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HAEMATOLOGICAL CHANGES IN *Oreochromis niloticus* (OSTEICHTHYES: CICHLIDAE) WITH GILL ICHTHYOPHTHIRIASIS AND SAPROLEGNIOSIS

[Alterações hematológicas em *Oreochromis niloticus* (Osteichthyes: Cichlidae) com ictioftiríase branquial e saprolegniose]

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ABSTRACT

The effects of gill ichthyophthiriasis and saprolegniosis in haematological parameters and relative condition factor (Kn) of *Oreochromis niloticus* (Nile tilapia), naturally infected with *Ichthyophthirius multifiliis* and *Saprolegnia* sp. were studied, in comparison with those of healthy fish. Results show no difference in relative condition factor between the two groups. However, red blood cells count (RBCC), lymphocytes percentage, hemoglobin concentration, hematocrit and mean corpuscular hemoglobin concentration were significantly lower when compared with the healthy group ($P<0.01$). In addition, mean corpuscular volume, neutrophils and monocytes percentage were significantly higher ($P<0.01$) in the infected fish. Thrombocytes showed no differences ($P>0.05$). No relation between the thrombocytes and the mechanism of organic defense was observed. These results suggest that the parasitism induced anemia and increase in the percentage of circulating neutrophils and monocytes.

Key words: haematology; *Ichthyophthirius multifiliis*; *Oreochromis niloticus*; parasites; *Saprolegnia* sp.

RESUMO

Neste trabalho estão relatados os efeitos da ictioftiríase branquial e da saprolegniose sobre as variáveis hematológicas e fator de condição relativo (Kn) de espécimes de tilápia-do-Nilo, *Oreochromis niloticus*, naturalmente infectado com *Ichthyophthirius multifiliis* e *Saprolegnia* sp., em comparação com peixes sadios. Os resultados não demonstraram diferenças estatisticamente significativas quanto ao fator de condição relativo entre os dois grupos. Entretanto, a contagem de eritrócitos, o percentual de linfócitos, a concentração de hemoglobina, o hematócrito e a concentração de hemoglobina corpuscular média (CHCM) nos peixes infectados foram significativamente ($P<0,01$) menores que os observados no grupo controle. Por outro lado, o volume corpuscular médio (VCM) e o percentual de neutrófilos e monócitos foram significativamente ($P<0,01$) superiores nos peixes infectados. O percentual de trombócitos não foi alterado significativamente ($P>0,05$) pelo parasitismo. Os resultados sugerem que o parasitismo provocou anemia e aumento percentual de neutrófilos e monócitos.

Palavras-chave: hematologia; *Ichthyophthirius multifiliis*; *Oreochromis niloticus*; parasitos; *Saprolegnia* sp.

Introduction

The presence of parasites in the water or in the fish tissue has been responsible by the occurrence of diseases in fishfarms and feefishing in Brazil (THATCHER and BRITES-NETO, 1994; MARTINS, 1998), specially in the East Region. The parasites are responsible by 83.0% of diseases in fish culture (Békési, 1992).

One of the most important parasites of cultured fishes is *Ichthyophthirius multifiliis* Fouquet, 1876 (MARTINS, 1998). It is a pathogenic protozoan with a world-wide distribution and low parasitic specificity (Eiras, 1994), which can cause severe mortality rate during the cold season (CECCARELLI *et al*., 1990; THATCHER and BRITES-NETO, 1994; MARTINS and ROMERO, 1996; MARTINS, 1998).

Mycotic disease caused by S*aprolegnia* sp. is also extremely frequent in freshwater fish. Its primary signs are the whitish or brownish masses on the body surface and gills, which may cause destruction of the epithelium. Saprolegniosis occurs in the cold season (BLY *et al*., 1996) or associated to handling stress and with other parasites (KRISHNA *et al*., 1990; SÖDERHÄL *et al*., 1991; NOGA, 1996).

The standardization of the haematological parameters is difficult in fish because these

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parameters can be influenced by deficient diet, diseases and environmental stress situations (SILVEIRA and RIGORES, 1989). However, the analysis of these parameters may improve the diagnosis of fish health (BLAXHALL and DAISLEY, 1973; ANDERSON, 1974; ALDRIN; MESSAGER; LAURENCIN, 1982).

Oreochromis niloticus Linnaeus, 1558 naturally infected with *I. multifiliis* (Protozoa) and *Saprolegnia* sp. (Fungus) was examined in order to determine the values of hematocrit, haemoglobin concentration, mean corpuscular haemoglobin, differential count of defense cells (leucocytes and thrombocytes) and to evaluate the histopathological changes.

Material and Methods

Culture characteristics and fish – Fourty Nile tilapia, *Oreochromis niloticus* were collected at a ponds (30 m^2) at a fishfarm situated in Franca, São Paulo State (20° 32' 03" S - 47° 24' 38" W; mean altitude of 1,040 m). Nile tilapia was extensively cultivated for one year, in a stocking density of three fishes per m^2. The addition of 200 g/m^2 of horse dry manure was performed four days before fish storage, for to increase primary production; after that, the pond was fertilized only by fruit and foliage proceeding from the surrounding trees. During 1997, the water quality was analyzed, according to GOLTERMAN; CLYMO; OHNSTAD(1978). In May 1997, a decrease in the water temperature (from 23 to 17°C) caused behavior changes in 80.0% of the cultivated tilapia. Twenty of these fish, which exibited behavior changes and infections by *I. multifiliis* and *Saprolegnia* sp., and another twenty healthy fish from a different pond (control) were collected for examination.

Haematological parameters and relative condition factor (Kn) – The fish were previously anaesthetized with benzocain (1g/15L) and blood samples (0.5 mL) were taken from the caudal vessels into heparinized syringes (5000 I.U.) before sacrifice. Red blood cell counts (RBCC) (mean number/mL of blood) were performed in a Neubauer chamber after dilution in NaCl solution (0.65%) and neutral red (1%). Hematocrit (Hct) was determined in microhematocrit tubes, according to GOLDENFARB *et al.* (1971). Haemoglobin concentration in blood (g/dL) was determined by the cyanomethaemoglobin method, according to COLLIER (1944), with the aid of an absorbance spectrophotometer (Analyser 500S). Differential counts of the blood cells of organic defense (leucocytes and thrombocytes) were performed in blood smears stained according to the ROSENFELD (1947) method. Two hundred cells were counted in each sample. The presence of immature leucocytes was evaluated but not quantified. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to WINTROBE (1934). After blood collection, the total weight and the standard length were measured for each fish. These data allowed the calculation of the relative condition factor (LE CREN, 1951).

Parasitological examination - During the necropsy, fragments of gills, kidney, liver, spleen and heart were excised and maintained into Petri dish with 0.65% saline solution. Pieces of the organs were mounted on cover glasses and studied microscopically. The same was performed with skin mucus and fins. Parasites were fixed and identified according to THATCHER (1991), MARTINS and ROMERO (1996) and MARTINS (1998).

Histopathological examination – Small pieces of tissues were fixed in 10% buffered formalin solution and embedded in paraffin-block. Cross sections 6 mm thick, stained with hematoxylin and eosin, were observed under microscope.

Statistical analysis - Fish were distributed into two groups: one, composed of healthy fish (control), and another, of infected fish. Groups were compared by the *F* test with 5% of probability, according to BANZATO and KRONKA (1995).

Results

The water quality analyzed showed the following parameters: water temperature 17°C to 25°C, pH 8.0 to 8.3 and dissolved oxygen 3.0 to 4.0 mg/L.

Haematological parameters and relative condition factor (Kn) - Values of the erythrocyte series and condition factor of infected tilapia are shown in Table 1. RBC, haemoglobin, hematocrit and MCHC were significantly different ($P<0.05$) when compared to those of healthy fish. MCV values of the healthy fish were lower($P<0.05$) than those observed in infected fish. Weight, length and alometric condition factor did not show any difference.

The percentage of the blood cells of organic defense (leucocytes and thrombocytes) showed an increase ($P<0.05$), particularly of neutrophils and monocytes, and a significant decrease of lymphocytes ($P<0.05$) in infected fish, in relation to that observed in healthy fish. No significant difference in thrombocytes percentage between the groups ($P>0.05$) was observed (Table 2).

Table 1. Mean values ± standard deviation and range (values in parentheses) of fish measurements, relative condition factor (Kn) and erythrogram in healthy *Oreochromis niloticus* and with ichthyophthiriasis or saprolegniosis

	Fish conditon		
Parameter	Healthy (n=20)	Infected (n=20)	*F* test
Weight (g)	157.0 ± 35.0	242.0 ± 154.0	12.10 ^{NS}
	(97.3 – 249.6)	(101.0 – 558.0)	
Length (cm)	16.6 ± 0.6	18.8 ± 4.0	9.32 ^{NS}
	(14.0 – 19.5)	(14.0 – 29.5)	
Kn	1.00 ± 0.01	1.00 ± 0.02	5.68^{NS}
	(0.98 – 1.02)	(0.96 – 1.04)	
RBCC (10^6/µL)	2.473 ± 0.74	1.402 ± 0.56	31.07*
	(1.730 – 4.830)	(0.670 – 2.450)	
Haemoglobin (g/dL)	8.5 ± 1.9	5.8 ± 1.4	9.61*
	(5.4 – 11.5)	(4.4 – 8.5)	
Haematocrit (%)	30.6 ± 5.0	24.9 ± 6.3	24.19*
	(23.0 – 41.0)	(8.0 – 37.0)	
MCV (fL)	133.7 ± 31.8	196.6 ± 87.3	8.66*
	(70.8 – 205.0)	(64.0 – 375.0)	
MCHC (g/dL)	28.9 ± 9.7	23.4 ± 4.3	5.10*
	(16.8 – 55.2)	(14.1 – 29.3)	

NS - not significant (P>0.05); *- significant (P<0.05)

Table 2. Percentage of the defense blood cells in healthy *Oreochromis niloticus* and in that with ichthyophthiriasis and saprolegniosis. Mean values ± standard deviation and range (values in parentheses)

	Fish conditon		
Parameter	Healthy (n=20)	Infected (n=20)	*F*- test
Thrombocytes (%)	59.8 ± 14.0	53.6 ± 12.8	2.15 ^{NS}
	(21.0 – 81.0)	(30.0 – 71.0)	
Lymphocytes (%)	21.7 ± 8.1	9.4 ± 6.1	33.04*
	(11.0 – 42.0)	(3.0 – 30.0)	
Neutrophils (%)	10.7 ± 6.3	20.5 ± 14.6	6.41*
	(3.0 – 27.0)	(1.0 – 52.0)	
Monocytes (%)	7.5 ± 6.8	16.3 ± 5.7	21.35*
	(0.0 – 32.0)	(4.0 – 26.0)	

NS - not significant (P>0.05); *- significant (P<0.05)

Behavior and histopathological and parasitological examination – Sick fish showed erratic swimming, agglomeration near the water inflow and excessive mucus production. Petechiae and white spot (1.0 mm diameter) on the body surface were also observed. Cotton-like structures of brownish color were observed on the scales. Gills showed brownish mucus, petechiae and bloody aspect. Some animals presented pale liver, with increased volume. Microscopical observation of gills and mucus revealed a ciliate protozoan with 0.5 to 1.0 mm diameter, presenting a characteristic horseshoe nucleus, which was identified as *Ichthyophthirius multifiliis* Fouquet, 1876 (NOGA, 1996; MARTINS, 1998).

The examination of the cotton-like structures showed the existence of asseptated hiphae of different sizes provided by the sporangious, being identified as *Saprolegnia* sp. (NOGA, 1996; MARTINS, 1998).

Histopathological analysis revealed the presence of a great number of *I. multifiliis* in the gills between the secondary lamellae, which presented various degrees of hyperplasia of the epithelium and goblet cells that often occupied interlamellar spaces. Several parasites were observed in the hyperplasic epithelium, causing fusion of the secondary lamellae extremities. In addition, there was sinusoidal cappilar congestion and telangiectasis. Mononuclear inflammatory infiltration was also observed in some cases. Ulcers and erosions of the skin epithelium were associated with mononuclear inflammatory infiltration (Figure 1). The observation of other tissues of infected or healthy fish did not present structural changes.

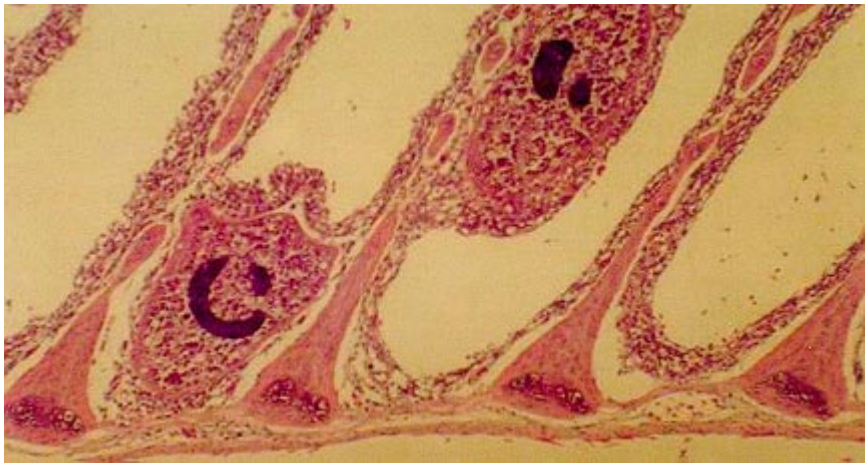

Figure1. Gill filaments in the primary lamellae of *Oreochromis niloticus* infected with *Ichthyophthirius multifiliis*. Stain HE x 1,365

Discussion

The determination of condition factor has been utilized most effectively in comparing two or more fish populations living under similar or different feeding, density, climate (WEATHERLEY and GILL, 1987), and health (MURAD and MUSTAFA, 1988) conditions. In the present study, the relative condition factor (Kn) of infected and non infected tilapia was similar. This result showed that the parasitism level did not occur in sufficient time to prejudice the fish development. Such observations confirm those of RANZANI-PAIVA *et al*. (1997), in mullets *Mugil platanus* parasitized by monogenean, copedods and Trichodinea, and also those of TAVARES-DIAS *et al*. (1999a) in *Piaractus mesopotamicus* and *Leporinus macrocephalus* parasitized by the monogenean *Lernaea cyprinacea* and *I. multifiliis*. MURAD and MUSTAFA (1988), KUROVSKAYA and OSADCHAYA (1993) and TAVARES-DIAS *et al*.(2000a) observed in parasitized fish lower values of condition factor when compared to those of the nonparasitized ones, probably due to the infection degree and time of parasitism.

Haematological studies may improve the evaluation of the health of a fish subjected to deficient diet, diseases and changes in the environmental conditions (BLAXHALL and DAISLEY, 1973, ACHUTHAN-NAIR and BALAKRISHNAN-NAIR, 1983). In the present study, the lowest values of erythrocytes number, hematocrit and haemoglobin indicate that the parasitized fish had a severe anemia. The blood indices MCV and MCHC were influenced by the parasitism in *O. niloticus*, and suggest the existence of macrocytic-hypochromic anemia. Changes in the

cellular composition of the blood as well as in the electrolytes values have been shown to be associated with infection (STOSKOPF,1993; YILDIZ, 1998). Decrease in the number of erythrocytes together with the increase of its volume is a sort of compensation for the haemoglobin oxygen transport. Reduction in the erythrocyte number, haemoglobin concentration and CHCM in *O. niloticus* with ichthyophthiriasis and saprolegniosis was observed. On the other hand, the increase of VCM may be the first response of fish in order to compensate the decrease in the number of erythrocytes. The greatest erythrocytes may carrier high quantities of haemoglobin.

Other studies have reported anemic process and macrocytic-hypochromic anemia in *Channa striatus* infested by the isopod, *Alitropus typus* (ACHUTHAN-NAIR and BALAKRISHNAN-NAIR, 1983) and normocytic-hypochromic anemia in *Heteropneustes fossilis* with metacercariae of *Diplostomulum* sp. (MURAD and MUSTAFA, 1988). On the other hand, KUROVSKAYA and OSADCHAYA (1993) did not report changes in erythrocyte and haemoglobin values of the carp *Cyprinus carpio* infected with *I. multifiliis*. It may be considered that the utilized fish was provenient of extensive culture, where *I. multifiliis* and *Saprolegnia* sp. were naturally present. The cited authors have studied the cultivated fish fed with balanced diet and experimentally infected with *I. multifiliis*. Such conditions may have stimulated fish resistance to the parasite.

Factors such as low temperature or bad water quality may be responsible for deficient development in tilapia (PAIVA *et al.*, 1988; CASTAGNOLLI, 1992). Low temperature may reduce fish activity, feeding behavior and metabolism (BOYD, 1990). Non infected tilapia (control) showed haematological values similar to or higher than those related in the literature to the same fish genus (DABROWSKA; MEYER-BURGDORFF; GÜNTHER, 1989; SILVEIRA and RIGORES, 1989; ALLEN, 1993a,b; SUN; CHEN; CHANG, 1994; NUSSEY; VAN VUREN; DU PREEZ, 1995a,b). Thus, the anemia observed in the fish of this study was probably caused by parasitism. This is especially true because no factors responsible for anemic process were observed.

Leucocyte number changes may occur in infectious diseases (BLAXHALL and DAISLEY, 1973; ANDERSON, 1974; ALDRIN; MESSAGER; LAURENCIN, 1982) and significant changes in the leucocyte number related to the degree of ichthyophthiriasis infection in *C. carpio* were mentioned (KUROVSKAYA and OSADCHAYA, 1993). In the present study, decrease in lymphocytes percentage and increase in those of neutrophils and monocytes were observed. Similar results, such as lymphocytopenia, neutrophilia and monocytosis in *Schizodon intermedius* infested by *L. cyprinacea* were related (SILVA-SOUZA; ALMEIDA; MACHADO, 2000). TAVARES-DIAS; MARTINS; KRONKA (1999) reported the occurrence of thrombocytopenia and monocytosis in *P. mesopotamicus* parasitized with *Argulus* sp. The assintomatic fish showed neutrophilia and relative lymphopenia, similar to that observed by HINES and SPIRA (1973) in carp with ichthyophthiriasis. In the present work, lymphopenia and relative neutrofilia were also observed, corroborating the foundings of the authors cited anteriorly. Changes in the numbers of lymphocytes and neutrophills are considered as secondary responses in different kinds of stress (CARNEIRO and URBINATI, 1998; MARTINS *et al.*, 2000). Although other parameters, such as glucose and cortisol levels, were not evaluated, it may be possible that infection provokes physiological disturbs.

Thrombocyte may be considered blood cell of defense (for details see TAVARES-DIAS *et al.*(1999b,c) and Tavares-Dias *et al.* (2000b,c,d). Because it is involved in the organic defense mechanism (Yokoiama, 1960 and Fange, 1968, *apud* ELLIS, 1976; PENHA; DIAS; MALUCELLI, 1996; MATUSHIMA and MARIANO, 1996; MARTINS, 2000). When the organic defense mechanism is concerned, the counts of the blood defense cells include leucocytes and thrombocytes (TAVARES-DIAS *et al.*,1999b,c and TAVARES-DIAS *et al.*, 2000b,c,d). This concept is based in the pathology aspects but not in the phisiological ones.

No significant difference were observed in the thrombocytes percentages of infected and healthy tilapia. Such cells do not have their origin in a white blood cell, but are present in inflammatory exudate and their phagocytic activity in the organic defense is comproved (GRECCHI; SALIBA; MARIANO, 1980; ISHIDA *et al.*,1985; KAJIGAYA *et al.*, 1985; SUZUKI, 1986; DIAS and SINHORINI, 1991; MATUSHIMA and MARIANO, 1996; PENHA; DIAS; MALUCELLI, 1996; HILL and ROWLEY, 1996). MATUSHIMA and MARIANO (1996) reported a predominance of thrombocytes (80.0%) in the inflammatory exudate provoked by carrageenin injected into the air cavity of Nile tilapia. These results were confirmed by MARTINS (2000). Nevertheless, AFONSO; ELLIS; SILVA (1997) showed

lymphocytes, macrophages, neutrophils, and, very rarely, thrombocytes as the predominant cells in the unstimulated peritoneal cavity of *Oncorhynchus mykiss*. The abundance of thrombocytes in blood of healthy fish (CHONDAR, 1982; MURRAY, 1984; LEA-MASTER *et al*., 1990, TAVARES-DIAS and FAUSTINO, 1998; TAVARES-DIAS *et al*., 1999b,c; TAVARES-DIAS *et al*., 2000b,c,d) and in the infected ones (TAVARES-DIAS *et al*., 1999a, TAVARES-DIAS; MARTINS; KRONKA, 1999; TAVARES-DIAS, 2000) was reported and this fact was considered normal. This kind of cell is erroneously included in the leukocyte differential count when the phisiological point of view is concerned (GARDNER and YEVICH, 1969; HINES and YASHOUV, 1970; CHONDAR, 1982; MURRAY, 1984; LEA MASTER *et al*., 1990; QUENTEL and OBACH, 1992; HOUSTON; DOBRIC; KAHURANANGA, 1996). To avoid any doubt concerning the present study, the authors included thrombocytes in the count of defense blood cells (lymphocytes, monocytes, neutrophils and trombocytes), considering the pathological aspect, although infected tilapia did not present changes in the thrombocytes percentage, when compared to healthy fish.

The immune fish response is negatively influenced by the decline in the environmental temperature, because the stress of handling in the cold season is responsible for secondary infections (BULLOCK, 1964; SNIESKO, 1964; WEDEMEYER, 1971; FINN and NIELSEN, 1971; KURATA *et al*., 1995; TAVARES-DIAS; MARTINS; MORAES, 2001). *I. multifiliis* and *Saprolegnia* sp. have appeared more frequently in the winter or under inadequate handling of fish culture, caused by denutrition and poor water quality (CECCARELLI *et al*., 1990; THATCHER and BRITES-NETO, 1994; NOGA, 1996; MARTINS and ROMERO, 1996; MARTINS, 1998). This outbreak occurred in the winter when the water inlets are lower than in the summer. For this reason, before the cold season, fish farmers must concentrate attention in prophylaxis, avoiding stress and unbalance of the host-parasite-environment system which may be reached with suplementation of vitamin C (PETRIC, 2000; MARTINS, 2000)

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References

ACHUTHAN-NAIR, G. and BALAKRISHNAN-NAIR, N. 1983 Effect of infestation with the isopod, *Alitropus typus* M. Edwards (Crustacea: Flabellifera: Aegidae) on the haematological parameters of the host fish, *Channa striatus* (Bloch). *Aquaculture*, *30*: 11-19.

AFONSO, A.; ELLIS, A.E.; SILVA, M.T. 1997 The leucocyte population of the unstimulated peritoneal cavity of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Imunol*., *7*: 335-348.

ALDRIN, J.F.; MESSAGER, J.L.; LAURENCIN, F.B. 1982 La biochimie clinique en aquaculture. Intérêt et perspective. *CNEXO Actes Colloq.*, *14*: 219-326.

ALLEN, P. 1993a Determination of haematological parameters of *Oreochromis aureus* (Steindachner) and the effects of heparin on these. *Comp. Biochem. Physiol.*, *106A*: 355-358.

_____ 1993b Effects of acute exposure to cadmium (II) chloride and lead (II) chloride on the haematological profile of *Oreochromis aureus* (Steindachner). *Comp. Biochem. Physiol.*, 105C: 213-217.

ANDERSON, D.P. 1974 *Fish Immunology*. Neptune: T.F.H. Publications, New Jersey. 239p.

BANZATO, D.A. and KRONKA, S.N. 1995 *Experimentação Agrícola*. 3ª ed. Jaboticabal: Ed. FUNEP. 247p.

BÉKÉSI, L. 1992 Evaluation on data of ichthyopathological analyses in the Brazilian northeast. *J. Braz. Assoc. Advanc. Sc.*, *44*: 400-403.

BLAXHALL, P.C. and DAISLEY, K.W. 1973 Routine hematological methods for use with fish blood. *J. Fish. Biol.*, *5*: 771-781.

BLY, J.E.; QUINIOU, S.M.A.; LAWSON, L.A.; CLEM, L.W. 1996 Therapeutic and prophylatic measures for winter saprolegniosis in channel catfish. *Dis. Aquat. Organ.*, *24*: 25-33.

BOYD, C. 1990 *Water Quality in Ponds for Aquaculture*. Alabama: Birmingham Publishing Co. 482p.

BULLOCK, G. 1964 Pseudomonadales as fish pathogens. *Develop. Indust. Microbiol.*, *5*: 101-106.

CARNEIRO, P.C.F and URBINATI, E.C. 1998 Alterações metabólicas, hematológicas e osmoregulatórias do matrinxã *Brycon cephalus* causadas pelo estresse de transporte. *Aqüicultura Brasil'98, 2*:609-620.

CASTAGNOLLI, N. 1992 *Piscicultura de Água Doce*. Jaboticabal: FUNEP. 189p.

CECCARELLI, P.S.; FIGUEIRA, L.B.; FERRAZ-LIMA, C.B.L.; OLIVEIRA, C.A. 1990 Observações sobre a ocorrência de parasitos no CEPTA entre 1983 e 1990. *Bol. Téc. CEPTA, 3*: 43-54.

COLLIER, H.B. 1944 The standardization of blood haemoglobin determinations. *Canad. Med. Assoc. J., 50*: 550-552.

CHONDAR, S.L. 1982 The haematology of *Gudusia chapra* (Pisces: Clupeidae). *J. Inland Fish. Soc. India, 14*: 1-10.

DABROWSKA, H.; MEYER-BURGDORFF, K.; GÜNTHER, K.D. 1989 Interaction between dietary protein and magnesium level in tilapia (*Oreochromis niloticus*). *Aquaculture*, *76*: 277-291.

DIAS, J.L.C. and SINHORINI, I.L. 1991 Qualitative evaluation of the inflammatory response modulated by temperature in tadpoles of *Rana catesbeiana*. *J. Braz. Assoc. Advanc. Sci., 43*: 304-306.

EIRAS, J.C. 1994 *Elementos de Ictioparasitologia*. Porto: Fundação Eng. Antônio de Almeida. 339p.

ELLIS, A.E. 1976 Leucocytes and related cells in the plaice, *Pleuronectes platessa*. *J. Fish Biol., 8*: 143-156.

FINN, J.P. and NIELSEN, N.O. 1971 The effect of temperature variation on the immune response of rainbow trout. *J. Pathol., 105*: 257-268.

GARDNER, R.G. and YEVICH, P.P. 1969 Studies on the blood morphology of three estuarine cyprinodontoform fishes. *J. Fish. Res. Board. Can., 26*: 433-447.

GOLDENFARB, P.B.; BOWYER, F.P.; HALL, E.E.; BROSIOUS, E. 1971 Reproducibility in the hematology laboratory: the microhematocrit determination. *Amer. J. Clin. Pathol., 56*: 35-39.

GOLTERMAN, H.L.; CLYMO, R.S.; OHNSTAD, M.A.M. 1978 *Methods for Physical and Chemical Analysis of Freshwaters*. London: Blackweel Sci. Publ., IBP. Handbook number 8. 214p.

GRECCHI, R.; SALIBA, A.M.; MARIANO, M. 1980 Morphological changes, surface receptors and phagocytic potencial of fowl mononuclear phagocytes and trombocytes *in vivo* and *in vitro*. *J. Pathol., 130*: 23-31.

HILL, D.J. and ROWLEY, A.F. 1996 The thromboxane mimetic, U-46619, induces the aggregation of fish thrombocytes. *Brit. J. Haematol., 92*: 200-211.

HINES, R. and YASHOUV, A. 1970 Differential leukocyte counts and total leukocyte and erythrocyte counts for same normal israeli mirror carp. *Bamidgeh, 22*: 106-113.

HINES, R.S. and SPIRA, D.T. 1973 Ichthyophthiriasis in the mirror carp.II. Leukocytes response. *J. Fish Biol., 5*: 527-534.

HOUSTON, A.H.; DOBRIC, N.; KAHURANANGA, R. 1996 The nature of hematological response in fish. *Fish Physiol. Biochem., 15*: 339-347.

ISHIDA, Y.; SUGYIAMA, M.; KAJIGAYA, H.; UMEDEA, M.; ISODA, M. 1985 Inflammatory reactions on Japanese quails muscular tissue caused by sutures. *Bull. Nippon. Vet. Zootech. Coll., 31*: 39-46.

KAJIGAYA, H.; KAMEKURA, M.; TANAHARA, N.; OTHA, A.; SUZUKI, H.; SUGIYAMA, M.; ISODA, M. 1985 Acute and chronic inflammation induced by silk sutures in chicken mesentery. *Bull. Nippon Vet. Zootech. Coll., 34*: 35-38.

KRISHNA, L.; GUPTA, V.K.; KATOCH, R.C.; SINGH, D. 1990 Saprolegniosis in indian major carps -an investigation. *Indian Vet. J., 67*: 554-555.

KURATA, O.; OKAMOTO, N.; SUZUMURA, E.; SANO, N.; IKEDA, Y. 1995 Accommodation of carp natural killer-like cells to environmental temperatures. *Aquaculture, 129*: 421-424.

KUROSVSKAYA, L.N. and OSADCHAYA, S.A. 1993 The influence of *Ichthyophthirius multifiliis* on underyearling carp, *Cyprinus carpio*. *J. Ichthyol., 33*: 81-92.

LEA-MASTER, B.R.; BROCK, J.A.; FUJIOKA, R.S.; NAKAMURA, R.M. 1990 Hematologic and blood chemistry values for *Sarotherodon melanotheron* and a red hybrid tilapia in freshwater and seawater. *Comp. Biochem. Physiol., 97A*: 525-529.

LE CREN, E.D. 1951 The length-weight relationship and seasonal cycle in gonadal weight and condiction in the perch (*Perca fluviatilis*). *J. Anim. Ecol., 20*: p. 201-219.

MARTINS, M.L. 1998 *Doenças Infecciosas e Parasitárias*

de peixes. Jaboticabal: FUNEP. 65p.

MARTINS, M.L. 2000 *Efeito da suplementação com vitamina C sobre a reação inflamatória em* ***Piaractus mesopotamicus*** *Holmberg, 1887 estressados*. Jaboticabal, Centro de Aqüicultura da UNESP. 130p. (Tese de Doutoramento. Universidade Estadual Paulista, CAUNESP).

_____; MORAES, F.R.; MORAES, J.R.E.; MALHEIROS, E.B. 2000 Falha na resposta do cortisol ao estresse por captura e por carragenina em *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae). *Acta Scientiarum, 22*: 545-552.

_____ and ROMERO, N.G. 1996 Efectos del parasitismo sobre el tejido branquial en peces cultivados: estudio parasitologico e histopatologico. *Revta bras. Zool., 13*: 489-500.

MATUSHIMA, E.R. and MARIANO, M. 1996 Kinetics of the inflammatory reaction induced by carrageenin in the swimbladder of *Oreochromis niloticus* (Nile tilapia). *Braz. J. vet. Res. anim. Sci., 33*: 5-10.

MURAD, A. and MUSTAFA, S. 1988 Blood parameters of catfish, *Heteropneustes fossilis* (Bloch), parasitized by metacercariae of *Diplostomulum* sp. *J. Fish Dis., 11*: 365-368.

MURRAY, S.A. 1984 Hematological study of the bluegill, *Lepomis macrochirus* Raf. *Comp. Biochem. Physiol., 78A*: 787-791.

NOGA, E.J. 1996 *Fish Disease. Diagnosis and Treatment*. St. Louis: Mosby. 367p.

NUSSEY, G.; VAN VUREN, J.H.J.; DU PREEZ, H.H. 1995a Effects of copper on the haematological and osmoregulation of the Mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). *Comp. Biochem. Physiol., 111C*: 369-380.

_____; _____; _____ 1995b Effects of copper on the differential white cell couts of the Mozambique tilapia, *Oreochromis mossambicus*. *Comp. Biochem. Physiol., 111C*: 381-388.

PAIVA, P.; VERANI, J.R.; MAINARDES-PINTO, C.S.R.; TABATA, Y.A. 1988 Studies on the growth and reproduction in the cichlid *Oreochromis niloticus* (Linnaeus, 1757) in a earthern pond. *B. Inst. Pesca, 15*: 109-114.

PENHA, M.L.; DIAS, J.L.C.; MALUCELLI, B.E. 1996 Influence of low environmental temperature on the phagocytic activity of bullfrog (*Rana catesbeiana*) thrombocytes. *Braz. J. vet. Res. anim. Sci., 33*(1):15-18.

PETRIC, M.C. 2000 *Efeito da suplementação alimentar com vitamina C sobre a formação de gigantócitos em lamínulas de vidro implantadadas no tecido subcutâneo de pacus (****Piaractus mesopotamicus*** Holmberg, 1887*)*. Jaboticabal, Departamento de Patologia Veterinária. 86p. (Dissertação de Mestrado. Faculdade de Ciências Agrárias e Veterinárias- Unesp).

QUENTEL, C. and OBACH, A. 1992 The cellular composition of the blood and haematopoietic organs of turbot *Scophthalmus maximus* L. *J. Fish Biol., 41*: 709-716.

RANZANI-PAIVA, M.J.; ISHIKAWA, C.M.; CAMPOS, B.E.S.; EIRAS, A.C. 1997 Haematological characteristics associated with parasitism in mullets, *Mugil platanus* Günther, from the estuarine region of Cananéia, São Paulo, Brazil. *Revta bras. Zool., 14*: 329-339.

ROSENFELD, G. 1947 Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. *Mem. Inst. Butantan, 20*: 329-334.

SILVA-SOUZA, A.T.; ALMEIDA, S.C.; MACHADO, P.M. 2000 Effect of the infestation by *Lernaea cyprinacea* Linnaeus, 1758 (Copedoda, Lernaeidae) on the leucocytes of *Schizodon intermedius* Garavello & Britski, 1990 (Osteichthyes: Anostomidae). *Rev. Brasil. Biol., 60*(2): 217-220.

SILVEIRA, R. and RIGORES, C. 1989 Caracteristicas hematologicas normales de *Oreochromis aureus* em cultivo. *Rev. Latinoam. Acuicul., 39*: 54-56.

SNIESZKO, S.F. 1964 Remarks on some effects of bacterial fish diseases. *Develop. Indus. Microbiol., 5*: 97-102.

SÖDERHÄLL, K.; DICK, M.W.; CLARK, G.; FÜRST, M.; CONSTANTINESCU, O. 1991 Isolation of *Saprolegnia parasitica* from the crayfish *Astacus leptodactylus*. *Aquaculture, 92*: 121-125.

STOSKOPF, M.K. 1993 *Fish medicine*. Philadelphia: W.B., Saunders Company. 882p.

SUN, L.T.; CHEN, G.R.; CHANG, C.F. 1994 Characteristics of blood parameters and gill Na^+-K^+-ATPase in chilled comatosa tilapia cultured in various salinities. *Comp. Biochem. Physiol., 107A*: 641-646.

SUZUKI, K. 1986 Morphological and phagocytic characteristics of peritoneal exudate cells in tilapia, *Oreochromis niloticus* (Trewavas), and carp, *Cyprinus carpio* L. *J. Fish Biol., 29*: 349-364.

TAVARES-DIAS, M. 2000 *Estudos parasitológico e hematológico em peixes oriundos de "pesque-pagues" do município de Franca, São Paulo*. Jaboticabal, Centro de Aqüicultura da UNESP. 130p (Dissertação de Mestrado. Universidade Estadual Paulista - CAUNESP).

_____ and FAUSTINO, C.D. 1998 Parâmetros hematológicos da tilápia-do-Nilo *Oreochromis niloticus* (Cichlidae) em cultivo extensivo. *Ars Veterinaria, 14*: 254-263.

_____; SCHALCH, S.H.C.; MARTINS, M.L.; SILVA, E.D.; MORAES, F.R.; PERECIN, D. 1999a Hematologia de teleósteos brasileiros com infecção parasitária. I. Variáveis do *Leporinus macrocephalus* Garavello & Britski, 1988 (Anostomidae) e *Piaractus mesopotamicus* Holmberg, 1887 (Characidae). *Acta Scientiarum*, *21*: 337-342.

_____; TENANI, R.A.; GIOLI, L.D.; FAUSTINO, C.D. 1999b Características hematológicas de teleósteos brasileiros. II. Parâmetros sangüíneos do *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae) em policultivo intensivo. *Revta bras. Zool.*, *16*: 423-431.

_____; FRASCÁ-SCORVO, C.M.D.; MORAES, F.R.; CAMPOS-FILHO, E. 1999c Características hematológicas de teleósteos brasileiros. IV. Parâmetros eritroleucométricos, trombométricos e glicemia do matrinxã *Brycon cephalus* Günther, 1869 (Osteichthyes: Characidae). *Ars Veterinaria*, *15*(3): 149-153,.

_____; MARTINS, M.L.; KRONKA, S.N. 1999d Evaluation of the haematological parameters in *Piaractus mesopotamicus* Holmberg (Osteichthyes: Characidae) with *Argulus* sp (Crustacea, Branchiura) infestation and treatment with organophosphate. *Revta. Bras. Zool.*, *16*: 553-555.

_____; _____ .; MORAES, F.R.; KRONKA, S.N. 2000a Fator de condição e relação hepato e esplenossomática em teleósteos de água doce naturalmente parasitados. *Acta Scientiarum, 22*(2): 533-537.

_____ ; SCHALCH, S.H.C.; MARTINS, M.L.; ONAKA, E.M.; MORAES, F.R. 2000b Haematological Characteristics of Brazilian Teleosts. III. Parameters of the Hybrid Tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*) (Osteichthyes: Characidae). *Revta bras. Zool., 17*(4): 899-926.

TAVARES-DIAS, M.; FRASCÁ-SCORVO, C.M.D.; NOVATO, P.F.C.; MORAES, F.R. 2000c Haematological Characteristics of Hybrid Florida Red Tilapia, *Oreochromis urolepis hornorun x O. mossambicus* under intensive rearing. In: TILAPIA AQUACULTURE IN THE 21st CENTURY, 3-7 set., Rio de Janeiro, 2000. *Anais*... Rio de Janeiro (Proceedings International Symposium on Tilapia Aquaculture). p.533-541.

_____; SCHALCH, S.H.C.; SILVA, E.D.; MARTINS, M.L.; MORAES, F.R. 2000d Características Hematológicas de *Oreochromis niloticus* (Osteichthyes: Cichlidae) Cultivada Intensivamente em "Pesque-Pague" do Município de Franca, São Paulo, Brasil. *Ars Veterinaria, 16*(2): 76-82.

_____; MARTINS, M.L.; MORAES, F.R. 2001 Fauna parasitária de peixes oriundos de "pesque-pague" do município de Franca, São Paulo, Brasil. I. Protozoários. *Revta bras. Zool., 18*(supl. 1): 67-79.

THATCHER, V.E. 1991 Amazon fish parasites. *Amazoniana, 11*(3/4): 263-572.

_____ and BRITES-NETO, J. 1994 Diagnóstico, prevenção e tratamento das enfermidades de peixes neotropicais de água doce. *Rev. Bras. Med. Vet.*, *16*: 111-128.

WEATHERLEY, A.H. and GILL, H.S. 1987 *The biology of fish growth*. New York: Academic Press. 443p.

WEDEMEYER, G. 1971 The stress of formalin treatments in rainbow *trout* (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *J. Fish Res. Board. Can.*, *28*: 1899-1904.

WINTROBE, M.M. 1934 Variations on the size and hemoglobin content of erythrocytes in the blood of various vertebrates. *Fol. Haematol.*, *51*: 32-49.

YILDIZ, H.Y. 1998 Effects of experimental infection with *Pseudomonas fluorescens* on different blood parameters in carp (*Cyprinus carpio* L.). *Israeli J. Aquaculture - Bamidgeh, 50*:82-85.