity, the environment in which the fish live, and those of parasitism.

CONCLUSION

Parasitism, in the degree found in this study, does not influence the studied blood parameters of *Mugil platanus* in natural conditions.

ACKNOWLEDGEMENTS. Thanks go to laboratory technicians Jose Plaza and Helio Sanches Mariscal, for their valuable collaborations in the collections of the fish and to graphic artist Regina Celia Barbosa da Silva, who drew the graphs. An IP-SAA/SECIRM joint project.

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Recebido em 30.XI.1995; aceito em 10.VI.1997.