Physiological and haematological response of *Oreochromis niloticus* (Osteichthyes: Cichlidae) exposed to single and consecutive stress of capture

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ABSTRACT. This work is a sequence of studies on tropical fish of economic importance that evaluated the effects of two different stress of handling on the physiology and haematology of *Oreochromis niloticus* L. acclimated for 10 days before the essay. The stress consisted in net capture of all fish from each aquarium for 30s emersion. Fish exposed to single stress (SS) the samples were collected in the times 0, 10, 20, 30, 40, 50, 60, 120, 180, 240 and 300min. after stress. In the consecutive stress (CS) the samples were collected in the times 0; 15min. after the first stress; 15min. after the second stress; 15min. after the third stress and 15, 30, 45, 60, 120, 180 e 240min. after the fourth stress totalizing four stimuli every 60min. Fish exposed to SS showed increased cortisol and glucose concentrations at 60min. as well as in the leucocytes number and hematocrit at 50min. after stress. Cortisol did not alter in fish exposed to CS, but glucose increased 15min. after the third stress. On the other hand, CS provoked reduction in the leucocytes number and later hematocrit increasing. Neutrophilia and lymphopenia were related to SS and CS.

Key words: *Oreochromis niloticus*, consecutive stress, cortisol, glucose, haematology.

RESUMO. Resposta fisiológica e hematológica de *Oreochromis niloticus* (Osteichthyes: Cichlidae) exposto ao estresse único e consecutivo de captura. Este trabalho é seqüência de estudos com peixes tropicais de importância econômica avaliando os efeitos de dois tipos de estresse sobre a fisiologia e hematologia de *O. niloticus* L, aclimatados durante 10 dias antes do experimento. O estresse consistiu na captura de todos os peixes do aquário com rede e emersão por 30 s. Nos animais submetidos ao estímulo único de captura (EU) as amostras foram coletadas nos tempos 0, 10, 20, 30, 40, 50, 60, 120, 180, 240 e 300min. após o estresse. No estímulo consecutivo (EC) as amostras foram coletadas nos tempos 0; 15min. após o primeiro estresse; 15min. após o segundo estresse; 15min. após o terceiro estresse e 15, 30, 45, 60, 120, 180 e 240min. após o quarto estresse totalizando quatro estímulos a cada 60min. Os peixes expostos ao EU apresentaram aumento nas concentrações de cortisol e glicose 60min., bem como no número de leucócitos e hematócrito 50min. após o estresse. Não houve alteração do cortisol nos peixes do EC, mas a glicose aumentou 15min. após o terceiro estresse. Por outro lado, o EC provocou redução no número de leucócitos e aumento tardio do hematócrito. Observou-se neutrofilia e linfopenia após o EU e EC.

Palavras-chave: *Oreochromis niloticus*, estresse consecutivo, cortisol, glicose, hematologia.

Introduction

In Aquaculture the main causes of stress have been related to transport, low water quality, nutritional deficiency, overcrowding and the lack of prophylaxis. Consequently, changes in the organism homeostasis have provoked infectious and parasitic diseases that can result in economic losses to fish production in Brazil, according to Martins *et al*. (2002a). This fact is due to an interaction between environment and immunological system of fish. Disease resistance is directly related to hypothalamic/pituitary/interrenal axis. As a result of environmental disturbed or stress of handling, changes in cortisol and catecolamines are responsible for immunosupression (Yada and Nakanishi, 2002). Corticosteroid levels may depend on severity and type of stressor stimulus varying for a period of one to 24 hours (Schreck, 1981). The stress of handling, transport or anoxia has been responsible for increasing in the cortisol and glucose concentration (Korovin *et al*., 1982; Woodward and Strange, 1987; Salonius and Iwama, 1993; Barry *et al*., 1993; Demers and Bayne, 1997; Ortuño *et al*., 2001;

McCormick *et al*., 2003). On the other hand, consecutive stress of capture was studied in *Salmo gairdneri* (Barton *et al*., 1980; Barton and Schreck, 1987), *Oncorhynchus tshawytscha* (Barton *et al*., 1986), *Oncorhynchus mykiss* (Barton *et al*., 1987; Forsman *et al*., 1998), *Oncorhynchus kisutch* (Stratholt *et al*., 1997) and *Salmo salar* (McCormick *et al*., 1998). Little is known about in what conditions the cortisol levels do not respond as normally or decrease their concentration in fish as related by Swift (1983). Clearance or the lack of an elevation in plasma cortisol have been discussed as an auto depuration of this hormone in the organism, evaluated in *Oreochromis niloticus* larvae and *O. mykiss* (Hwang *et al*., 1992) and in marine teleost *Hemitripterus americanus* (Vijayan and Moon, 1994).

In tropical fish the effects of stress were related in *Piaractus mesopotamicus* (Krieger-Azzolini *et al.*, 1989; Martins *et al*., 2000), *O. niloticus* (Barcellos *et al*., 1997; Vijayan *et al*., 1997), *Brycon cephalus* (Carneiro and Urbinati, 1998), *Rhamdia quelen* (Barcellos *et al*., 2001), hybrid tambacu *P. mesopotamicus* x *Colossoma macropomum* (Martins *et al*., 2001; 2002b) and *C. macropomum* (Tavares-Dias *et al*., 2001, Gomes *et al*., 2003). As a result of stressor factors, haematological changes have been related in *Prochilodus lineatus* (Ranzani-Paiva and Godinho, 1986), *C. macropomum* and *Hoplosternum littorale* (Moura *et al.*, 1994), *O. niloticus* (Sun *et al.*, 1992), *Oreochromis mossambicus* (Okimoto *et al.*, 1994), *B. cephalus* (Carneiro and Urbinati, 1998; Carneiro *et al*., 2002), hybrid tambacu (Martins *et al.*, 2002b), *C. macropomum* (Tavares-Dias *et al*., 2001) and *Tilapia rendalli* (Tavares-Dias and Moraes, 2003).

This work is a sequence of studies on tropical fish of economic importance that aims at evaluating the effects of a single and consecutive stress of capture on the physiological and haematological response of *O. niloticus*.

Material and methods

Fish maintenance

This work was developed at the Pathology Laboratory of Aquatic Organisms, Aquaculture Center, Unesp, Jaboticabal, São Paulo State, Brazil. Specimens of *O. niloticus* Linnaeus, 1758 (Osteichthyes: Cichlidae) with 295.6±58.2g mean weight and 25.2±2.4cm total length were distributed in 250L aquaria with constant water flow, six animals in each and two replications. Fish were acclimated for 10 days before essay, fed daily with a commercial diet. During this period water temperature was 29.0±1.5ºC; pH 7.0±0.5, electric conductivity 146.5±66.7 µS/cm and dissolved oxygen 4.8±0.9mg/L.

Induction of stress and samples collection

The stress consisted in capture with net of all fish from each aquarium and emerged out of the water for a period of 30s to simulate a stress of handling normally used in fish farms (Davis and Schrech, 1997; Martins *et al*., 2000). In the animals exposed to single stress the samples were collected in the times 0: initial collection; 10, 20, 30, 40, 50, 60, 120, 180, 240 and 300min. after stress. In those exposed to consecutive stress the samples were collected in the 0: initial collection; 15min. after the first stress; 15min. after the second stress; 15min. after the third stress and 15, 30, 45, 60, 120, 180 and 240min. after the fourth stress totalising four stimuli every 60min.

Haematological analysis

Blood was withdrawn (2.0mL) from the fishes' caudal vein into a syringe containing a drop of a 10% EDTA solution to cortisol by radioimmunoessay (DPC-Diagnostic Products Corporation), glucose (King and Garner, 1947), blood smears and staining (Rosenfeld, 1947), differential counting of leucocytes, hematocrit percentage (Goldenfarb *et al.*, 1971) and erythrocytes number in haemocytometer. Total leucocytes number was calculated by the formula:

Leucocytes/µL = (leucocytes number in the blood smears x erythrocyte number/µL) / 2000 erythrocytes counted in the blood smears

Statistical analysis

The average comparison was performed by Tukey test at 5% probability and the percentage from differential counting of the blood cells were transformed in arc sin ($\sqrt{P+0.5}$) (Snedecor and Cochran, 1974).

Results

Haematological characteristics of tilapia exposed to single stress

The plasma cortisol levels showed a slight reduction, but not significant at 20min., but significantly increased 60min. after stress. After this time its concentration returned to an initial level. On the other hand, plasma glucose increased 20min. after stress with a maximum value at 60min. Total leucocytes number showed a maximum value at 50min., after that returned to an initial value. The number of erythrocytes was significantly reduced at 120min. Nevertheless, hematocrit showed a significant decrease in relation to 180min. The differential counting of leucocytes presented increasing in the monocyte percentage from time 0 to 300min. post-stress. Into this time intervals its values were constant. Reduction in the lymphocytes percentage and increasing in the neutrophils were related at 120min. after stress. The percentage of basophils was significantly different in the times 0, 10 and 20min. with a maximum value at 60min.

after stress. The presence of immature cells in the blood smears reaching a maximum value between 20 and 50min. after stress was also observed (Figures 1-3, Table 1).

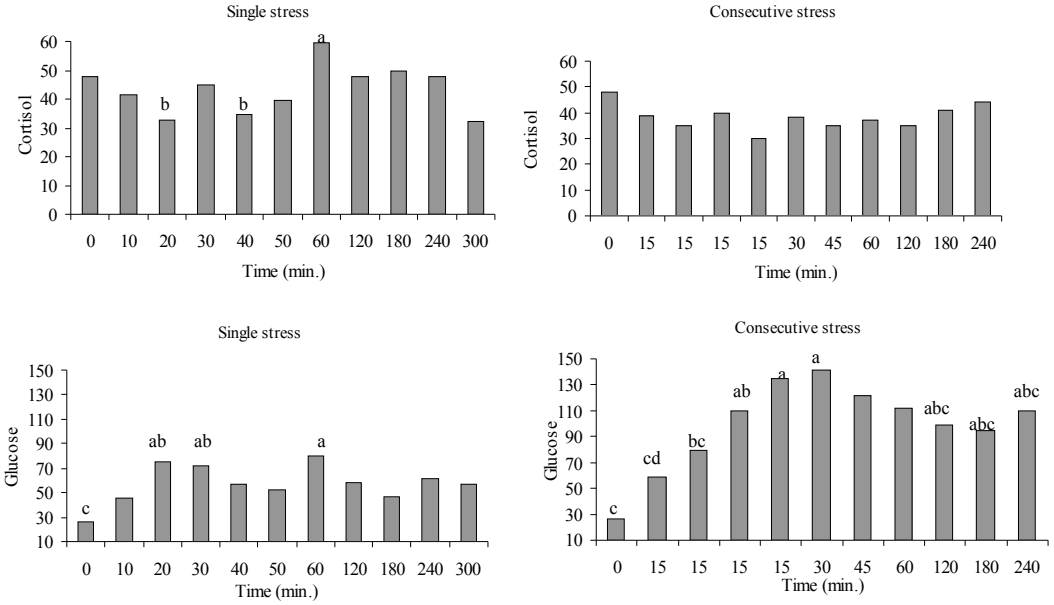

Figure 1. Haematological characteristics of *Oreochromis niloticus* exposed to single (samples collected minutes after stress) and consecutive stress (samples collected 15 minutes after each stress and the respective samples after the fourth stress) of capture. Different letters indicate significant difference into the samples (P<0.01).

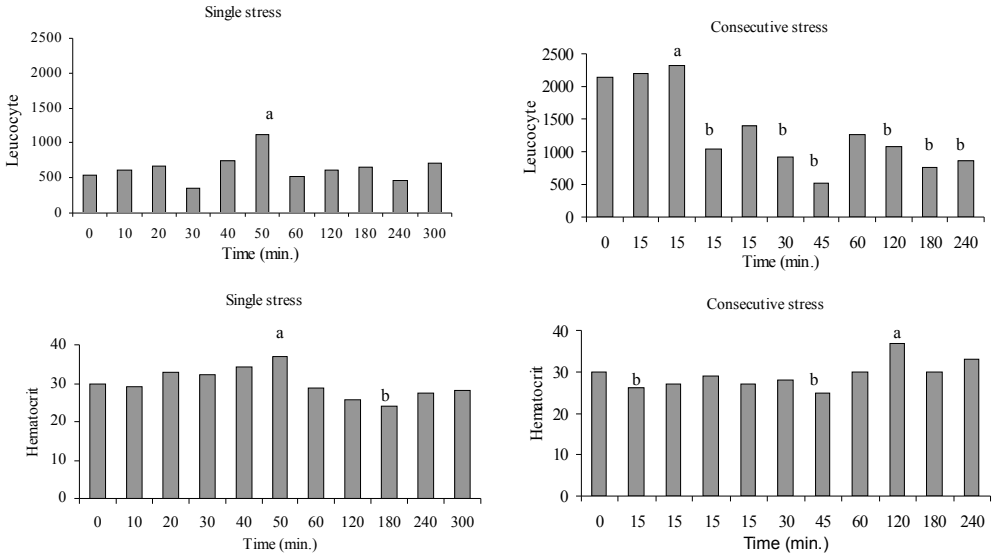

Figure 2. Haematological characteristics of *Oreochromis niloticus* exposed to single (samples collected minutes after stress) and consecutive stress (samples collected 15 minutes after each stress and the respective samples after the fourth stress) of capture. Different letters indicate significant difference into the samples (P<0.01).

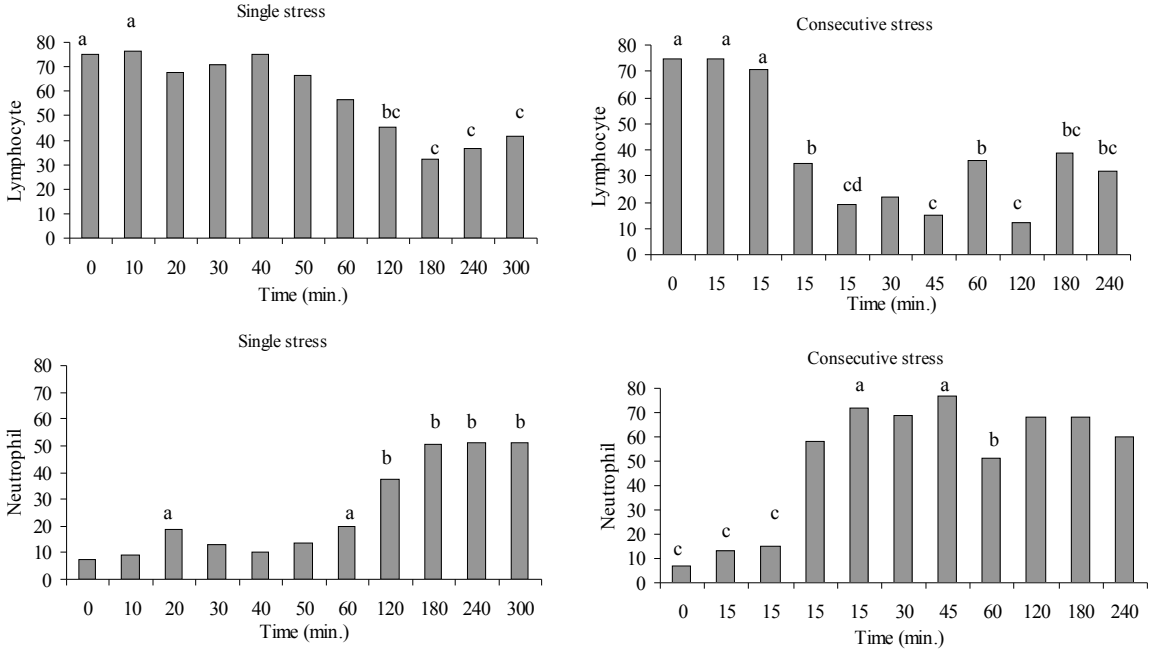

Figure 3. Haematological characteristics of *Oreochromis niloticus* exposed to a single (samples collected minutes after stress) and consecutive stress (samples collected 15 minutes after each stress and the respective samples after the fourth stress) of capture. Different letters indicate significant difference into the samples (P<0.01).

Table 1. Mean values of erythrocytes number (x 10^3/µl) and monocytes, basophils and immature cells (IC) percentages in the differential counting of leucocytes in the blood of *Oreochromis niloticus* exposed to a single stress of capture in the different sampling times.

Time (min.)	Erytrocytes	Monocyte	Basophils	IC
0	1732.9 a	17.7 a	0.5 b	0.2 c
10	1925.0 a	14.4 ab	0 b	0.4 c
20	1946.0 a	13.0 ab	0.3 b	9.7 a
30	2058.0 a	16.0 a	1.0 ab	5.0 ab
40	1562.0 ab	14.0 ab	0.8 ab	7.0 ab
50	1696.0 ab	18.4 a	1.3 ab	3.8 b
60	1443.3 ab	19.7 a	4.5 a	0.2 c
120	938.3 b	16.5 ab	2.7 ab	0 c
180	1495.0 ab	15.4 ab	1.8 ab	0.2 c
240	1458.3 ab	9.4 ab	3.2 ab	3.2 c
300	1586.0 ab	6.6 b	0.8 ab	0 c

Different letters indicate difference statistically significant between the sampling times (**P<0.01).

Haematological characteristics of tilapia exposed to consecutive stress

Erythrocytes number and the cortisol levels did not show significant changes along the period in which the stimuli were applied. However, glucose concentration revealed cumulative increasing when the stressor stimulus was applied reaching a maximum value at 30min. after the fourth stress. Total counting of leucocytes showed a significant reduction 15min. after the third stress remaining at low levels until 240min. after the fourth stress. On the other hand, increased hematocrit percentage was related 15min. after the first stress when compared to 120min. after the fourth stress. Monocytes and immature cells from the blood smears were not different between the samples. Once more, increased neutrophils percentage accompanied by decreased lymphocytes was observed 15min. after the third stress. These cells remained with this behaviour until the finish of samples. A little variation in the basophils percentage was noted by increasing only from the first to the second stressor stimulus (Figures 1-3, Table 2).

Table 2. Mean values of erythrocytes number (x 10^3/µl) and monocytes, basophils and immature cells (IC) percentages in the differential counting of leucocytes in the blood of *Oreochromis niloticus* exposed to consecutive stress of capture in the different sampling times.

Time (min.)	Erythrocytes	Monocytes	Basophils	IC
0	1732.9 a	17.2 a	0.5 ab	0.2 a
15	1592.0 a	11.5 a	0 b	0 a
15	1854.0 a	11.7 a	2.5 a	0 a
15	2102.0 a	7.0 a	1.8 ab	0 a
15	2095.0 a	7.2 a	2.5 ab	0 a
30	1736.0 a	7.2 a	2.2 ab	0 a
45	1946.0 a	8.4 a	0.4 ab	0 a
60	2090.0 a	12.4 a	1.0 ab	0 a
120	1908.3 a	17.7 a	0.5 ab	0.7 a
180	1801.4 a	9.1 a	0.1 ab	0.3 a
240	1983.3 a	7.3 a	0.5 ab	0 a

Different letters indicate difference statistically significant between the sampling times (**P<0.01).

Discussion

Morphological, biochemical and physiological changes as a result of stress constitute the so called General Adaptation Syndrome, that can be divided in three phases: alarm, resistance or adaptation (Selye, 1950). The responses of stress may be classified in primary, secondary and tertiary differing between fish species, the environment in which they are maintained

and the type of handling stress (Mazeaud *et al*., 1977). The cumulative effects of stress in Aquaculture have already been commented by Barton and Iwama (1991). Cronical exposition to stressors elevated the concentration of cortisol and glucose as well as haematological changes that prejudice the fish immune system favouring the pathogen installation (Yada and Nakanishi, 2002). The present work is a sequence of papers with Brazilian fish of economic importance such as *P. mesopotamicus* (pacu) and the hybrid tambacu. The aim is to determine the intensity and quickness that fish respond to a single and consecutive stress of handling by analysing physiological and haematological characteristics.

Pickering *et al*. (1982) have related in *Salmo trutta* the maximum values of cortisol and glucose 2 and 4 hs after handling. On this view, Woodward and Strange (1987) demonstrated that wild trout had more elevated levels of cortisol and glucose than the fish from the hatchery. In the present essay, the later increasing (60min.) in the circulating levels of cortisol and glucose may have been related to a low duration (30s emersion) of stress and to tilapia rusticity, as commented by the last authors in wild and cultured trout. Nevertheless, Rotlant and Tort (1997), Ruane *et al*. (2001) and Sloman *et al*. (2001) verified increasing in the cortisol and glucose levels respectively in *Pagrus pagrus*, *Cyprinus carpio* and *O. mykiss* 30min. after stress. On the other hand, *Sparus aurata* have shown increased concentration immediately after stress (Ortuño *et al*., 2001). These observations differ from the present study where changes have occurred only 60min. after single stress.

Studies on fish exposed to consecutive stress of handling are scarce. Cortisol remained at an elevated level when trout was submitted to consecutive stress (Barton *et al*., 1980). Consequently, Barton *et al*. (1986) also reported increased cortisol and glucose levels when salmon was submitted to 30s stress applied at 3h intervals. Similarly, three consecutive stress separated by 30min. intervals caused increasing in the cortisol and oxygen consumption rate in trout (Barton and Schreck, 1987). Later, Stratholt *et al*. (1997) studied the effects of mechanical disturbance on *O. kisutch* daily over a period of two weeks. Similar results were obtained in *S. trutta* exposed weekly to four stress stimuli (Forsmann *et al*., 1998). The present study differs from those authors. When tilapia was exposed to consecutive stress the cortisol did not alter significantly between the samples, although the glucose has shown cumulative effect 30min. after the fourth stress. There is an example that cortisol concentration can vary according to the fish species and the degree of the stress response being proportional to both severity and stressor duration. In fact, the low response of tilapia to stressor in this case must be explored. Not only changes in the glucose but also in the haematology may contribute to decreased organism resistance. The lack of an elevation in plasma cortisol was also observed by Salonius and Iwama (1993) in *O. kisutch* and *O. tshawytscha* and by Vijayan and Moon (1994) in *H. americanus*. Decreased cortisol 20min. after the single stress corroborated the results obtained in *S. salar* (McCormick *et al*., 1998).

In tropical fish, Martins *et al*. (2000) have reported significant reduction in cortisol of *P. mesopotamicus* exposed to the same consecutive stress of capture, corroborating the results of McCormick *et al*. (1998), but differing from this work with *O. niloticus*. According to Martins *et al*. (2000) the glucose was increased after the third stress such as in the present study. Contrarily, Krieger-Azzolini *et al*. (1989) and Barcellos *et al*. (2001) observed increased cortisol and glucose 1h after handling respectively for *P. mesopotamicus* and *R. quelen*. In the present essay glucose increased 20min. after the single and the third consecutive stress. Similar results obtained by Tavares-Dias *et al*. (2001) in *C. macropomum* submitted to 40s emersion stress were related. In this work, *O. niloticus* exposed to single stress the glucose levels increased more rapidly than the observed by those authors. On the other hand, increased cortisol and glucose levels were reported in *C. macropomum* immediately after stress of transport (Gomes *et al*., 2003).

While in the hybrid tambacu (Martins *et al*., 2002b) was not observed cortisol changes, in this essay tilapia have shown significant increasing 60min. after the same stress. After single stress glucose in tilapia increased more rapidly (20min.) than the hybrid tambacu (3 hs after stress) as reported by Martins *et al*. (2002b). The elevation in the cortisol and mainly glucose here observed was according to Vijayan *et al*. (1997) and Nolan *et al*. (1999) in *O. mossambicus*. Impaired response in cortisol of tilapia exposed to consecutive stress was according to Barcellos *et al*. (1997). Nevertheless, it must be emphasized that tilapia submitted to single stress of anoxia had an increase in the cortisol 60min. after stress. This was contrary to what Barcellos *et al*. (1997) observed.

The knowledge of the haematological characteristics is an important tool as well as parasitological analysis that can infer the fish population health. Fish respond in a different way depending on the stressor stimuli as observed by Carneiro and Urbinati (1998), Martins *et al*. (2000, 2001) and Gomes *et al*. (2003). Martins *et al*. (2002b) observed increased leucocytes number, decreased hematocrit and increased neutrophil percentage corroborating this work. Contrarily to that found in *C. macropomum* (Tavares-Dias *et al*., 2001), we observed increased hematocrit in tilapia exposed to single and consecutive stress.

The study of normal haematological characteristics

in *Tilapia zilli* revealed predominance of lymphocytes and neutrophils followed by monocytes and eosinophils (Ezzat *et al*., 1974). On the other hand, in *O. aureus* there was high percentage of neutrophils, monocytes and lymphocytes (Silveira and Rigores, 1989) and in *O. niloticus* also in normal conditions predominated neutrophils and lymphocytes followed by monocytes and rarely basophils and eosinophils (Ueda *et al*., 1997). Increased neutrophils and hematocrit percentages contrasted with those normally found (Tavares-Dias *et al*., 2000). Moreover, increased percentage of neutrophils and decreased percentage of lymphocytes in the circulating blood of *Anguilla anguilla* (Johanson-Sjöbeck *et al.*, 1978) and *Stizostediom vitreum* (Barton and Zitzow, 1995) corroborated these results. Contrarily to the observed in this essay, Salonius and Iwama (1993) related increased lymphocytes percentage in wild and cultivated salmon 4 hours after stress.

Following this topic, an increase in the haematological values during stress of capture and transport has been related to erythrocytes number, leucocytes, hematocrit and haemoglobin rate (Sopinska, 1985); in *Esox lucius* 20min. after exercise stress (Soivio and Oikari, 1976); in *Ictalurus punctatus* after stress of handling (Ellsaesser and Clem, 1986); in trout and *Carassius auratus* exposed to cronical and hypoxia stress (Kebus *et al*., 1992; Houston *et al*., 1996); and in *Morone saxatilis* submitted to acute stress (Young and Cech, 1993) that confirm the present investigation with tilapia except for the reduction in the erythrocytes number 120min. after single stress. The high percentage of immature cells that occurred in tilapia submitted to single stress between the times 20 and 50min. was neither cleared nor related to the analysed parameters.

Finally, this work confirmed the observations of Modrá *et al*. (1998) in which values of the differential counting of leucocytes in fish can vary according to the species, age and sex characteristics. Nevertheless, we also demonstrated that during consecutive stimulus of stress tilapia did not response when cortisol was analysed, contrasting with results previously obtained in *P. mesopotamicus* and the hybrid tambacu. The lack of cortisol response is probably due to fish being in the stage of resistance of Selye's General Adaptation Syndrome. The rusticity of tilapia was once more proved in relation to the other fish species. It is interesting to observe that after a unique stress tilapia showed an increase in the cortisol concentration that until then was not observed in tambacu (Martins *et al*., 2002b). However, glucose levels and cells of the differential counting in the circulating blood showed similar behaviour with previously related. In conclusion, whether cortisol is important for central nervous system activation increasing blood glucose, as suggested by Mommsen *et al*. (1999), in this study its influence was not clear. By supporting the above statements, future studies must be carried out to explain the influence not only of the cortisol but also of the glucose and leucocytes when Brazilian fish are exposed to stressors. Consequently, fish culturists must be able to recognize that some fishes may be more sensitive to stress at certain phases of their life cycle and in the environment in which are maintained.

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