

**Haematological characteristics of Brazilian Teleosts.
III. Parameters of the hybrid tambacu (*Piaractus
mesopotamicus* Holmberg x *Colossoma macropomum*
Cuvier) (Osteichthyes, Characidae)**

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ABSTRACT. Fifty six specimens of the hybrid “tambacu” (*Piaractus mesopotamicus* Holmberg, 1887 male x *Colossoma macropomum* Cuvier, 1818 female) were collected from fishfarm of Guariba, São Paulo, to evaluate their haematology. Fishes presented 400.0 to 3,100.0 g total weight and 20.0 to 52.0 cm total length. Haemoglobin, haematocrit, mean corpuscular haemoglobin content (MCHC) and percentage of defense blood cells including leucocytes and thrombocytes, were studied. Statistical analysis showed positive correlation ($P<0.01$) between haematocrit, MCHC and haemoglobin rate. Nevertheless, thrombocytes and lymphocytes showed negative correlation ($P<0.01$).

KEY WORDS. tambacu hybrid, haematology, haemoglobin, haematocrit, leucocytes, thrombocytes

Brazilian aquaculture initiated with the Northeast settlement Netherland colonizers. Still, the culture of native species was limited for a long period due to the lack of technology of fry production (ZANIBONI-FILHO 1997). Recent development of technics to increase the economy of fishfarming was responsible for the investments on some native species like the “tambaqui” *Colossoma macropomum* Cuvier, 1818 and the “pacu” *Piaractus mesopotamicus* Holmberg, 1887 (CASTAGNOLLI 1992). Environmental factors bring about limitations in the culture of native species. However, hybridization was a solution for this problem, culminating with the hybrid “tambacu”. “Tambacu” has a great economical importance but little is known about their physiology and haematology. Standardization of methods is important for determination of the effects of diet, diseases, handling stress and other environmental factors upon fishes (SILVEIRA & RIGORES 1989).

Recent reports on haematological parameters of intensively cultivated *P. mesopotamicus* showed similar values when compared to *C. macropomum* (TAVARES-DIAS *et al.* 1998, 1999a,b).

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In the present work, haematological parameters of tambacu hybrids reared in the commercial fishfarm, mean values of haematocrit, haemoglobin, mean corpuscular haemoglobin concentration, differential count of defense cells (leucocytes and thrombocytes) were evaluated.

MATERIAL AND METHODS

With the aid of line and hook, 56 juvenile specimens of the hybrid "tambacu" (*Piaractus mesopotamicus* x *Colossoma macropomum*) were collected between May to October 1997. Fish were reared in a 13,000 m² reservoir, in Guariba, State of São Paulo, Brazil (21°19'17"S and 48°12'37,8'W), and were fed with commercial food. Fish were anaesthetized with 1:15 benzocaine solution for collection of 1.0 ml blood from the caudal vein with syringes containing EDTA (10%) for the following analyses: haematocrit (Ht) according to GOLDENFARB *et al.* (1971), haemoglobin (Hb) according to COLLIER (1944) and mean corpuscular haemoglobin concentration (MCHC) according to WINTROBE (1934). Blood smears were stained by ROSENFELD (1947) method for the differential counts of the defense cells. During the experiment, water pH was maintained at 7.1±0.2; electric conductivity at 507.0±140.0 µS/cm and mean water temperature at 21.8±1.8°C. According to BANZATO & KRONKA (1995) the results were analyzed by the linear regression and F test, to the 1% probability level. Necroscopical examination was performed to confirm the state of health of the studied animals.

RESULTS

Erythrocyte parameters

Haematological parameters of the red blood cells of "tambacu" are shown in table I. Linear regression analysis showed significant positive correlation ($P < 0.05$) between haematocrit and MCHC with the haemoglobin concentration (Fig. 1) as for the following equations: $Ht = 20.451 + 1.5538 (Hb)$; $r = 0.75$ and $MCHC = 14.719 + 1.3155 (Hb)$; $r = 0.75$.

Table I. Haematological parameters of the hybrid tambacu. The data are expressed in mean values ± standard deviation and amplitude variation (Ax) in 56 specimens.

Parameters	Mean values	Ax
Weight (g)	1,007.5 ± 554.5	400.0 - 3,100.0
Length (cm)	37.9 ± 6.2	20.0 - 42.0
Haemoglobin (g/dL)	12.7 ± 3.7	5.1 - 18.9
Hematocrit (%)	40.2 ± 7.7	20.0 - 57.0
MCHC (g/dL)	31.5 ± 6.5	19.7 - 44.0

Morphological characteristics of defense blood cells

Blood smears of "tambacu" revealed the presence of thrombocytes, neutrophils, lymphocytes, monocytes, special granulocytic cells and eosinophils in circulating blood. Special granulocytic cells (SGC) were spherical with abundant cytoplasm, rich in light translucent and spherical granulation and uniformly distributed. The nucleus of this cell was small, with gross chromatin without nucleoli (Fig. 2A). Monocytes showed spherical shape with basophilic vacuolated or not vacuolated

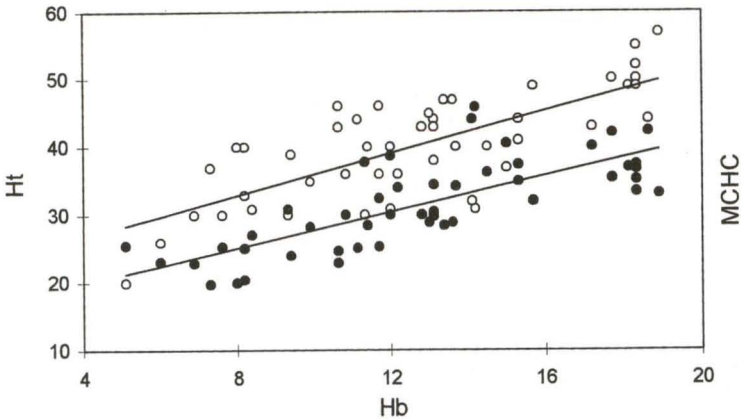


Fig. 1. Relation between haematocrit (O) and mean corpuscular haemoglobin concentration (●) with haemoglobin in 56 specimens of "tambacu".

cytoplasm. The nucleus was frequently eccentric, elongated, sometimes spherical (Fig. 2B,E). Thrombocytes were elongated spindle-shaped cells, with acidophilic cytoplasm without granulation. The nucleus were elongated showing a spindle-shaped and deeply stained structure (Fig. 2C). Eosinophils were spherical and small when compared with the other granulocytic cells presenting an acidophilic cytoplasm rich in eosinophilic granulation. Their round nucleus occupies a great proportion of the cell (Fig. 2C). Lymphocytes were predominantly spherical with variable size showing a deeply basophilic cytoplasm without granulation and nucleus slightly shaped like the human kidney with dense chromatin. Their relation with the cytoplasm was narrow (Fig. 2C). Neutrophils were round-shaped cell with acidophilic cytoplasm with fine granulation occupying a great proportion of the cytoplasm. Their nucleus was segmented, rod-shaped and in eccentric position (Fig. 2D).

Mean values and change intervals of differential counts are shown in table II. Thrombocytes and lymphocytes were the most frequently observed cells in circulating blood of "tambacu": $66.8 \pm 12.5\%$ and $21.0 \pm 10.6\%$, respectively. Linear regression showed negative correlation ($P > 0.01$) between the percentage of such cells (Fig. 3) indicating that an increase in thrombocyte was responsible for a decrease in lymphocyte number. This was demonstrated by the following equation: $\text{Lymphocyte} = -0.6301 (\text{Thrombocyte}) + 63.077$; $r = 0,74$.

Statistical analysis of the other defense cells showed no significant correlation.

Table II. Percentage of defense blood cells in the hybrid tambacu. The data are expressed in mean values \pm standard deviation and change amplitude (Ax) in 56 specimens.

Parameters (%)	Mean values	Ax
Thrombocytes	66.8 ± 12.5	44.0 - 91.0
Lymphocytes	21.0 ± 10.6	1.0 - 43.0
Neutrophils	6.4 ± 6.2	0.0 - 24.0
Monocytes	3.1 ± 3.3	0.0 - 14.0
Eosinophils	1.5 ± 2.4	0.0 - 13.0
SGC	1.2 ± 1.8	0.0 - 11.0

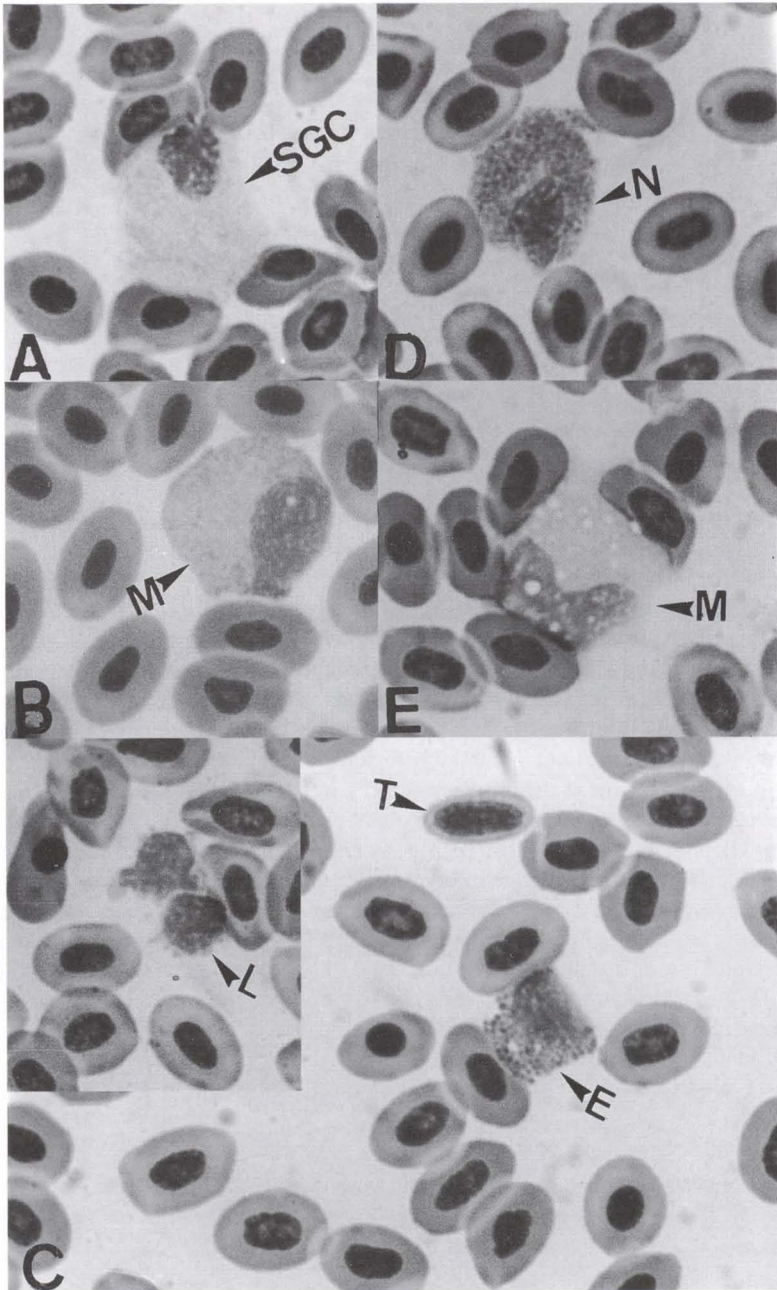


Fig. 2. Defense blood cells of the hybrid tobacco. (A) Special granulocitic cell (SGC); (B) monocyte (M); (C) lymphocyte (L), thrombocyte (T) and eosinophil (E); (D) neutrophil (N) and (E) monocyte (M). Stained with ROSENFELD (1947).

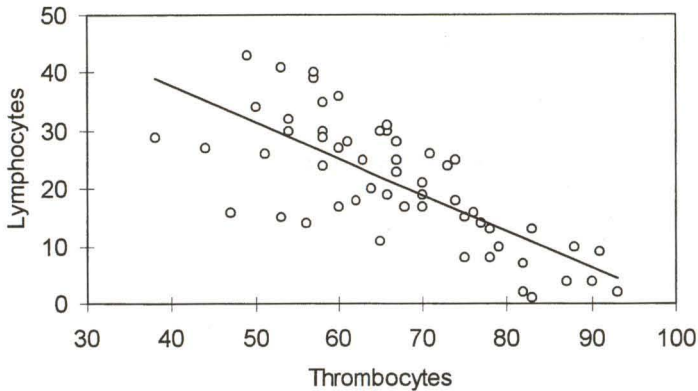


Fig. 3. Relation between percentage of thrombocytes and lymphocytes in 56 specimens of "tambacu".

DISCUSSION AND CONCLUSIONS

Organic factors such as weight (LARSSON *et al.* 1976; RANZANI-PAIVA 1991) or length (PITOMBEIRA *et al.* 1968; LARSSON *et al.* 1976; RANZANI-PAIVA 1991) may be responsible for the morphological and quantitative changes in the blood parameters. Mean values of haematocrit, haemoglobin and MCHC of "tambacu" showed increase when compared to *C. macropomum* (TAVARES-DIAS *et al.* 1998; RANZANI-PAIVA *et al.* 1998/1999) and *P. mesopotamicus* (RANZANI-PAIVA *et al.* 1998/1999; TAVARES-DIAS *et al.* 1999a). Nevertheless, it must be considered that fishes studied in the present work have a bigger size.

The correlation between weight and haematocrit was demonstrated in *Oncorhynchus mykiss* Walbaum, 1792 and *Dicentrarchus labrax* Linnaeus, 1758 (GARCIA *et al.* 1992); between weight, haematocrit and haemoglobin in *O. mykiss* (MARTÍNEZ *et al.* 1994); between length, haemoglobin and erythrocyte count in *Salmo gairdneri* Richardson, 1836 (Salmonidae) (LOWE-JINDE & NIIMI 1983); between haemoglobin and haematocrit in *C. macropomum* (TAVARES-DIAS *et al.* 1998). In the present paper, haemoglobin presented positive correlation with haematocrit and MCHC in blood of "tambacu". The same correlation was related in bluegill, *Lepomis macrochirus* Rafinesque (MURRAY 1984) and *Clarias isheriensis* Sydenham (Clariidae) (KORI-SIAKPERE 1985).

Defense blood cells identified in "tambacu" were morphologically similar to the ones described in *Hoplias malabaricus* Bloch, 1794 (CAMARGO *et al.* 1986/1987), *Cyprinus carpio* Linnaeus, 1758 (RANZANI-PAIVA *et al.* 1987), *Brycon* sp. (Characidae) (RANZANI-PAIVA 1996), *P. mesopotamicus* (TAVARES-DIAS *et al.* 1999a) and *C. macropomum* (TAVARES-DIAS *et al.* 1999b). The low presence of eosinophils in the blood of "tambacu" was observed. The same result was related in *Prochilodus scrofa* Steindachner, 1881 (RANZANI-PAIVA *et al.* 1998/1999). However, higher eosinophils values was described in *P. mesopotamicus* and *C. macropomum* (RANZANI-PAIVA *et al.* 1998/1999).

Thrombocytes were the predominant defense blood cells in the smears of "tambacu" followed by lymphocytes, neutrophils, monocytes, eosinophils and special

granulocytic cells. Elevated percentage of thrombocytes was described in bluegill. (MURRAY 1984), in *Gudusia chapra* Hamilton, 1822 (CHONDAR 1982), *Sarotherodon melanotheron* Ruppel, 1852 (LEA MASTER et al. 1990), *Oreochromis niloticus* Linnaeus, 1758 (TAVARES-DIAS & FAUSTINO 1998), *P. mesopotamicus* (TAVARES-DIAS et al. 1999a,c,d), *C. macropomum* (TAVARES-DIAS et al. 1999b), *Leporinus macrocephalus* Garavello & Britski, 1988 (TAVARES-DIAS et al. 1999d) and *Brycon cephalus* Günther, 1869 (Characidae) (TAVARES-DIAS et al. 1999e) when compared to the others cells. On the other hand, lymphocytes number is higher than thrombocytes in *C. carpio* (HINES & YASHOUV 1970) and *O. mykiss* (HOUSTON et al. 1996).

Thrombocytes function in fish is questionable (LOPES et al. 1997). In birds, reptiles (GARCIA-NAVARRO & PACHALY 1994; PENHA et al. 1996), amphibians and fish the involvement of thrombocytes in coagulation is known as their analogy with platelets (PENHA et al. 1996; TAVARES-DIAS et al. 1999d). PENHA et al. (1996) demonstrated thrombocytes activity in bullfrog (*Rana catesbeiana* Shaw, 1802) injected with colloidal carbon. In spite of leucocytic cells, their importance in inflammatory exsudate and phagocytosis indicate an organic defense relation (GRECHI et al. 1980; ISHIDA et al. 1985; KAJIGAYA et al. 1985; SUZUKI 1986; DIAS & SINHORINI 1991, 1992; MATUSHIMA & MARIANO 1996). On the other hand, some authors include, erroneously, thrombocytes in the differential count of leucocytes (WEINBERG et al. 1973; RAIZADA & SINGH 1981; CHONDAR 1982; MURRAY 1984; LEA MASTER et al. 1990; HOUSTON et al. 1996).

In teleost the exact function of each kind of defense blood cells is obscure. In the circulating blood eosinophils occurs in small number but abundant in the intestinal submucosa, in the peritoneal liquid, mesentery and gills (RANZANI-PAIVA 1991). In *Argulus* sp. (Crustacea) infested *C. carpio* a great number of leucocytes have been reported (RANZANI-PAIVA et al. 1987). RANZANI-PAIVA et al. (1987) observed the frequency of special granulocytic cells and eosinophils in parasitized fish. Infestation with *Argulus* sp. decreased thrombocytes percentage of *P. mesopotamicus* and increased monocytes and special granulocytic cells percentage (TAVARES-DIAS et al. 1999c). In the present work, "tambacu" did not show parasites but special granulocytic cells and eosinophils were present. Further studies must be carried out with this hybrid to determine haematological parameters of parasitized "tambacu" that is one of the most cultivated fish in Brazilian fishfarm.

REFERENCES

- BANZATO, D.A. & S.N. KRONKA. 1995. **Experimentação agrícola**. Jaboticabal, FUNEP, 3ª ed., 247p.
- CAMARGO, F.T.; J.R.V. COSTA; A.C. SOUZA CRUZ; W. ROCHA & V.V. VIGNOLI. 1986/1987. Estudo das séries hematológicas da traíra *Hoplias malabaricus* (Pisces – Teleostea). **Rev. Esc. Farm. Odont. Alfenas** 9: 63-69.
- CASTAGNOLLI, N. 1992. **Piscicultura de água doce**. Jaboticabal, FUNEP, 189p.
- CHONDAR, S.L. 1982. The haematology of *Gudusia chapra* (Pisces – Clupeidae). **Jour. Inland Fish. Soc. India**. 14 (1): 1-10.
- COLLIER, H.B. 1944. The standardization of blood haemoglobin determinations. **Can. Med. Ass. Jour.** 50: 550-552.
- DIAS, J.L.C. & I.L. SINHORINI. 1991. Qualitative evaluation of the inflammatory response modulated by temperature in tadpoles of *Rana catesbeiana*. **Ciênc. Cult** 43: 304-6.

- . 1992. Aspectos ultra-estruturais de células inflamatórias de girinos de *Rana catesbeiana* (rã-touro-gigante). **Ciênc. Cult.** **44** (Supl.): 730.
- GARCÍA, M.P.; G. ECHEVARRIA; F.J. MARTÍNEZ & S. ZAMORA. 1992. Influence of blood sample collections on the haematocrit value of two teleosts: rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax* L.). **Comp. Biochem. Physiol.** **101A**: 733-736.
- GARCIA-NAVARRO, C.E. & J.R. PACHALY. 1994. **Manual de hematologia veterinária**. São Paulo, Livraria Varela, 169p.
- GOLDENFARB, P.B.; F.P. BOWYER; E. HALL & E. BROSIOS. 1971. Reproducibility in the hematology laboratory: the microhematocrit determination. **Amer. Jour. Clin. Path.** **56**: 35-39.
- GRECCHI, R.; A.M. SALIBA & M. MARIANO. 1980. Morphological changes, surface receptors and phagocytic potential of fowl mononuclear phagocytes and trombocytes *in vivo* and *in vitro*. **Jour. Pathol.** **130**: 23-31.
- HINES, R. & A. YASHOUV. 1970. Differential leukocyte counts and total leukocyte and erythrocyte counts for same normal israeli mirror carp. **Bamidgeh** **22**: 106-113.
- HOUSTON, A.H.; N. DOBRIC & R. KAHURANANGA. 1996. The nature of hematological response in fish. **Fish Physiol. Biochem.** **15** (4): 339-347.
- ISHIDA, Y.; M. SUGIYAMA; H. KAJIGAYA; M. UMEDEA & M. ISODA. 1985. Inflammatory reactions on Japanese quails muscular tissue caused by sutures. **Bull. Nippon Vet. Zoot. Col.** **31**: 39-46.
- KAJIGAYA, H.; M. KAMEKURA; N. TANAHARA; A. OTIHA; H. SUZUKI; M. SUGIYAMA & M. ISODA. 1985. Acute and chronic inflammation induced by silk sutures in chicken mesentery. **Bull. Nippon Vet. Zoot. Col.** **34**: 35-8.
- KORI-SIAKPERE, O. 1985. Haematological characteristics of *Clarias isheriensis* Sydenham. **Jour. Fish Biol.** **27**: 259-263.
- LARSSON, A.; M.J. JOHANSSON-SJOBECK & R. FANGE. 1976. Comparative study of some haematological and biochemical blood parameters in fishes from Shagerak. **Jour. Fish Biol.** **9**: 425-440.
- LEA MASTER, B.R.; J.A. BROCK; R.S. FUJIOKA & R.M. NAKAMURAS. 1990. Hematologic and blood chemistry values for *Sarotherodon melanotheron* and a red hybrid tilapia in freshwater and seawater. **Comp. Biochem. Physiol.** **97A** (4): 525-529.
- LOPES, R.; M.A. SALA; T. PAULA-LOPES; T.M.C. OGASAWARA; L.S. WATANABE & M. SEMPRINI. 1997. Estudo hematológico de peixes brasileiros. XXXVII. As células sanguíneas do carapeba *Diapterus rhombeus* Valenciennes 1830 (Pisces, Gerridae), do Município de Iguape – SP, Brasil. **Rev. Esc. Farm. Odont. Alfenas** **19**: 27-32.
- LOWE-JINDE, L. & A.J. NIIMI. 1983. Influence of sampling on the interpretation of haematological measurements of rainbow trout, *Salmo gairdneri*. **Can. Jour. Zool.** **61**: 396-402.
- MARTÍNEZ, F.J.; M.P. GARCÍA-RIERA; M. CANTERAS; J. COSTA & S. ZAMORA. 1994. Blood parameters in Rainbow Trout (*Oncorhynchus mykiss*): Simultaneous influence of various factors. **Comp. Biochem. Physiol.** **107A**: 95-100.
- MATUSHIMA, E.R. & M. MARIANO. 1996. Kinetics of the inflammatory reaction induced by carrageenin in the swimbladder of *Oreochromis niloticus* (Nile tilapia). **Braz. Jour. Vet. Anim. Sci.** **33** (1): 5-10.
- MURRAY, S.A. 1984. Hematological study of the bluegill, *Lepomis macrochirus* Raf. **Comp. Biochem. Physiol.** **78A** (4): 787-791.
- PENHA, M.L.; J.L.C. DIAS & B.E. MALUCELLI. 1996. Influence of low environmental temperature on the phagocytic activity of bullfrog (*Rana catesbeiana*) thrombocytes. **Braz. Jour. Vet. Anim. Sci.** **33** (1): 15-18.
- PITOMBEIRA, M.S.; J.M. MARTINS & E. FURTADO. 1968. Hematology of the Atlantic thread herring, *Opisthonema oglinum* (Le Sueur). **Arq. Est. Biol. Mar. Univ. Fed. Ceará** **8** (2): 111-116.
- RAIZADA, M.N. & C.P. SINGH. 1981. Seasonal variations in the differential leucocyte counts (DLC) of *Cirrhinus mrigala* (Ham). **Indian Jour. Zoot.** **22**: 147-150.
- RANZANI-PAIVA, M.J.T. 1991. Hematologia de peixes. p. 65-70. *In*: H.S.L. SANTOS (Ed.). **Histologia de Peixes**. São Paulo, FCAV-UNESP, 83p.
- RANZANI-PAIVA, M.J. 1996. Células sanguíneas e contagem diferencial de leucócitos em

- pirapitinga-do-sul, *Brycon* sp., sob condições experimentais de criação intensiva. **Rev. Ceres** 43 (250): 685-695.
- RANZANI-PAIVA, M.J.; C.M. ISHIKAWA; M.C. PORTELLA & R.J. CELIBERTO. 1987. Hematologia da carpa comum *Cyprinus carpio*, infestada por *Argulus* sp. e após um tratamento com fosfato de 0,0-dimetil-oxi-2,2,2 - tricloretoilo (Neguvon). **Bol. Inst. Pesca** 14: 83-92.
- RANZANI-PAIVA, M.J.T.; F.A. SALLES; J.C. EIRAS; A.C. EIRAS; C.M. ISHIKAWA & A.C. ALEXANDRINO. 1998/1999. Análise hematológica de curimbatá (*Prochilodus scrofa*), pacu (*Piaractus mesopotamicus*) e tambaqui (*Colossoma macropomum*) das estações de piscicultura do Instituto de Pesca, Estado de São Paulo. **Bol. Inst. Pesca** 25: 77-83.
- ROSENFELD, G. 1947. Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grunwald e do Giemsa num só corante de emprego rápido. **Mem. Inst. Butantan** 20: 329-334.
- SILVEIRA, R. & C. RIGORES. 1989. Características hematológicas normais de *Oreochromis aureus* em cultivo. **Rev. Lat. Acui** 39: 54-56.
- SUZUKI, K. 1986. Morphological and phagocytic characteristics of peritoneal exudate cells in tilapia, *Oreochromis niloticus* (Trewavas), and carp, *Cyprinus carpio* L. **Jour. Fish Biol.** 29 (3): 349-364.
- TAVARES-DIAS, M. & C.D. FAUSTINO. 1998. Parâmetros hematológicos da tilápia-do-Nilo *Oreochromis niloticus* (Cichlidae) em cultivo extensivo. **Ars Veterinaria** 14 (3): 254-263.
- TAVARES-DIAS, M.; E.F.S. SANDRIM & A. SANDRIM. 1998. Características hematológicas do tambaqui (*Colossoma macropomum*) Cuvier, 1818 (Osteichthyes: Characidae) em sistema de monocultivo intensivo. I. Série eritrocitária. **Rev. Brasil. Biol.** 58 (2): 197-202.
- TAVARES-DIAS, M.; R.A. TENANI; L.D. GIOLI & C.D. FAUSTINO. 1999a. Características hematológicas de teleosteos brasileiros. II. Parâmetros sangüíneos do *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae) em policultivo intensivo. **Revta bras. Zool.** 16 (2): 423-431.
- TAVARES-DIAS, M.; E.F.S. SANDRIM & E. CAMPOS-FILHO. 1999b. Características hematológicas do tambaqui *Colossoma macropomum* Cuvier (Osteichthyes: Characidae) em sistema de monocultivo intensivo. II. Leucócitos. **Revta bras. Zool.** 16 (1): 175-184.
- TAVARES-DIAS, M.; M.L. MARTINS & S.N. KRONKA. 1999c. 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 (2): 553-555.
- TAVARES-DIAS, M.; S.H.C. SCHALCH; M.L. MARTINS; E.D. SILVA; F.R. MORAES & D. PERECIN. 1999d. Hematologia de teleosteos brasileiros com infecção parasitária. I. Varáveis do *Leporinus macrocephalus* Garavello & Britski, 1988 (Anostomidae) e *Piaractus mesopotamicus* Holmberg, 1887 (Characidae). **Acta Scientiarum** 21 (2): 337-342.
- TAVARES-DIAS, M.; C.M.D. FRASCÁ-SCORVO; F.R. MORAES; E. CAMPOS-FILHO. 1999e. Características Hematológicas de Teleosteos Brasileiros. IV. Parâmetros Eritroleucométricos, Trombométricos e Glicemia do Matrinxã *Brycon cephalus* Günther, 1869 (Osteichthyes: Characidae). **Ars Veterinaria** 15 (3): 149-153.
- WEINBERG, S.R.; C.D. SIEGEL & A.S. GORDON. 1973. Studies on the peripheral blood cell parameters and morphology on the red paradise fish, *Macropodus opercularis*. Effect of food deprivation on erythropoiesis. **Anat. Rec.** 175: 7-14.
- WINTROBE, M.M. 1934. Variations on the size and hemoglobin content of erythrocytes in the blood various vertebrates. **Fol. Haematol.** 5: 32-49.
- ZANIBONI-FILHO, E. 1997. O desenvolvimento da piscicultura brasileira sem a deterioração da qualidade de água. **Rev. Brasil. Biol.** 57 (1): 3-9.