ACTA
AMAZONICA

Sodium chloride added to transport water and physiological responses of Matrinxã *Brycon amazonicus* (Teleost: Characidae)

Elisabeth Criscuolo URBINATI[1] and Paulo César Falanghe CARNEIRO[2]

RESUMO

A adição de sal à água tem sido utilizada para a mitigação de estresse e aumento da taxa de sobrevivência em peixes. O presente estudo avaliou o efeito do cloreto de sódio (0,0; 1,0; 3,0 e 6.0 g/l) nas concentrações de cortisol plasmático, glicemia, triglicerídios, proteínas total plasmática, hematócrito, hemoglobina, número de eritrócitos, glicogênio e lipídio hepáticos, e lipídio muscular em matrinxã *Brycon amazonicum* adultos após quatro horas de transporte e durante período de recuperação de 96 h. Amostras foram coletadas antes e depois do transporte, bem como 24 e 96 h após a chegada. O nível de cortisol plasmático estava mais elevado logo após o transporte quando comparado à condição inicial (pré-transporte), exceto para os peixes transportados com sal nas concentrações 3,0 e 6,0 g/l. Comportamento semelhante foi observado para a glicemia, porém os peixes dos tratamentos 0,0, 1,0 e 3,0 g/l necessitaram de período superior a 24 h para recuperar a condição inicial. Foram registrados níveis mais baixos de glicogênio hepático em peixes do tratamento controle (0,0 g/l). Os parâmetros hemoglobina, número de eritrócitos, proteína plasmática total e lipídio hepático não apresentaram alterações durante o período experimental. Os valores de hematócrito diminuíram logo após o transporte em todos os tratamentos, retornando aos níveis iniciais após 24 h. Todos os tratamentos apresentaram redução nos níveis de lipídio muscular e triglicerídios durante o período de recuperação. Os resultados sugerem que a adição de 6,0 g/l de sal na água de transporte reduz as alterações fisiológicas de estresse e que é necessário período de 96 h após o transporte para a recuperação da condição inicial de matrinxãs transportados sem a adição de sal.

PALAVRAS-CHAVE

Sal, transporte, metabolismo.

Adição de cloreto de sódio à água de transporte e respostas fisiológicas do matrinxã Brycon amazonicus *(Teleost: Characidae)*

ABSTRACT

*The addition of salt to the water has been used to mitigate stress and improve survival in fishes. This study investigated the effects of sodium chloride (0.0, 1.0, 3.0 and 6.0 g/l) on levels of plasma cortisol, glucose, tryacilglycerol, total protein, hematocrit, hemoglobin, erythrocyte number, liver glycogen and lipid, and muscle lipid in adult matrinxã (*Brycon amazonicum*) after a 4-h transport and during a 96-h recovery period. Fish were sampled before and after transport, and 24 and 96 h of the recovery period. Plasma cortisol was higher than initial condition immediately after transportation, except in fish transported in 3.0 and 6.0 g/l of salt. A similar pattern was observed for blood glucose but fish transported in water with 0.0, 1.0 and 3.0 g/l of salt needed more than 24 h to return to the initial condition. Liver glycogen was lower after transport in fish not exposed to salt. Hemoglobin, erythrocyte number, total plasma protein and liver lipid did not change during the experiment but hematocrit was lower after transport in all treatments and returned to pre-transport values in 24 h. Reductions of muscle lipid and plasma tryacilglycerol were observed during the recovery period in fish from all treatments. The results show that 6.0 g/l NaCl added to the transport water reduce the stress responses and a 96-h recovery period is needed if no salt is used to mitigate the stress.*

KEY WORDS

Salt, transport, metabolism.

[1] Centro de Aqüicultura da Universidade Estadual Paulista – CAUNESP. Via de Acesso Prof. Paulo Donato Castelane, 14884-900, Jaboticabal, SP – Brasil. bethurb@caunesp.unesp.br. Tel.: +55 16 3203-2110. Fax: +55 16 3203-2268. Corresponding author.

[2] Pontifícia Universidade Católica do Paraná – PUCPR. Centro de Ciências Agrárias e Ambientais – CCAA. CP 129, 83010-050, São José dos Pinhais, PR – Brasil. paulo.carneiro@pucpr.br.

INTRODUCTION

Stress is defined as the non-specific response of the body to any demand upon it (Selye, 1973). The physiological reactions caused by stressors evolve adaptive mechanisms that allow fish to maintain homeostasis in the presence of adverse stimuli by mobilization of energy reserves (Sumpter, 1997).

Aquacultural practices, such as handling and transport, are known to induce stress in fish (Carmichael *et al.*, 1983; Carneiro & Urbinati, 2002) and are usually associated with fish losses (Hattingh *et al.*, 1975). If the fish are not permitted enough time to recover completely after stress, a second stressor can be fatal (Carmichael, 1984).

Sodium chloride (salt) is one of the most used stress-reduce substances added to transport water for many fish species (Hattingh *et al.*, 1975, Carmichael *et al.*, 1984; Carmichael & Tomasso, 1988, Weirich *et al.*, 1992). The addition of salt to the water increases the salinity to levels similar to those of the fish blood, diminishing the ionic gradient (McDonald & Milligan, 1997) and metabolic and hormonal responses (Sumpter, 1997).

Matrinxã (*Brycon amazonicum*) is a freshwater stenohaline fish from the Amazon Basin, of increasing importance to the aquaculture industry in Brazil, and is very sensitive to transport operations (Urbinati & Carneiro, 2004). This study evaluated the effect of sodium chloride added to the water on metabolic responses of adult matrinxã during transport and recovery.

MATERIAL AND METHODS

Fish were food restricted for 48 h prior to the transport. One hundred-twenty matrinxãs (mean body weight 1.0 kg ± 0.2 SEM) were transported in four 200-l plastic tanks for four hours on a pickup truck. Each tank hauled 30 fish (150 $kg.m^{-3}$) at different NaCl concentrations: 0.0, 1.0, 3.0, and 6.0 g/l. Water temperature, pH, un-ionized ammonia nitrogen (NH_3-N), CO_2 and oxygen were measured every 30 min during the transport. Oxygen levels were maintained above 6 $mg.l^{-1}$ by injection of liquid oxygen.

Fish were sampled before (initial condition) and after transport (arrival), and 24 and 96 h later (recovery period). For the recovery period fish were maintained in four 300-m^2 earthen ponds. In each period, five fish of each treatment were anesthetized (benzocaine, 50 $mg.l^{-1}$) and heparinized blood was drawn from the caudal vessel. Blood glucose (King and Garner, 1947), plasma cortisol (Radioimunoassay Coat-a-Count kit, Diagnostic Products Corporation), tryacilglycerol (Labtest[a] kit), total protein (Gornall *et al.*, 1949), hemoglobin (Labtest[a] kit) and erythrocyte number (Neubauer chamber) were determined. A blood sample collected in capillary tubes was centrifuged to determine hematocrit. Fish were killed and liver and white muscle were removed and stored at -20 °C for glycogen (Moon *et al.*, 1989) and lipid (Bligh & Dyer, 1959) analysis.

A split-plot plus control (initial condition) design was employed and data were analyzed by two-way analysis of variance (ANOVA), with salt treatments (0.0, 1.0, 3.0 and 6.0 $g.l^{-1}$) and sampling times (arrival, 24 and 96 h) as the factors. Where F values indicated significance ($P<0.05$), means were compared using Tukey test. Results are presented as means ± SEM. All data were analyzed with the statistical software SAS 6.12.

RESULTS

During the 4-h transport, un-ionized ammonia nitrogen (NH_3-N) and CO_2 increased from 0.22 $ug.l^{-1}$ and 18 $mg.l^{-1}$ to 19.43 $ug.l^{-1}$ and 136 $mg.l^{-1}$, respectively, while pH dropped from 7.4 to 5.9. Water temperature increased from 26.3 to 29.5 °C after 4 h of transport in all the experimental units. No mortality was observed during the whole experimental period.

After transport, plasma cortisol levels of fish from 0.0 and 1.0 $g.l^{-1}$ treatments were higher than inicial condition ($P<0.01$ and $P<0.05$, respectively), returning to the initial condition 24 h later (Figure 1). A similar pattern was verified for blood glucose but the fish from 0.0, 1.0 and 3.0 g/l treatments needed more than 24 h to return to the initial condition. Only fish transported with 6.0 $g.l^{-1}$ did not show alteration on blood glucose after transport when compared to initial condition (Figure 2). The liver glycogen changed inversely to the glucose. In fish not exposed to salt liver glycogen decreased after transport ($P=0.037$). Fish from 0.0 and 1.0 $g.l^{-1}$ treatments showed lower ($P<0.01$) glycogen levels than those exposed to 6.0 g/l of salt at the end of transportation. After 24 h the initial condition was re-established in all fish (Figure 3).

There were no significant differences among treatments on the parameters presented in Table 1. Therefore the average values of the four treatments, in each sampling time, were listed for comparisons among sampling times. No significant differences were observed in plasma protein, liver lipid, hemoglobin and

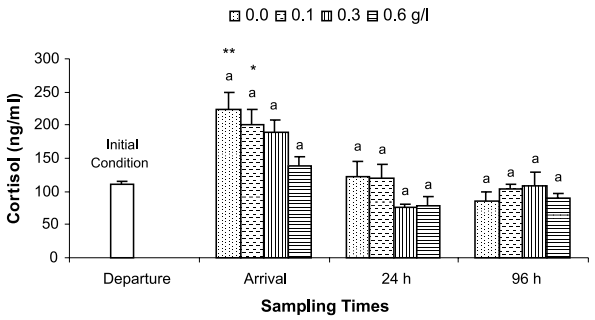

Figure 1 - Plasma cortisol of matrinxã exposed to NaCl during transport. Different letters indicate differences ($P<0.05$) among treatments within the sampling time. Single and double asterisks indicate differences ($P<0.05$ and $P<0.01$, respectively) between treatments and initial level (open bar); all treatments share the same initial condition. Vertical bars represent SEM ($N=5$).

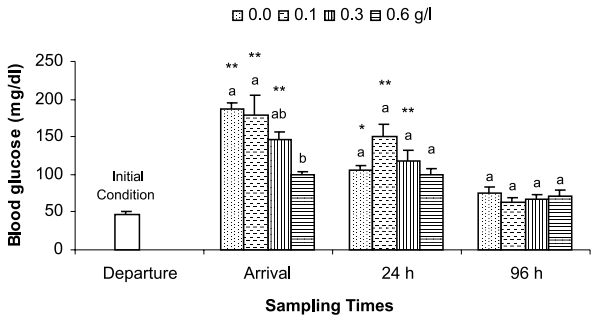

Figure 2 - Blood glucose of matrinxã exposed to NaCl during transport. See Figure 1 for explanation of letters and asterisks in the bars.

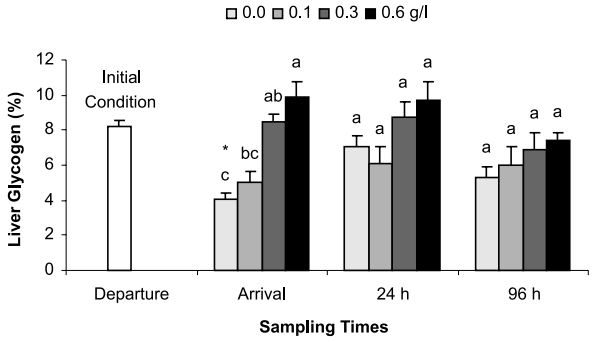

Figure 3 - Liver glycogen of matrinxã exposed to NaCl during transport. See Figure 1 for explanation of letters and asterisks in the bars.

erythrocyte number. The amount of muscle lipid was lower ($P<0.05$) 96 h after transportation in fish from all treatments and tryacilglycerol levels decreased continually ($P<0.05$) during the experiment. Fish also showed decrease in hematocrit after transport, recovering the initial condition in 24 h (Table 1).

DISCUSSION

Stress in fish involves the activation of two types of endocrine responses, the adrenergic system (adrenaline/noradrenaline) and the hypothalamo-pituitary axis (cortisol). These hormones are related to glucose regulation by glycolysis, glycogenolysis and glucogenesis. The blood glucose elevation is started and sustained by the action of adrenaline and cortisol on tissues such as liver and muscle. The greater the stress intensity, the longer the period required by fish to recover homeostasis and the resting condition (Carmichael *et al.*, 1984; McDonald & Milligan, 1997; Sumpter, 1997; Iwama *et al.*, 2006).

Matrinxã transported at 0.0, 1.0 and 3.0 $g.l^{-1}$ of salt showed stress responses characteristic to most of the teleost fishes submitted to an acute stress, e.g. cortisol and/or glucose elevation (Sumpter, 1997). The blood glucose levels after transport in fish exposed to 6.0 $g.l^{-1}$ of salt suggest no stress response. We cannot state that glucose levels did not change in those fish, because blood samples were not done during transportation, but if that

Table 1 - Liver and muscle lipid (%), plasma triacylglycerol (mg/dl) and total protein (mg/ml), hemoglobin (g/dl), erythrocyte number per mm^3 (x 10^3) and hematocrit (%) of transported matrinxã. SEM are within parenthesis (N=5 – arrival; N=20 – other sampling times). Different letters in the same line indicate significant differences ($P<0.05$).

	Sampling Times			
	Departure	Arrival	24 hours	96 hours
Liver lipid	17.13 (3.28) a	18.29 (1.48) a	16.32 (1.37) a	20.94 (2.11) a
Muscle lipid	2.49 (0.14) ab	2.54 (0.17) a	2.65 (0.14) a	2.02 (0.08) b
Triacylglycerol	525 (27.47) a	386 (15.88) b	338 (12.87) c	337 (11.27) c
Plasma protein	9.24 (0.48) a	7.91 (0.23) a	8.12 (0.25) a	8.21 (0.35) a
Hemoglobin	12.60 (0.37) a	11.91 (0.46) a	13.39 (0.34) a	-----
Erythrocyte	3.638 (212.1) a	3.748 (100.2) a	3.917 (86.9) a	-----
Hematocrit	49.10 (1.05) a	42.18 (0.85) b	48.70 (0.94) a	47.90 (1.07) a

happened the salt probably helped matrinxã to recover the initial condition during the transport period. The positive effect of salt added to the transport water in reducing plasma cortisol and blood glucose level has been demonstrated (Hattingh *et al.*, 1975; Carmichael *et al.*, 1984). However an increase in plasma cortisol was observed in striped bass *Morone saxatilis* transported in water with 10 $g.l^{-1}$ of added salt (Harrell, 1992). A 24-h recovery period for plasma cortisol levels was also described for coho salmon *Oncorhynchus kisutch* transported for 4 or 12 h in water without added salt (Specker & Schreck, 1980).

Blood glucose elevation in matrinxã exposed to 0.0, 1.0 and 3.0 $g.l^{-1}$ of salt was similar to those observed in *Labeo capensis* that presented an increase of 168% after a 4-h transport with no added salt, and a 24-h recovery (Hattingh, 1976). Unchangeable levels of blood glucose in fish exposed to 6.0 $g.l^{-1}$ of salt indicate that ambient salinity can efficiently reduce glucose-induced changes after transport. Breakdown of liver glycogen was observed mainly in matrinxã transported without salt. Liver glycogen decrease and blood glucose increase were also registered in tilapia *O.mossambicus* submitted to confinement stress (Vijayan et al., 1997).

Weirich et al. (1992) reported no osmoregulatory dysfunction in hybrid white bass x striped bass *M. chrysops x M. saxatilis* transported in 8.0 $g.l^{-1}$ salt added water, contrarily to what was observed in 1.0, 16.0 and 24 $g.l^{-1}$. These authors suggested that 8 $g.l^{-1}$ is similar to the internal osmotic pressure of that species, diminishing the ionic gradient between external and internal environments. This fact allows the fish to save energy that is used for ion balance during stressful situations. However, every fish species present a different preference in terms of salinity and Hattingh *et al.* (1975) suggested that salt concentrations between 3 and 7 $g.l^{-1}$ improve health conditions of several fish species during transport. The present study showed that 6.0 $g.l^{-1}$ of salt can help matrinxã to maintain homeostasis after transport stress, avoiding energetic spending as observed by the blood glucose and liver glycogen profiles.

Lipids are mainly storred in fish body as triacylglycerol in several organs and tissues like liver, muscle and visceral fat. The 4-h transport did not affect the tissue lipid content in any treatment. During the recovery, lipid reserves were mobilized evidenced by diminished muscle lipid and blood tryacilglycerol. Once cortisol had already returned to the resting levels at the recovery period, it is supposed that other hormones, such as glucagon, are involved in lipolysis. This supposition is supported by the fact that glucagon is released during starvation period (Suarez & Mommsen, 1987) and that matrinxãs were not fed during the experimental period.

Stress responses are related to increase in energy demand, which includes higher need of glucose and oxygen (Schreck, 1981). The increased oxygen supply to the tissues during acute stressful situation is achieved by artery expansion and increase of blood volume (Gratzek & Reinert, 1984). When this happens, alterations in hematological parameters, such as hematocrit, hemaglobin and erythrocytes, are expected, as shown in mudfish *Labeo umbratu* after capture and transport (Hattingh & van Pletzen, 1974). However, no changes in hemoglobin and erythrocyte were observed in matrinxã although hematocrit decreased after transport, similarly to that observed in *Labeo capensis* after a 4-hour transport (Hattingh, 1976).

The results show the stressing effects of the transport practice in metabolic and hormonal responses of matrinxã and their prevention by adding salt ($6.0 g.l^{-1}$) in the water.

LITERATURE CITED

Bligh, E.G.; Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology,* 37: 911-917.

Carmichael, G.J.; Wedmeyer, G.A.; McCraen, J.D.; Millard, J.L. 1983. Physiological effects of handling and hauling stress on smallmouth bass. *Progressive Fish-Culturist*, 45: 110-113.

Carmichael, G.J.; Tomasso, J.R.; Simco, B.A.; Davis, K.B. 1984. Characterization and alleviation of stress associated with hauling largemouth bass. *Transaction of the American Fisheries Society*, 113: 778-785.

Carmichael, G.J.; Tomasso, J.R. 1988. Survey of fish transportation equipment and techniques. *Progressive Fish-Culturist,* 80: 155-159.

Carneiro, P.C.F.; Urbinati, E.C. 2002. Transport stress in matrinxã, *Brycon amazonicum* (Teleostei: Characidae), at different densities. *Aquaculture International*, 10: 221-229.

Gornall, A.G.; Bardawill, C.J.; David, M.M. 1949. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, 4: 751-766.

Gratzek, J.B.; Reinert, R. 1984. Physiological response of experimental fish to stressful conditions. *Natl. Cancer Inst. Monogr*, 65: 187-193.

Harrell, R.M. 1992. Stress mitigation by use of salt and anesthetic for wild striped bass captured for brood stock. *Progressive Fish-Culturist,* 54: 228-233.

Hattingh, J.; Van Pletzen, J.J. 1974. The influence of capture and transportation on some blood parameters of fresh water fish. *Comparative Biochemistry and Physiology*, 49A: 607-609.

Hattingh, J.; Fourie, F.L.R.; Van Vuren, J.H.J. 1975. The transport of freshwater fish. *Journal of Fish Biology*, 7: 447-449.

Hattingh, J. 1976. Blood sugar as an indicator of stress in the freshwater fish, *Labeo capensis* (Smith). *Journal of Fish Biology*, 10: 191-195.

Iwama, G.K.; Afonso, L.O.B.; Vijayan, M.M. 2006. Stress in fishes. *In*: Evans, D.H.; Claiborne, J.B. (Eds). *The physiology of fishes*. 3ª ed. CRC, New York, p. 319-342.

King, E.J.; Garner, R.J. 1947. Colorimetric determination of glucose. *Journal of Clinical Pathology,* 1: 30-33.

McDonald, G.; Milligan, L. 1997. Ionic, osmotic and acid-base regulation in stress. *In*: Iwama, G.W.; Pickering, A.D.; Sumpter, J.P.; Schreck, C.B (Eds). *Fish stress and health in aquaculture*. University Press, Cambridge, p. 119-144.

Moon, T.W.; Foster, W.; Plisetskaya, E.M. 1989. Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. C*anadian Journal of Zoology*, 67: 2189-2193.

Schreck, C.B. 1981. Stress and compensation in teleostean fishes: Response to social and physical factors. In: Pickering, A. D. *Stress and Fish*. Academic Press, London, p. 295-322.

Selye, H. 1973. Homeostasis and heterostasis. *Perspectives of Biological Medicine*, 16: 441-445.

Specker, J.L.; Schreck, C.B. 1980. Stress response to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. *Canadian Journal of Fisheries and Aquatic Science*, 37: 765-769.

Suarez, R.K.; Mommsen, T.P. 1987. Gluconeogenesis in teleost fishes. *Canadian Journal of Zoology*, 65: 1869-1882.

Sumpter, J.P. 1997. The endocrinology of stress. *In*: Iwama, G.W., Pickering, A.D., Sumpter, J.P., Schreck, C.B (Eds). *Fish stress and health in aquaculture*. University Press, Cambridge, p. 95-118.

Urbinati, E. C.; Carneiro, P. C. F. 2004. Práticas de manejo e estresse dos peixes em piscicultura intensiva. *In*: Cyrino, J.E.P.; Urbinati, E.C.; Fracalossi, D.M., Castagnolli, N. (Eds). *Tópicos especiais em piscicultura de água doce tropicas intensiva.* TecArt, São Paulo, p.171-194.

Vijayan, M.M.; Pereira, C.; Grau, E.G.; Iwama, G.K. 1997. Metabolic responses associated with confinement stress in tilapia: The role of cortisol. *Comparative Biochemistry and Physiology,* 116C: 89-95.

Weirich, C.R.; Tomasso, J.R.; Smith, T.I.J. 1992. Confinement and transport-induced stress in white bass *Morone chrysops* x striped bass *M. saxatilis* hybrids: Effect of calcium and salinity. *Journal of the World Aquaculture Society*, 23: 49-57.

Recebido em 10/05/2006
Aceito em 27/112006