

Stress in *Salminus brasiliensis* fingerlings due to different densities and times of transportation

[*Estresse em alevinos de dourado (Salminus brasiliensis) em diferentes densidades e tempos de transporte*]

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ABSTRACT

The stress in dorado fingerlings (*Salminus brasiliensis*) caused by transportation at densities of 5, 10, and 15g/l after 4, 8, and 12h was evaluated by the concentration of tissue cortisol measured by ELISA. The conditions of transportation were simulated on an orbital table shaker with horizontal movements, inside 15 litres plastic bags filled with 4 litres of water and pure oxygen. Cortisol concentrations increased in all densities after 4h of transportation converging to a common concentration at the end of the tested times. Electrical conductivity of water increased with density and transportation time. The transportation caused stress on fish, but the increase on density and in times of transportation did not cause mortality to fingerlings. The transportation of *S. brasiliensis* fingerlings can be done without mortality or apparent injuries to animals until the maximum analyzed density of 15g/l and up to 12h.

Keywords: *Salminus brasiliensis*, fish, stress, transportation, cortisol

RESUMO

O estresse em alevinos de dourado (Salminus brasiliensis) causado pelo transporte nas densidades 5, 10 e 15g/l após 4, 8 e 12h, foi avaliado por meio dos níveis de cortisol tecidual quantificado por ELISA. O transporte foi simulado em uma mesa agitadora com movimento orbital, em sacos plásticos de 15 litros, contendo 4 litros de água e oxigênio puro. As concentrações de cortisol aumentaram em todas as densidades após 4h de transporte e convergiram para uma mesma concentração ao final dos tempos de transporte. A condutividade elétrica da água aumentou com a densidade e com os tempos de transporte. O transporte causou estresse aos peixes, mas o aumento da densidade e do tempo de transporte não causou mortalidade nos alevinos. O transporte de alevinos de S. brasiliensis pode ser realizado até a densidade máxima avaliada de 15g/l e o tempo máximo de 12h sem que haja mortalidade ou danos aparentes aos animais.

Palavras-chave: Salminus brasiliensis, peixe, estresse, transporte, cortisol

INTRODUCTION

The transportation of fish is done in closed systems, using plastic bags which are filled with water and pure oxygen, or in opened systems, with specific containers that receive oxygen or air (Berka, 1986).

Despite the greatest use of closed systems, the method can become a limiting factor, as well as an important stressing factor due to the accumulation of metabolites in water, as carbon dioxide and total ammonia, and also due to the decreasing of dissolved oxygen concentrations and changes of water PH (Amend et al., 1982; Berka, 1986; Gomes et al., 2003a).

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The stress produced by fish transportation is classified as acute, since it causes an increase of cortisol levels into the plasma of the animals (Barton and Iwama, 1991). Cortisol releasing is soon activated when fish feels a homeostatic change, which is triggered by several conditions that stimulate the hypothalamus-pituitary-interrenal axis. External stimuli, caused by stressing agents, act out on hypothalamus that produces the releasing factor of corticotrophin, which acts out on the pituitary gland that, on its turn, produces adrenocorticotrophic hormone (ACTH), that reaches the interrenal tissue in the head kidney where cortisol is released into the blood vessels (Donaldson, 1981).

To evaluate the stress caused by transportation and transferring of fingerlings, plasma or tissue concentration of cortisol had been collected from several species of fish, as *Salmo trutta* (Pickering, 1984), *Paralichthys olivaceus* (De Jesus et al., 1991), *Oreochromis niloticus* (Hwang et al., 1992), *Seriola quinqueradiata* (Sakakura et al., 1998), *Salmo salar* (Sandodden et al., 2001), *Ictalurus punctatus* (Davis et al., 2002; Bilodeau et al., 2003), *Colossoma macropomum* (Gomes et al. 2003a,b), and *Rhamdia quelen* (Barcellos et al., 2001; Barcellos et al. 2004).

According to Amend et al. (1982) and Berka (1986), the best results during transportation were achieved when fishes were submitted to a fasting of 24h to promote the cleaning of digestive tract before the beginning of transportation, which avoid water quality degradation. According to these authors, the correct stocking density and time of transportation prevent the establishment of undesirable concentrations of metabolites into the water. A high density associated with a very long time of transportation can stress fish, impair transportation efficiency, and cause mortality, as well as cause negative effects on animals performance (Amend et al., 1982). Hence, an acute stress during transportation can predispose fish to pathologies after the stock-term due to immunosuppression caused by stress (Barton and Iwama, 1991; Wendelaar Bonga, 1997).

Although transportation of fish is an ordinary activity at aquaculture (Carmichael et al., 2001), it is also seen as an important stressing factor (Iversen et al., 1998); but few studies had been

made regarding stress on Brazilian native fish during transportation (Carneiro et al., 2002a,b; Golombieski et al., 2003; Gomes et al., 2003a,b; Urbinati et al., 2004).

This experiment studied the stress on dorado fingerlings (*Salminus brasiliensis* Cuvier, 1816) at different densities and times of transportation, considering this species has become important to Brazilian aquaculture.

MATERIAL AND METHODS

This trial was carried out at the Fish Culture Station of São Carlos (SC) in February 2005. *S. brasiliensis* fingerlings lengthing 3.75 ± 0.53 cm (mean=standard deviation) and weighting 0.71 ± 0.53 g were submitted to fast during 48h; then, they were stocked at a density of 5, 10, and 15g/l, equivalent to 7, 14, and 21 fish/l, respectively. The experimental units were composed by 15 litres plastic bags, with 4 litres of water and pure oxygen as the remaining part. The plastic bags were carefully closed and conditioned in a polystyrene box to avoid mechanical shocks and help on maintenance of temperature during the whole period of transportation.

The simulations of transportation were done on an orbital table shaker with horizontal movement, 40cm of amplitude and 1080 cycles/h. Each density of transportation was tested during 4, 8, and 12h. Due to the impossibility of using the shaker simultaneously with all experimental units, this trial was carried out according to a randomized block design, with three replications that ran at different times, which constituted the blocks distributed throughout the studied times.

At the beginning of the trial and at the end of transportation, water temperature, pH, electrical conductivity, and dissolved oxygen concentrations were measured in each experimental unit, using an YSI-55 digital oxymeter and one YSI-63 digital multi-parameter. Water samples of each experimental unit were also collected for analysis of total ammonia and nitrite concentrations, according to the methodologies described by Koroleff (1976) and Golterman et al. (1978), respectively. The non-ionized ammonia fraction in water was

calculated using Johansson and Wedborg (1980) equation.

Cortisol concentration was recorded in tissues from five fingerlings sampled in fish ponds prior the beginning of the experiment and from tissue extracted from samples of five fishes collected at each experimental unit just after the end of each transportation time. For cortisol extraction, each fish sample (n=5) was pooled and the whole fishes were grinded together prior the analysis and mixed with a phosphate buffered solution (PBSG). This mixture was washed with ether, evaporated on a heated bath, and sonicated after addition of PBSG (De Jesus et al., 1991). After extraction, the concentration was determined by ELISA using the Adaltis® EIAgem-Cortisol kit.

Survival was recorded in each experimental unit at the end of time of transportation. Water quality variables, survival, and tissue cortisol concentration were analyzed by regression (Zar, 1996) to establish the relationship of each variable with transportation time at the different densities. ANOVA (Zar, 1996) was used to compare the initial concentration of these variables at the beginning of transportation with the concentration after four hours of transportation. Each regression analysis was tested ($\alpha=0.05$) and only valid regressions were presented as lines in the figures showed in the next pages.

RESULTS AND DISCUSSION

Temperature and pH of water did not show significant differences ($P>0.05$) among densities and times of transportation, presenting mean values (\pm standard error) of $20.6\pm 0.8^\circ\text{C}$ and 6.8 ± 0.1 . The dissolved oxygen concentration in water at the end of each time always remained above 20mg/l in all the experimental units, exceeding oxymeter capacity, indicating a supersaturation of gas into the water.

At the beginning of this trial, total ammonia concentration was not present in the water; however, the concentrations showed significant increase ($P<0.05$) along the time at the 10g/l density (Fig. 1a). The low total ammonia concentration, registered at 5g/l, did not change with time and the same happened at 15g/l density, in which the highest concentrations were

registered after the first four hours of transportation. The lowest density did not produce ammonia to alter its concentration in water, but with the double of biomass (10g/l) the concentration increase was noticeable. The non-ionized ammonia concentrations in water always remained below 0.01mg/l and probably did not stress fish, once concentrations below 0.02mg/l were considered safe for the majority of fish species (Vinatea, 1997; Baldisserotto, 2002; Foss et al., 2003). The presence of nitrite in water was not detected at the initial readings, but its concentration increased ($P<0.05$) during transportation at 5 and 10g/l densities, whereas the concentrations found at 15g/l remained high during all times of transportation (Fig. 1b).

At the beginning of transportation, the electrical conductivity of water ($14.0\pm 1.7\mu\text{Scm}^{-1}$) increased significantly ($P<0.05$) during the first four hours in all densities. After that, it remained constant at 5 and 15g/l densities and there was a significant increase ($P<0.05$) with time at the 10g/l density (Fig. 2). The increase of water conductivity in the experimental units was caused by ions release, mainly Na^+ and K^+ , through gills, since fish presented exchanges on osmoregulation during stressing conditions (Wendelaar Bonga, 1997).

The mean concentration of cortisol (\pm standard deviation) on tissue of *S. brasiliensis* fingerlings at the beginning of transportation was $45.0\pm 21.2\text{ng/mg}$, a value lower ($P<0.05$) than the one registered at all tested densities after 4 hours of transportation, which were on average four times superior to the initial value. Later, cortisol concentrations stayed high at 15 and 10g/l densities, but decreased at the lowest density (Fig. 3).

The initial increase of cortisol concentration was also registered by Gomes et al. (2003a) in the transportation of *Colossoma macropomum* juveniles at different densities; however, concentration decreased as time passed by and when submitted to a period of recovery. After the first four hours of transportation, it was recorded an increase of cortisol for *Salmo salar* (Sandodden et al., 2001) and *Perca fluviatilis* (Acerete et al., 2004), when they were under stress due to transportation.

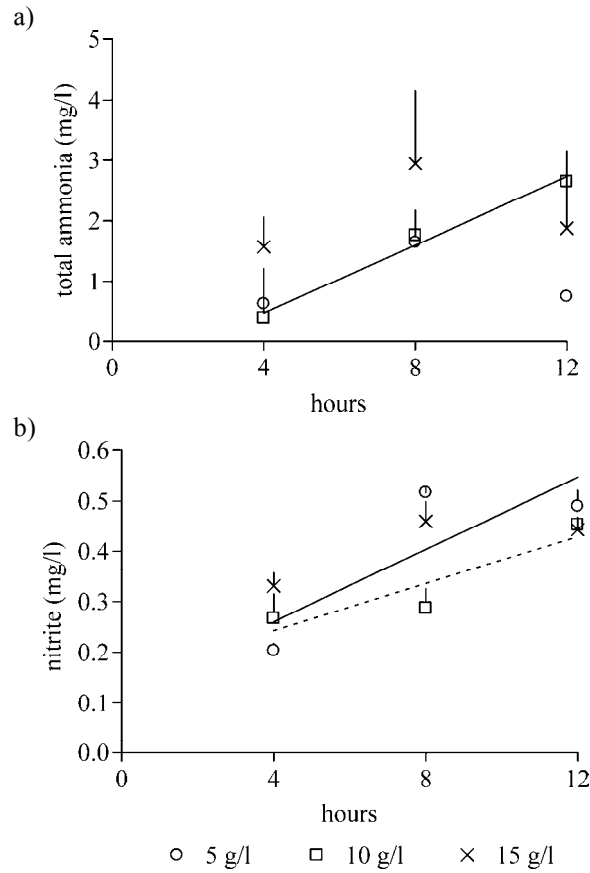


Figure 1. Total ammonia (a) and nitrite (b) concentrations (mean \pm standard error) in water during transportation of *S. brasiliensis* fingerlings in three times and densities. (a) filled line indicates regression between total ammonia and time at the density 10g/l; (b) filled line indicates regression between nitrate and time at the density 5g/l and dotted line at the density 10g/l.

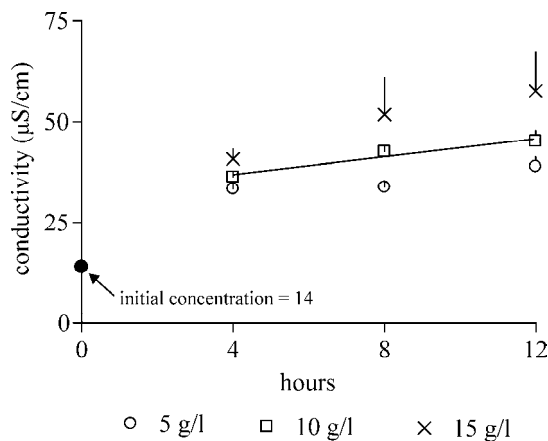


Figure 2. Conductivity (mean \pm standard error) of water during transportation of *S. brasiliensis* fingerlings in three times and densities. Filled line indicates regression between conductivity and time at the density 10g/l.

Stress in *Salminus brasiliensis* fingerlings...

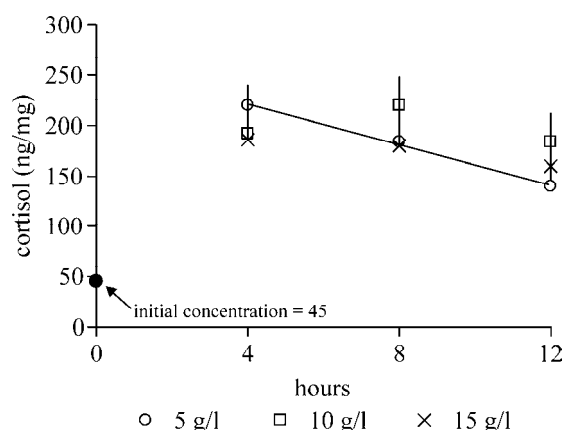


Figure 3. Cortisol concentrations (mean \pm standard error) in tissue of *S. brasiliensis* during transportation of *S. brasiliensis* fingerlings in three times and densities. Filled line indicates regression between cortisol and time at the density 5g/l.

The inverse relation among cortisol concentrations and density of transportation along the time were observed in *Brycon cephalus* fingerlings under stress transportation (Urbinati et al., 2004). Robertson et al. (1988) analyzed the plasmatic cortisol variation in *Sciaenops ocellatus* juveniles during transportation in water with 32‰ of salinity and registered the increase in cortisol levels after 1h 30min, when compared to the initial concentration. After 5h 30min, fish were transferred to recovery tanks and, after two days, concentrations decreased to values next to the initial ones. These authors also tested the 2h 30min time of transportation in 4‰ salinity water and observed that, after 30min, the plasmatic cortisol increased from almost zero to 150ng/ml, decreasing at the end of the trial to values near to 50ng/ml. According to Barcellos et al. (2001), the increase of plasmatic cortisol in *Rhamdia quelen* happened one hour after its transference to growing tanks, which could explain the fast effect registered in this study.

S. brasiliensis fingerlings were evaluated until the end of transportation times, since cortisol was not recorded during the recovering period, even though a reduction in concentration to levels near basal ones was expected. Nevertheless, after 8 and 12h of transportation, the reduction of cortisol concentrations showed the same pattern already observed by Barcellos et al. (2001) after 4, 12, and 24h, despite there was a different response concerning the duration of the stressing stimulation.

After the initial increase, cortisol concentrations from different densities converged to a common value at the end of the tested times of transportation ($P < 0.05$). These variation presented similarity to the standard response to other stimuli that cause acute stress, which tended to return to basal levels after the initial increase.

The densities and times of transportation produced stress on *S. brasiliensis* fingerlings but did not cause mortality, suggesting that conditions that could compromise the survival of fingerlings are beyond the tested levels. Therefore, until the maximum analyzed density of 15g/l and time of 12h, the transportation of *S. brasiliensis* fingerlings can be done without mortality or apparent injuries to animals.

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Stress in Salminus brasiliensis fingerlings...

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