Boletim do Instituto de Pesca, São Paulo, 27 (1): 43 - 48, 2001

PHYSIOLOGICAL RESPONSES OF "TAMBAQUI" *Colossoma macropomum* (CHARACIDAE) TO ACUTE STRESS

[Resposta fisiológica do tambaqui Colossoma macropomum (Characidae) ao estresse agudo]

Marcos TAVARES-DIAS[1], Elziane Ferreira da Silva SANDRIM[2], Flávio Ruas de MORAES[1,3], Paulo César Falanghe CARNEIRO[1]

[1]Laboratório de Patologia de Organismos Aquáticos, Centro de Aqüicultura da Universidade Estadual Paulista (CAUNESP).
[2]Piscicultura São Geraldo
[3]Departamento de Patologia Veterinária, FCAV-UNESP
[4]Endereço/Address: Via de Acesso Prof. Paulo Donato Castellane, s/n, 14870-000 - Jaboticabal, São Paulo, Brasil. E-mail:fruas@fcav.unesp.br

ABSTRACT
In this study some physiological responses of "tambaqui" *Colossoma macropomum* submitted to capture and handling stress were evaluated. Changes were observed in red blood cell (RBC), white blood cell (WBC), hematocrit percentage, hemoglobin concentration, mean corpuscular volume (MCV), blood glucose and plasma cortisol. After stress, red blood cell, hemoglobin, and hematocrit decreased significantly while blood glucose, plasma cortisol, MCV, white blood cell number, lymphocytes and neutrophils numbers increased. MCHC, monocytes and special granulocitic cells numbers (SGC) did not show any change after the disturbance.
Key words: "tambaqui", *Colossoma macropomum*, stress, hematology

RESUMO
No presente trabalho avaliaram-se as respostas fisiológicas do tambaqui, *Colossoma macropomum*, submetido ao estresse de captura e manejo. Foram analisados o eritrograma, o leucograma, o hematócrito, a hemoglobina, o volume corpuscular médio (VCM), a concentração da hemoglobina corpuscular média (CHCM), a glicemia sangüínea e o cortisol plasmático. Após estresse, observou-se decréscimo do número total de eritrócitos, da concentração da hemoglobina e do hematócrito e elevação no VCM, da glicemia sangüínea e do cortisol plasmático. Os leucócitos totais, o número de linfócitos e de neutrófilos também aumentaram significativamente após o estímulo estressante. Não foram registradas alterações significativas no CHCM e no número de monócitos e de células granulocíticas especiais (CGE).
Palavras-chave: "tambaqui", *Colossoma macropomum*, estresse, hematologia

Introduction

Capture, transport, and handling are aquaculture procedures normally employed in intensive culture systems (KRIEGER-AZZOLINI *et al*., 1989; NARNAWARE; BAKER; TOMLINSON, 1994). Those practices may cause morphological, biochemistry, or physiological changes, which may lead to metabolic disturbance, enzymatic dysfunction, and several other malfunctions in the fish organism (KUROVSKAYA and OSADCHAYA, 1993).

Hypersecretion of corticosteroids, mainly cortisol (by the interrenal cells), and catecholamines (by the chromaffin cells), such as adrenaline and noradrenaline, are considered primary responses to stress (MAZEAUD; MAZEAUD; DONALDSON, 1977; REID and PERRY, 1994). Hyperglycemia is considered one of the secondary responses, induced by the greater glycogen depletion caused by the increasing of catecholamines concentration (MAZEAUD; MAZEAUD; DONALDSON, 1977; PICKERING, POTTINGER; CHRISTIE, 1982). Blood glucose and plasma cortisol concentrations are good indicators of stress in teleost fishes. The magnitude and duration of such response are strongly related to the species and nutritional status (NAKANO and TOMLINSON, 1967; WENDT and SAUNDERS, 1973; PICKERING, POTTINGER; CHRISTIE, 1982; VIJAYAN and MOON, 1992).

The negative effect of high plasma cortisol levels is the suppression of some aspects of the immune system, which interferes on the number of leukocytes

Artigo Científico: Recebido em 08/05/00 - Aprovado em 22/03/01

and proportion of different types of white cells (TOMASSO; SIMCO; DAVIS, 1983; BARTON and ZITZOW, 1995; JENEY *et al*., 1997). A preliminary study suggested a close relation between infestation of parasites and high plasma cortisol concentration in tambaqui (TAVARES-DIAS and SANDRIM, 1998).

"Tambaqui", *Colossoma macropomum*, is an economically important tropical freshwater fish from the Amazon Basin (CASTAGNOLLI, 1992), with few scientific studies on stress responses. The aim of this study was to determine the physiological response of "tambaqui" submitted to stress of capture and handling.

Material and Methods

Thirty juvenile "tambaqui", *Colossoma macropomum*, (mean weight 585 g ± 100 g and mean length 27 cm ± 3 cm) were brought from a commercial fish farm in Sertãozinho, SP, Brazil and maintained in an aerated tank. Blood was rapidly collected (within 60 s) from the caudal vein of each animal with syringes and needles containing EDTA (10%). A blood aliquot was used for red blood cell (RBC) and leukocyte determinations. Plasma was separated by centrifugation for further cortisol analyses. Another blood sample, collected with syringes containing EDTA and fluoride, were obtained for blood glucose determination.

After the first sampling, fish were kept in a 36-m^3 cement tank, with earthen bottom, and fed normally for 72 hours. After this period, all 30 fish were captured individually and subjected to stress by allowing them to struggle out of the water for 40 seconds (for details see BARTON and ZITZOW, 1995; DAVIS and SCHRECK, 1997). Another sampling, similar to the first one, was performed again. During the experimental period, temperature, pH and dissolved oxygen were monitored.

Red blood cells (RBC) were determined with an automatic blood cell counter. Hemoglobin and hematocrit were obtained according to COLLIER (1944) and GOLDENFARB *et al.* (1971), respectively. White blood cells (WBC) were counted in Neubauer chamber after blood dilution in sodium chloride solution (0.65%) with gentian violet (1%) and red neutral (TAVARES-DIAS; SANDRIN; CAMPOS-FILHO, 1999). Blood smears, stained according to ROSENFELD (1947), were used for differential count of leukocyte under microscopic examination (TAVARES-DIAS; SANDRIN; CAMPOS-FILHO, 1999)

Blood glucose was measured by enzymatic method (Labtest kit) and cortisol determined by radioimmunoassay (Coat-a-Count Kit). Mean corpuscular volume (MVC) and mean corpuscular hemoglobin concentration (MCHC) were determined according to WINTROBE (1934).

The statistical design was entirely randomized, and the averages of the two treatments were compared by F-test ($P<0.01$). Data were also submitted to Pearson linear correlation (BANZATO and KRONKA, 1995).

Results

During the experimental period, water quality parameters presented the following value ranges: temperature, 23.0 - 25.0 °C; pH, 7.5 - 7.7; dissolved oxygen, 5.3 - 6.7 mg/L.

Red blood cell (RBC), hemoglobin concentration and hematocrit decreased significantly ($P<0.01$), while blood glucose, plasma cortisol and MCV increased ($P<0.01$) after stress (Table 1). The white blood cells (WBC), lymphocytes and neutrophils numbers were higher ($P<0.01$) after the disturbance (Table 2). Monocytes and special granulocitic cells numbers (SGC) did not change ($P>0.05$) after stress.

Significant linear correlation ($P<0.01$) between blood glucose and plasma cortisol were observed (Table 3). Significant linear correlation ($P<0.01$) were also reported among WBC, blood glucose, and plasma cortisol (Table 4).

Table 1. Average values ± standard deviation of blood characteristics of *C. macropomum* before and after acute stress

Blood parameters	Before stress	After stress	F-test
Blood glucose (mg/dL)	116.7 ± 30.8	166.9 ± 62.0	15.24**
Plasma cortisol (ng/mL)	182.1 ± 47.7	333.8 ± 95.9	58.20**
RBC ($10^6/\mu L$)	2.830 ± 0.56	1.868 ± 0.36	73.54**
Hemoglobin (g/dL)	11.3 ± 0.9	8.4 ± 1.0	138.4**
Hematocrit (%)	41.6 ± 6.9	29.8 ± 3.0	71.13**
MCV (fL)	150.0 ± 15.9	165.2 ± 25.7	7.31**
MCHC (g/dL)	27.7 ± 3.3	28.1 ± 2.2	0.02^{NS}

^{NS}- not significant ($P>0.05$); ** - significant ($P<0.01$)

Table 2. Average values ± standard deviation of leukocytes count of *C. macropomum* before and after acute stress

Leukocytes	Before stress	After stress	*F*-test
WBC (μL)	2663.3 ± 233.8	6910.0 ± 1023.6	41.94**
Lymphocytes (μL)	937.6 ± 81.3	2130.0 ± 195.6	55.04**
Neutrophils (μL)	1566.3 ± 137.2	4594.9 ± 890.5	35.24**
Monocytes (μL)	86.7 ± 22.5	121.4 ± 26.0	1.26^{NS}
SGC (μL)	72.8 ± 26.3	63.7 ± 27.6	0.08^{NS}

^{NS}- not significant (P>0.05); ** - significant (P<0.01)

Table 3. Pearson linear coefficient among red blood cell count (RBC), hemoglobin, hematocrit, blood glucose and plasma cortisol of *C. macropomum* after acute stress

Parameters	Glucose	Cortisol
RBC(μL)	0.317^{ns}	0.259^{ns}
Hemoglobin (g/dL)	-0.277^{ns}	-0.263^{ns}
Hematocrit (%)	-0.122^{ns}	0.277^{ns}
Glucose (mg/dL)	1.000	0.617**
Cortisol (ng/mL)	-	1.000

^{NS}- not significant (P>0.05); ** - significant (P<0.01)

Table 4. Pearson linear coefficient among leukocytes count and plasma cortisol of *C. macropomum* after acute stress

Parameters	WBC	Lymphocytes	Neutrophils	Monocytes	S.G.C.	Cortisol
WBC(μL)	1.000	0.628**	0.980**	0.600**	0.408^{NS}	-0.372^{NS}
Lymphocytes(μL)	-	1.000	0.468*	0.727**	0.432*	-0.197^{NS}
Neutrophils(μL)	-	-	1.000	0.483*	0.327^{NS}	-0.380^{NS}
Monocytes(μL)	-	-	-	1.000	0.540**	-0.109^{NS}
S.G.C. (μL)	-	-	-	-	1.000	-0.041^{NS}

^{NS}- not significant (P>0.05); * - significant (P<0.05); ** - significant (P<0.01)

Discussion

Aquaculture urges for more accurate information on stress control, in order to assure health of fish, once those animals are confined into artificial environment. Hematological parameters can be of great importance for fishfarmers, serving as indicators of the physiological status and helping on the prevention and control of pathologies related to stress (ALDRIN; MESSAGER; BAUDIN-LAURENCIN, 1982).

Fish respond to stress with characteristic acute increases in plasma levels of the catecholamines, adrenaline and noradrenaline, and slower, but more sustained, increases in plasma levels of the corticosteroid cortisol. Increases in catecholamine and corticosteroid levels are generally mirrored by increases in plasma levels of glucose generated by the glucose-mobilizing effects of both classes of hormone (WENDELAAR BONGA, 1997).

FEVOLDEN; REFSTIE; ROED (1991) observed positive correlation between blood glucose and plasma cortisol in Atlantic salmon *S. salar* submitted to confinement stress. Similar results were observed in tambaqui after capture and handling stress. Contrarily, pacu *Piaractus mesopotamicus* did not show increased plasma cortisol level after capture stress, even when high blood glucose was observed (MARTINS *et al*., 2000). DAVIS and PARKER (1986) reported increases in plasma cortisol levels in 13 teleost species after transport stress, with differences among the species.

This study demonstrates a significant reduction in RBC, hemoglobin and hematocrit in "tambaqui" immediately after submission to capture and handling stress. Similar studies with brown trout *Salmo trutta* (PICKERING; POTTINGER; CHRISTIE, 1982) and channel catfish *Ictalurus punctatus* (ELLSAESSER and CLEM, 1986) do not show significant alteration in the erythrocyte number. The lack of effect was also observed by other authors on hematocrit (ELLSAESSER and CLEM, 1986; CARNEIRO and URBINATI, 1998) and hemoglobin (CARNEIRO and URBINATI, 1998). Several stress sources induce erythrocyte releasing from the spleen (VIJAYAN and LEATHERLAND, 1989; PULSFORD *et al*., 1994) and increase erythrocyte fragility (MONTERO *et al*., 1999). HARMS *et al*. (1996) observed erythrocyte fragments in blood smears and anisocytosis and higher plasma cortisol levels in striped bass *Morone saxatilis* subjected to stress.

Stress can alter the standard hematological characteristic of teleosts, elevating plasma corticosteroids (FIN and NIELSON, 1971; MAZEAUD; MAZEAUD; DONALDSON, 1977; PICKERING; POTTINGER; CHRISTIE, 1982;TOMASSO; SIMCO; DAVIS, 1983; ELLSAESSER and CLEM, 1986; BARTON and IWAMA, 1991) and catecholamines (NARNAWARE; BAKER; TOMLINSON, 1994). When challenged with moderate to severe hypoxia, rainbow trout, *Onchorhynchus mykiss*, release catecholamine hormones, mainly adrenaline and noradrenaline, into the circulation. An increase in plasma catecholamine levels can enhance both oxygen transport in the blood and transfer across the gills (THOMAS and PERRY, 1992).

Stress might also lead to increase of susceptibility to infectious diseases (PICKERING; POTTINGER; CHRISTIE, 1982; PICKERING and POTTINGER, 1987; MAULE *et al*., 1989; WIIK *et al.*, 1989). Such decrease in disease resistance seems to be mediated by reduction of leukocytes number (PICKERING; POTTINGER; CHRISTIE, 1982; ELLSAESSER and CLEM, 1986; AINSWORTH; DEXIANG; WATERSTRATT, 1991) or suppression of its activities (PICKERING and POTTINGER, 1985; ELLSAESSER and CLEM, 1986). Stress also acts on many others components of immune system (PICKERING and POTTINGER, 1985; WIIK *et al*., 1989). However, in the present study it was not registered any significant correlation between plasma cortisol levels and circulating leukocytes. Neutrophils are the major elements in leukocyte cells in "tambaqui". As indicated by the Pearson correlation, there might be a dependence among neutrophils, lymphocytes and monocytes functions after stress.

In "tambaqui" subjected to stress, white blood cells (WBC), lymphocytes and neutrophils numbers showed significant increase ($P<0.01$) and those of SGC and monocytes exibited a slight alteration. Probably, the increase in leukocyte number in stressed "tambaqui" was caused by an increased leukocyte migration from the spleen to the blood circulation, which was observed in *Limanda limanda* (PULSFORD *et al.*, 1994).

BARTON and ZITZOW (1995) observed increase in lymphocytes in walleyes *Stizostedion vitreum* 1 hour after handling, and lymphopenia, 3 hours later, but no significant changes in neutrophils. MOURA; FARIAS; VAL (1994) studied the leukocyte response of "tambaqui" and "tamoatá" *Hoplosternum littorale* under different temperatures and found limphopenia in tambaqui and no changes in differential leukocyte count in "tamoatá". Lymphopenia and neutrophilia were observed in channel catfish 18 hours after intravenous inoculation of cortisol (ELLSAESSER and CLEM (1987).

In the present study, changes in plasma cortisol and blood glucose levels were similar to those showed by other teleosts (ROBERTSON; THOMAS; ARNOLD, 1988; KRIEGER-AZZOLINI *et al*., 1989; FEVOLDEN; REFSTIE; ROED, 1991;VIJAYAN and MOON, 1993; JENEY *et al*., 1997). The other analyzed parameters, as red blood cell count (RBC), leukocytes, hematocrit, and hemoglobin, altered differently in "tambaqui" when compared to other fish species. Handling stress might lead to important metabolic, osmoregulatory (BARTON and ZITZOW, 1995) and hematological (ELLSAESSER and CLEM, 1986; BARTON and ZITZOW, 1995; CARNEIRO and UURBINATI, 1998) changes, which may cause serious growth problems, susceptibility to disease and parasite infection and even death (WIIK *et al.*, 1989). Nevertheless, the magnitude of such stress responses may vary among fish species and may be influenced by the type of stimulus and time of exposure. In intensive culture systems, fish are submitted to environmental variations and stressful procedures and the knowledge on fish stress is still very incipient. Thus, future studies are required to find methods which could minimize such adverse effects, avoiding losses and damages to the fish producers.

References

AINSWORTH A.J.; DEXIANG, C.; WATERSTRATT, P.R. 1991 Changes in peripheral blood leukocytes percentages and function of neutrophils in stressed channel catfish. *J. Aquat. Anim. Health, 3*: 41-47.

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

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

BARTON, B.A. and IWAMA, G.K. 1991 Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Ann. Rev. Fish Dis*., *1*: 3-26.

BARTON, B.A. and ZITZOW, R.E. 1995 Physiological responses of juvenile walleyes to handling stress with recovery in saline water. *Prog. Fish-Culturist*, *57*: 267-276.

CARNEIRO, P.C.F. and UURNINATI, E.C. 1998 Alterações metabólicas, hematológicas e osmoregulatórias do matrinxã *Brycon cephalus* causadas pelo estresse de transporte. In: AQÜICULTURA BRASIL'98. *Anais*... Recife, 2:609-620.

CASTAGNOLLI, N. 1992 *Piscicultura de água doce*. FUNEP, Jaboticabal, 189p.

COLLIER, H.B. 1944 The standardization of blood hemoglobin determinations. *Can. Med. Ass. J*., *50*: 550-552.

DAVIS, E.L. and SCHRECK, C.B. 1997 The energetic response to handling stress in juvenile coho salmo. *Trans. Amer. Fish. Soc*., *126*:248-258.

DAVIS K.B. and PARKER, N.C. 1986 Plasma corticosteroid stress response of fourteen species of warmwater fish to transportation. *Trans. Amer. Fish. Soc*., *115*: 495-499.

ELLSAESSER, C.F. and CLEM, L.W. 1986 Haematological and immunological changes in channel catfish stressed by handling and transport. *J. Fish Biol*., *28*:511-521.

ELLSAESSER, C.F. and CLEM, L.W. 1987 Cortisol-induced hematologic and immunologic changes in channel catfish (*Ictalurus punctatus*). *Comp. Bioch. Physiol*., *87A*(2):405-408.

FINN, J.P. and NIELSON, N.O. 1971 The inflammatory response of the rainbow trout. *J. Fish Biol*., *3*:463-478.

FEVOLDEN, S.E.; REFSTIE, T.; ROED, K.H. 1991 Selection for high and low cortisol stress response in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *95*: 53-65.

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

HARMS, C.A; SULLIVAN, C.V.; HODSON, R.G.; STOSKOPF; M.K. 1996 Clinical pathology and histopathology characteristics of net-stressed striped bass with "red tail". *J. Aquat. Anim. Health*, *8*:82-86.

JENEY, G.; GALEOTTI, M.; VOLPATTI, D.; JENEY, Z.; ANDERSON, D.P. 1997 Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. *Aquaculture*, *154*:1-15.

KRIEGER-AZZOLINI, M.H.; DELATTRE, E.; CAROLSFELD, J.; CECCARELLI, P.C.; MENEZES, F.V. 1989 A time-course study of physiological indicators of handling stress in the tropical fish *Piaractus mesopotamicus* (Pacu). *Braz. J. Med. Biol. Res*., *22*:1019-1022.

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

MAULE, A.G.; TRIPP, R.A.; KAATTARI, S.L.; SCHRECK, C.B. 1989 Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). *J. Endocrinol*., *120*: 135-142.

MAZEAUD, M.M.; MAZEAUD, F.; DONALDSON, E.M. 1977 Primary and secondary effects of stress in fish: Some new data with a general review. *Trans. Amer. Fish. Soc*., *160*:201-211.

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

MONTERO, D.; BLAZER, V.S.; SOCORRO, J.; IZQUIERDO, M.S.; TORT, L. 1999 Dietary and culture influences on macrophage aggregate parameters in gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture*, *179*:523-534.

MOURA, M.A.F.; FARIAS, I.P.; VAL, A.L. 1994 Effects of temperature on leucocytes of *Colossoma macropomum* and *Hoplostermum littorale* (Pisces). *Braz. J. Med. Biol. Res*., *27*: 1589-1598.

NAKANO, T. and TOMLINSON, N. 1967 Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fish. Res. Bd. Can*., *24*: 1701-1715.

NARNAWARE, Y.K.; BAKER, I.B.; TOMLINSON, M.G. 1994 The effects of various stresses, corticosteroids and adrener-

gic agents on phagocytosis in the rainbow trout *Oncorhynchus mykiss*. *Fish Physiol. Bioch*., *13*: 31-40.

PICKERING, A.D. and POTTINGER, T.G. 1985 Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. *J. Fish Biol*., *27*: 611-619.

PICKERING, A.D. and POTTINGER, T.G. 1987 Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. *J. Fish Biol*., *30*:701-712.

PICKERING, A.D.; POTTINGER, T.G.; CHRISTIE, P. 1982 Recovery of the trout, *Salmo trutta* L., from acute handling stress: A time-course study. *J. Fish Biol*., *24*: 731-740.

PULSFORD, A.L.; LEMAIRE-GONY, S.; TOMLINSON, M.; COLLINGWOOD, N.; GLYNN, P.J. 1994 Effects of acute stress on the immune system of the dab, *Limanda limanda*. *Comp. Biochem. Physiol*., *109C*(2):129-139.

REID, S.G. and PERRY, S.F. 1994 Storage and differential release of catecholamines in rainbow trout (*Oncorhynchus mykiss*) and American eel (*Anguilla rostrata*). *Physiol. Zool*., *67*(1):216-237.

ROBERTSON, L.; THOMAS, P.; ARNOLD, C.R. 1988 Plasma cortisol secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transportation procedures. *Aquaculture*, *68*:115-130.

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. Instit. Butantan*, *20*: 329-334.

TAVARES-DIAS, M. and SANDRIM, E.F.S. 1998 Características hematológicas de teleósteos brasileiros. I. Série vermelha e dosagens de cortisol e glicose do plasma sangüíneo de espécimes de *Colossoma macropomum* em condições de cultivo. *Acta Scientiarum*, *20*: 157-160.

TAVARES-DIAS, M.; SANDRIM, E.F.S.; CAMPOS-FILHO, E. 1999 Características hematológicas do tambaqui *Colossoma macropomum* Cuvier (Osteichthyes: Characidae) em sistema de monocultivo intensivo. II. Leucócitos. *Revta Bras. Zool*., *16*(1):175-184, 1999.

THOMAS, S. and PERRY, S.F. 1992 Control and consequences of adrenergic activation of red blood cell Na^+/H^+ exchange on blood oxygen content and carbon dioxide transport in fish. *J. Exp. Zool*., *2633*:160-175.

TOMASSO, J.R.; SIMCO, B.A.; DAVIS, K.B. 1983 Circulating corticosteroid and leucocyte dynamics in channel catfish during net confinement. *Tex. J. Scien*., *35*:83-88.

VIJAYAN, M.M. and LEATHERLAND, J.F. 1989 Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon (*Oncorhynchus kisutch* Walbaum). *Can. J. Zool., 67*:2746-2750.

VIJAYAN, M.M. and MOON, T.W. 1992 Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci*, *49*:2260-2266.

VIJAYAN, M.M. and MOON, T.W. 1993 The response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Can. J. Zool*, *72*: 379-386.

WENDELAAR BONGA, S.E. 1997 The stress response in fish. *Physiological Reviews*, *773*: 591-625.

WENDT, C.A.G. and SAUNDERS, R.L. 1973 Changes in carbohydrate metabolism in young Atlantic salmon in response to various forms of stress. *Spec. Public. Internat. Atlantic Salmon Found*., *4*:55-82.

WIIK, R.; ANDERSEN, K.; UGLENES, I.; EGIDIUS, E. 1989 Cortisol-induced increase in susceptibility of Atlantic salmon, *Salmo salar*, to *Vibrio salmonicida*, together with effects on the blood cell pattern. *Aquaculture*, *83*: 201-215.

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