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Transport of *Astyanax altiparanae* Garutti and Britski, 2000 in saline water

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ABSTRACT. Two experiments were performed. The first aimed to assess the tolerance of fingerlings *Astyanax altiparanae* to water salinity. Fish were exposed to salinity of 0, 3, 6, 9, 12 or 15 g NaCl L^{-1} for 96 hours. The fish mortality was 0%, in the levels of 0, 3 and 6 g L^{-1}; 75% in the level of 9 g $L^{-1}$and 100% at 12 and 15 g L^{-1} of common salt. The second experiment aimed to assess the parameters of water quality, mortality and blood glucose during transport. For this, *A. altiparanae* were stored in plastic bags at 22, 30 and 37 g of fish L^{-1} stocking densities and salinity of 0, 3, 6 and 9 g L^{-1}, for. Fish showed similar mortality levels in the different salinities and stocking densities. The increase in fish density reduced the dissolved oxygen levels and salinity decreased the pH. The blood glucose levels were higher in those fish with 0 g L^{-1} salinity and higher stocking densities. The addition of salt to the water reduces the stress responses of *A. altiparanae* during transport.

Keyword: density, stress, glucose, oxygen, common salt.

Transporte de *Astyanax altiparanae* Garutti and Britski, 2000 em água salinizada

RESUMO. Foram realizados dois experimentos. O primeiro teve o objetivo de avaliar a tolerância de alevinos de *Astyanax altiparanae* a salinidade da água. Os peixes foram submetidos às salinidades de 0, 3, 6, 9, 12 ou 15 g de NaCl L^{-1} durante 96 horas. A mortalidade dos peixes foi de 0%, nos níveis de 0, 3 e 6 g L^{-1}; de 75% no nível de 9 g L^{-1} e de 100% em 12 e 15 g L^{-1} de NaCl. No segundo experimento, objetivou-se avaliar os parâmetros de qualidade de água, mortalidade e a glicose sanguínea durante o transporte. Para isso, *A. altiparanae* foram estocados em sacos plásticos nas densidades de 22, 30 e 37 g de peixe L^{-1} e salinidades de 0, 3, 6 e 9 g de NaCl L^{-1}. A mortalidade foi semelhante nas diferentes salinidades e densidades de estocagem. O aumento da densidade de peixes reduziu o nível de oxigênio dissolvido e a salinidade da água reduziu o pH. Os níveis de glicose sanguínea foram maiores nos peixes expostos a salinidade de 0 g L^{-1} e nas maiores densidades de estocagem. A adição de sal na água reduz as respostas de estresse em *A. altiparanae* durante o transporte.

Palavras-chave: densidade, estresse, glicose, oxigênio, sal comum.

Introduction

Commercial fish farming increases the quest for efficient handling practices to minimize the losses during the reproductive period (GROTTUM et al., 1997). Transport can trigger physiological responses in these animals due to stress, therefore carefully monitoring of such responses is essential to help the fish adapt to the handling operations (GOMES et al., 2006b).

Conditioning fish in plastic bags, despite the lack of a steady supply of dissolved oxygen is a transportation method for these animals, particularly in Brazil (GOLOMBIESKI et al., 2003; AZAMBUJA et al., 2011). However, the transportation time, temperature, fish density and the high concentrations of ammonia and other metabolites excreted into the water can induce stress in the fish during transport in closed systems (GOMES et al., 2006a).

The stress caused to the fish during transport includes physiological changes in the animals in order to adapt to the new conditions. These responses are categorized into three groups: primary; corresponding to the endocrinal changes, such as increased plasma catecholamines and corticosteroids (MAZEAUD et al., 1977; CARNEIRO et al., 2009). These changes trigger secondary responses such as spikes in the blood glucose levels, changes in the hematological parameters and increased membrane permeability (MAZEAUD et al., 1977; BARTON, 2002) which increases the

water uptake and blood electrolyte losses in freshwater fish (MAZEAUD et al., 1977; CECH et al., 1996). The tertiary response reduces growth, causes behavioral changes and increases the susceptibility to disease due to the depression of the immune system, which in turn may increase the mortality (WENDELAAR BONGA, 1997). The plasma cortisol or glucose, which varies with the type and duration of animal exposure to stressors, is a good indicator to assess the stress levels in fish (HASAN; BART, 2007; OBA et al., 2009).

Storage density can also cause stress in fish, especially during transport (TANG et al., 2009). Decreased density during transport reduces the stress as it minimizes the abrasive contact between the fish (PICKERING, 1993) and reduces the drop in water quality (GOLOMBIESKI et al., 2003). Common salt (NaCl) can attenuate the responses to stress during transport as reported for *Colossoma macropomum* (GOMES et al., 2003) as it reduces the osmotic gradient between the fish plasma and the water and also reduces the ion transport through the cell membranes by increasing the mucous production in the gill epithelia.

Astyanax altiparanae, one of the most common among the freshwater fish, is reared in various regions of Brazil. This fish is attractive for commercial production because of its rapid growth and easy handling (GONÇALVES et al., 2014), early sexual maturity and excellent meat for the consumer. However, knowledge regarding the responses of *A. altiparanae* to stress during transport is still meager.

The aim of this study was to evaluate the responses of the *A. altiparanae* fingerlings to stress during transport, using different stocking densities and common salt (NaCl) levels.

Material and methods

This experiment was approved by the Ethic Commission in Use of Production Animals of the Federal University of Viçosa (process number 28/2013). This certifies that the experiment is in agreement with the Ethical Principles for Animal Research established by the National Council of Animal Experimentation Control (CONCEA) and with present Brazilian legislation.

The experiment was conducted in the Laboratory of Fish Nutrition, Department of Animal Biology (DAB) of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil.

Common salt (NaCl) was the agent used to mitigate stress in the *Astyanax altiparanae* fingerlings, in two consecutive experiments (salinity tolerance and transport simulation). In the first, the acute tolerance of the fish to the salinity in the water was evaluated. In the second, the *A. altiparanae* responses to stress during transport were evaluated, using different stocking densities and salt levels. The final salt concentrations were those that provided the best survival rates of this fish in the salt tolerance test.

Salinity tolerance:

This experiment was performed by conducting six treatments (0, 3, 6, 9, 12 and 15 g L^{-1} of salt) and four replications in a randomized design.

Eight *Astyanax altiparanae* fingerlings (mean weight 0.37 g ± 0.05) were placed per aquarium (30 x 35 x 14cm) with 8 liters of water each, constant aeration and temperature of 24ºC controlled by a thermostat and heater (10 watts) with a 12-hour photoperiod. Twenty-four aquaria with 3mm screens to prevent the fish from escaping were used, each of which was considered as an experimental unit.

The *A. altiparanae* fingerlings were maintained without food for 96 hours and their mortality evaluated every three hours. Individuals were considered dead when spontaneous movements or responses to mechanical stimuli were lacking. The dead fish were removed from the aquarium, counted and discarded. The total number of dead fish per treatment was recorded at the end of this experiment and the averages evaluated by descriptive analysis.

Transport simulation:

This experiment was conducted in a completely randomized design in a factorial layout (4 x 3) with three replications. The treatments included concentrations of 0, 3, 6 and 9 g L^{-1} of salt and stocking densities of 22, 30 and 37 g fish L^{-1}.

The *Astyanax altiparanae* fingerlings were transferred to a tank in the Fish Farming Sector, DAB/UFV for depuration and starved for 24 hours to empty their digestive tracts. Following this period, they were distributed in densities of 22, 30 and 37 g fish L^{-1} per plastic bag (45 x 27 cm) with a quarter of its water capacity and two quarters of oxygen, introduced via a hose attached to an oxygen cylinder, in salinity concentrations of 0, 3, 6 and 9 g L^{-1} of salt. Plastic bags were sealed to prevent the escape of O_2 and placed in styrofoam boxes. Each plastic bag was considered an experimental unit.

The styrofoam boxes with plastic bags containing fish, were shaken manually every ten minutes for eight hours, to simulate the conditions of transport. Immediately after transport simulation, the fish were transferred from the plastic bags to the aquarium (30 x 35 x 14 cm) with 8L of freshwater

and controlled temperature (24°C) thermostat and heater (10 watts), constant aeration (7.0 to 7.5 mg L^{-1}) and 12-hour photoperiod. Fish mortality was observed after 24 hours.

For blood collection, the fish were anesthetized in benzocaine solution (50 mg L^{-1}), the caudal peduncle was cut with a scalpel and the blood was directly deposited on test strips of glucose. Blood samples of 10 fish per treatment were collected, prior to the commencement of the experiment and eight hours post transport simulation. Blood glucose was measured using a digital monitor Optium Xceed ™.

Temperature, pH, dissolved oxygen and ammonia dissolved in water were evaluated during the experimental period utilizing a common alcohol thermometer, potentiometer, portable oximeter and digital photometer, respectively.

Data were subjected to analysis of variance (ANOVA) and to regression at 5% probability ($p < 0.05$) for means with significant differences.

Results and discussion

Salinity tolerance

The concentrations of 0, 3 and 6 g L^{-1} of salt were found to induce no mortality in the *Astyanax altiparanae*. However, fish mortality began only after 12 hours and reached up to 75% after 96 hours in saline 9 g L^{-1}, and 100% at 12 and 15 g L^{-1} (Table 1).

Table 1. *Astyanax altiparanae* mortality (Mort.) of 3-96 hours in water with 0 to 15 g L^{-1}.

	Salinity (g L^{-1})					
Hour	0	3	6	9	12	15
3	–	–	–	–	50	100
6	–	–	–	–	100	100
12	–	–	–	9.4	100	100
15	–	–	–	18.8	100	100
18	–	–	–	21.9	100	100
30	–	–	–	25.0	100	100
33	–	–	–	34.4	100	100
42	–	–	–	40.6	100	100
54	–	–	–	46.9	100	100
57	–	–	–	50.0	100	100
69	–	–	–	53.1	100	100
75	–	–	–	56.3	100	100
87	–	–	–	59.4	100	100
90	–	–	–	65.6	100	100
93	–	–	–	71.9	100	100
96	–	–	–	75.0	100	100
Mort. (%)*	0	0	0	75	100	100

*Descriptive analysis

The survival values after placing *Astyanax altiparanae* individuals in concentrations of 0, 3 and 6 g L^{-1} of salt for 96 hours concur with the reports that a salinity of 6 g L^{-1} does not affect the survival of the freshwater fishes (ALTINOK; GRIZZLE, 2001; LUZ et al., 2008; LUZ; SANTOS, 2010). Moreover, the survival of all the *A. altiparanae* individuals for the first 12 hours of exposure to a concentration of 9 g L^{-1} of salt showed that this fish can be transported to short distances. Water salinity is used extensively to reduce stress in the fish during transport (CARNEIRO; URBINATI, 2001), with a positive or negative effect, depending on the salt concentration. The adaptation of any species to salinity changes is related to its ability to alter its hormone secretions and mechanisms to adjust the ion transport and permeability of the gills, kidneys and intestines to water, minimizing the changes in the plasma ion concentration (BALDISSEROTTO, 2009). *Acipenser medirostris* exhibited changes in the cellular structures of the gills and kidneys, the osmoregulatory organs, when placed in water with high salinity (POLETTO et al., 2013).

The high mortality of *A. altiparanae* with 9, 12 and 15 g L^{-1} of salt in the water can be explained by the osmoregulatory disorders due to the dissolved ions. Freshwater fish maintain a hyperosmotic condition, reducing the ion losses and eliminating the excess body water, water salinity reduces the osmotic and ionic differences between the fish and the water (RILEY et al., 2003). However, excess salt in the water induces difficulty in fish osmoregulation (JOMORI et al., 2012). Hypo and hyperosmotic treatment may or may not cause stress and/or affect the metabolic rate of the animals (STIEGLITZ et al., 2012), although the concentrations of 9, 12 and 15 g L^{-1} of salt were observed to increase the osmotic imbalance in the fingerlings *A. altiparanae*. Tolerance to salinity varies with the species, developmental stage (FASHINA-BOMBATA; BUSARI, 2003) and fish exposure time. The *Lophiosilurus alexandri* fry, *Ctenopharyngodon idella* fingerlings and *Pseudoplatystoma corruscans* fry tolerate salinities of 2, 10 and 2 g L^{-1}, respectively (YILDIZ; UZBILEK, 2001; SANTOS; LUZ, 2009). This reveals the importance of evaluating tolerance to salinity of the fish based on its species and developmental stage.

Transport simulation

The salinity and stocking density were found to have no effect upon the mortality of *Astyanax altiparanae* during transport (Table 2). The stocking density reduced the dissolved oxygen level in the water ($y = -0.1397x + 24.018$, $R^2 = 0.9969$), especially with the 37 g fish L^{-1} (Table 3). The salinity of 8.14 g L^{-1} showed the lowest pH content of the water, based on the regression equation ($y = 0.0062x^2 - 0.1009x + 6.8482$, $R^2 = 0.9742$). The temperature and ammonia level did not differ among treatments (Table 3).

Table 2. *Astyanax altiparanae* mortality (mean ± standard deviation) after eight, 24 and 96 hours of transport, depending on water salinity and density storage.

	8h	24h	96h
[ns]Salinity (g L^{-1})		Mortality (%)	
0	0.30 ± 0.53	4.91 ± 4.79	7.14 ± 6.82
3	0.00 ± 0.00	2.35 ± 1.57	4.34 ± 3.04
6	0.00 ± 0.00	4.04 ± 4.04	5.56 ± 5.12
9	2.39 ± 3.72	5.78 ± 3.49	12.04 ± 7.88
[ns]Density (g fish L^{-1})		Mortality (%)	
22	1.52 ± 2.78	6.44 ± 4.80	11.27 ± 8.08
30	0.28 ± 0.51	3.89 ± 3.15	6.15 ± 4.27
37	0.23 ± 0.41	2.48 ± 2.06	4.39 ± 3.77

ns = not significant by analysis of variance, F test ($p > 0.05$)

Table 3. Levels of dissolved oxygen (DO) and pH (mean ± standard deviation) depending on stocking density and salinity in transport of *Astyanax altiparanae*.

	Density (g fish L^{-1})			
	22	30	37	
pH[ns]	6.66 ± 0.20	6.57 ± 0.18	6.54 ± 0.12	
DO[1]	20.98 ± 1.34	19.76 ± 0.80	18.88 ± 1.53	
	Salinity (g L^{-1})			
	0	3	6	9
pH[2]	6.86 ± 0.14	6.57 ± 0.12	6.50 ± 0.06	6.43 ± 0.11
DO[ns]	20.23 ± 1.56	19.50 ± 1.29	19.52 ± 1.32	20.23 ± 1.64

ns = not significant by analysis of variance, F test ($p > 0.05$). [1]Significant difference at the 5% level of significance ($p < 0.05$) for the linear regression. [2]Significant difference at the 5% level of significance ($p < 0.05$) for quadratic regression.

Water salinity and stocking density affected the blood glucose of the fish (Table 4), with higher values being recorded in water without any salt and with higher stocking densities ($y = 11.2436x - 194.115$, $R^2 = 0.8578$). The salinity of 5.96 and 6.40 g L^{-1} revealed the lowest blood glucose values for the densities of 30 and 37 g fish L^{-1}, respectively ($y = 2.1759x^2 - 25.95x + 120.82$, $R^2 = 0.6475$; $y = 4.3518x^2 - 55.70x + 225.07$, $R^2 = 0.9109$, respectively), while the density of 22 g fish L^{-1} showed no interaction with salinity (Table 4).

Table 4. Blood glucose levels (mean ± standard deviation) of *Astyanax altiparanae* depending on water salinity and stocking density (salinity x density) eight hours after transport simulation.

	Salinity (g L^{-1}) X Density (g fish L^{-1})			
	0[1]	3[ns]	6[ns]	9[ns]
22[ns]	62.67 ± 18.22	77.00 ± 20.00	66.67 ± 26.44	66.33 ± 6.44
30[2]	123.00 ± 32.00	56.00 ± 12.67	50.00 ± 2.00	61.33 ± 14.89
37[2]	232.67 ± 8.44	74.33 ± 7.78	70.33 ± 9.11	68.67 ± 17.68

ns = not significant by analysis of variance, F test ($p > 0.05$). [1]Significant difference at the 5% level of significance ($p < 0.05$) for the linear regression. [2]Significant difference at the 5% level of significance ($p < 0.05$) for quadratic regression.

The similarity in the *Astyanax altiparanae* mortality with different salinity levels can be explained as a result of the reduction of stress induced by the common salt. Transport stress causes ion losses via the gills and kidneys, increases the catecholamine and corticosteroid synthesis and hence the blood flow (OBA et al., 2009), greatly raising the energy expenditure by the fish to maintain the osmotic equilibrium. The salt lowers the osmotic pressure gradient between the plasma and water enabling the fish to maintain a closer isosmotic state (RILEY et al., 2003). This reduces the energy expenditure with the osmoregulation processes (STIEGLITZ et al., 2012) and stress, which is advantageous to the live fish during the transport. The positive impact of salinity was also revealed by the low mortality of the juvenile *Arapaima gigas* during transport in 3 and 6 g L^{-1} of salt (BRANDÃO et al., 2008).

A similar mortality rate of the *A. altiparanae* with different stocking densities shows that the reduction of dissolved oxygen in the water transport did not harm this species. The water quality varies with the density and depends on the time duration of transportation (PENG et al., 2011). The addition of pure oxygen into the bags raises the dissolved gas levels to very high magnitudes (CARNEIRO et al., 2009). This is desirable because, in addition to consuming oxygen, breathing produces carbon dioxide (CO_2), which leads to the formation of carbonic acid, which can be dissociated into H^+ and HCO_3^-, lowering the pH and reducing the NH_3 formation, which is toxic ammonia (TOMASSO et al., 1980; TANG et al., 2009; SINK, 2010). The pH of the water decreased after 30 to 90 minutes of transporting *Salmo salar* (FARRELL, 2010). Similar pH levels with different stocking density helped in keeping the ammonia toxicity at low levels. Dissolved oxygen levels also decreased as the stocking density of *Pampus argenteus* increased (PENG et al., 2011). The pH range best suited for *A. altiparanae*, hovers between 6.5 and 8.0 (PORTO-FORESTI et al., 2010) and despite the fact that the pH levels varied with the saline water, they continued to remain within this range.

The higher glucose levels in the *A. altiparanae* blood at the higher densities (30 and 37 g fish L^{-1}) of this fish are related to the increase in the stress intensity without common salt dissolved during the water transport (0 g L^{-1}). The blood glucose levels did not vary when 3, 6 or 9 g $L^{-1}$of salt were added to the water transport, independent of the density used. Exposure to stressors such as transport increases the plasma glucose levels of the fishes depending on the catabolic responses after the stress (BARTON; IWAMA, 1991) and provides additional energy resources for the animal to overcome the disturbance. This increase is related to the higher blood cortisol levels (GOMES et al., 2006b), which reduces the growth and increases the susceptibility to disease, besides increasing fish mortality (OBA et al., 2009). The plasma glucose levels of the *Arapaima gigas* (GOMES et al., 2006b) were higher at the end of the transport, although the differences in salinities did not affect this fish. In this study, the

interaction between the stocking densities and different salinities clearly reveals the role of salt in the mitigation of stress during the transport of the fish.

Conclusion

Astyanax altiparanae fingerlings tolerate water salinity up to 6 g L^{-1}. High densities increase the stress response and the addition of salt to the water reduces the stress responses of *A. altiparanae* during transport.

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