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Transport of jundiá *Rhamdia quelen* juveniles at different loading densities: water quality and blood parameters

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Fish transportation is a common practice on fish farms and is considered to be a stressor that could negatively affect fish health. The objective of this study was to evaluate several physiological responses of stress in jundiá caused by transport at different loading densities. Jundiá juveniles were placed in plastic bags on a mechanical transport simulator for four hours at four different loading densities (75, 150, 250 and 350 g L^{-1}) and then transferred to sixteen 80-L plastic boxes for 96 hours after transport. Water samples were collected before and after transport to measure dissolved oxygen, temperature, pH and ammonia levels. Blood samples were taken at departure and arrival, as well as at 24 and 96 hours after transport to monitor cortisol, glucose, ammonia, chloride and hematocrit levels. Water ammonia levels were found to increase gradually as loading densities increased. Plasma ammonia was higher after transport in fish from all treatments. Compared to initial values, substantial increases in plasma cortisol and ammonia levels were detected mainly in those fish submitted to the highest loading density. Blood glucose appeared to be positively influenced by the increase of transport densities. No statistical differences were observed in any of the other blood parameters. The costs in fish culture, as in other animal production systems, must be minimized and fish producers depend on optimal techniques to ensure better profit. Therefore, based on fish survival and the physiological indicators determined in the present study, especially during recovery, the best density at which to transport jundiá in plastic bags for four hours is about 350 g/L.

O transporte de peixes é uma prática comum em piscicultura e considerado como um agente estressor que causa efeitos negativos na saúde do peixe. O objetivo deste estudo foi avaliar algumas respostas fisiológicas de estresse no jundiá causadas pelo transporte em densidades diferentes. Juvenis de jundiá foram transportados em sacos plásticos num simulador de transporte por quatro horas em diferentes densidades (75, 150, 250 e 350 g L^{-1}) e transferidos para 16 caixas plásticas de 80 L por 96 horas após o transporte. Amostras de água foram coletadas antes e após o transporte para determinações de oxigênio dissolvido, temperatura, pH e amônia. Além dos momentos da saída e da chegada, amostras de sangue foram retiradas 24 e 96 horas após o transporte para monitorar os níveis de cortisol, glicose, amônia, cloreto e hematócrito. A amônia na água aumentou gradualmente acompanhando o aumento das densidades. A amônia plasmática estava elevada após o transporte nos peixes de todos os tratamentos. Comparando com os valores iniciais, aumentos substanciais nos níveis plasmáticos de cortisol e amônia foram registrados principalmente nos peixes submetidos à densidade de transporte mais elevada. Os níveis glicêmicos parecem ter sido influenciados pelo aumento nas densidades de transporte. Não foram registradas diferenças significativas nos demais parâmetros sanguíneos. O custo da criação de peixes, da mesma forma que de outros animais, deve ser minimizado e os produtores dependem de técnicas que permitam lucros maiores. Portanto, com base nos indicadores fisiológicos e na taxa de sobrevivência obtidos no presente estudo, especialmente considerando o período de recuperação, sugere-se que a melhor densidade para o transporte do jundiá em sacos plásticos por quatro horas seja de aproximadamente 350 g/L.

Key words: Stress, Cortisol, Glucose, Ammonia.

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Introduction

The success of fish manipulations depends on the control of adverse conditions within the captive environment. The great complexity of this issue makes it difficult to identify a single stressor during the routine activities of a fish farm. According to Barcellos *et al*. (2006), high densities, weaning and frequent net captures of fish are events that provoke chronic stress that, together with subsequent acute transport stress, may cause not only deleterious effects to fish health, but also death. According to Iwama *et al*. (2004), there are two types of stress responses: behavioral and physiological. Cortisol release from the interrenal glands is a primary fish response to stressful conditions that leads to a chain of physiological events such as glucose release into the bloodstream (Urbinati & Carneiro, 2004). These responses permit the fish to react to stressor-induced stimuli that demand energy from the physiological systems responsible for the production and release of glucose (Iwama *et al*., 2004).

The high cost of live fish transport is due to the large amount of water that needs to be transported, which necessitates increasing the fish loading rate for transportation. It is therefore important to identify adequate transport densities so as to minimize costs and mitigate the physiological stress responses of fish (Carneiro & Urbinati, 2002).

It is fundamentally important that studies concerning routine culture practices focus on fish species of local commercial importance. Jundiá, *Rhamdia quelen*, is a catfish with great potential for aquaculture in southern Brazil because of its fast growth, hardiness, and resistance to low temperatures that are typical of winters in the southern region of the country (Carneiro, 2004; Garcia *et al*., 2008). While water quality changes and the survival of juveniles during transport of this species in plastic bags have already been studied (Golombieski *et al*., 2003), no studies have addressed the effects of transport on blood chemistry or cortisol in jundiá. The objective of this study was therefore to evaluate the physiological responses of jundiá juveniles to transport stress at different loading densities.

Material and Methods

Fish were starved for 24 hours prior to transport so as to reduce oxygen consumption and ammonia excretion into the water. Jundiá juveniles (23.2 ± 5.3 g) were transferred to plastic bags with 1.8 L water and 4.0 L of pure oxygen, and then placed on a transport simulator where they remained for four hours at four different loading densities (in g L^{-1}): either 75 (6 fish), 150 (12 fish), 250 (18 fish), or 350 (26 fish). There were four replicates for each loading density. The transport simulator was built to reproduce the horizontal and vertical movements of a vehicle on a road, thus mimicking a commercial operation. The plastic bags with the fish were laid on a flat surface and protected from light and heat.

Water samples were collected before the plastic bags were closed and after the 4-hour transport period for determinations of dissolved oxygen and temperature (YSI 55 digital meter), pH (pHTester2, Oakton Instruments), and total ammonia (Boyd & Tucker, 1992). Un-ionized ammonia levels were calculated according to Emerson *et al*. (1975). Blood samples were collected to obtain physiological stress responses both before and after transport, as well as 24 and 96 hours later. For the first blood sample, four fish represented the initial condition before transfer to the plastic bags. The second blood sample was performed with four fish per treatment, each one from a different replicate after transport. From that point on, a recovery period was started; five fish from each plastic bag were transferred to each of the sixteen 80-L plastic boxes that were part of a recirculation system containing mechanical and biological filters, aeration and a controlled temperature.

Blood was collected from anesthetized fish (50 mg L^{-1} benzocaine) via the caudal vein. A portion of the blood was used for hematocrit determination; the remainder was centrifuged at 3,000 rpm for 10 min for plasma separation and determination of cortisol (enzyme-linked immunsorbent assay - ELISA), glucose (PAP Liquiform kit - Labtest®), chloride (enzymatic method - Biotecnica®) and ammonia (Verdouw *et al*., 1978). The cortisol assay was validated for jundiá by demonstrating the correlation between the standard curve and serial dilutions (1:2 - 1:512) of a pooled sample of sera from all individuals. The assay sensitivity based on a 96% binding rate, was 78 pg ml^{-1}. The mean inter-assay coefficient of variation for high (36% of binding) and low (74% of binding) was 8.5%. The intra-assay coefficient of variation was 3.5%. The results were submitted to ANOVA, and means were compared by Tukey's test when significant differences ($P < 0.05$) were detected. SAS 8.0 software was used for analysis. Data are expressed as means ± S.D.

Results

Dissolved oxygen (DO) increased to very high levels after the transport period due to the addition of pure oxygen in the plastic bags, a very common situation observed during the transport of fish in closed systems. Throughout the 4-hour transport period, DO was consumed by the fish; at the end of the period, the bags with the highest loading density exhibited the lowest DO level ($P < 0.01$). DO levels were significantly different among all treatments, and the oxygen consumption seemed to increase with the increase in density. After the transport period, only the fish submitted to the lowest loading density were in water with a DO level higher than 20 mg L^{-1} (Table 1).

Un-ionized ammonia (NH_3) increased significantly ($P < 0.01$) in the water of all treatments after the transport period; the highest levels were detected in the water in which fish were transported at the two highest loading densities (Table 1). The increase in waterborne NH_3 was accompanied by an increase in levels of this metabolite in the plasma of fish from

all treatments after transport (Table 2). When compared to initial levels, plasma ammonia was significantly higher ($P < 0.05$) in fish from all treatments after transport; initial levels were recovered within 24 hours. In contrast to waterborne NH_3 values, there were no differences in plasma ammonia levels between treatments at the end of the transport simulation (Tables 1-2). Temperature and pH did not show significant differences ($P > 0.05$) between treatments after transport. In contrast, the pH of all treatments showed significantly lower levels ($P < 0.05$) than the initial value (Table 1). Plasma chloride and hematocrit did not show any significant difference ($P > 0.05$), either among treatments or during the experimental period (Table 2).

The plasma cortisol of fish transported at the highest loading density (350 g L^{-1}) reached 173.18 ng mL^{-1} after transport, a value three times higher ($P < 0.001$) than that observed four hours prior - before transportation (Fig. 1). Fish submitted to the other loading densities showed plasma cortisol levels below 100 ng mL^{-1}, values that were significantly lower ($P < 0.05$) than those of the fish submitted to 350 g L^{-1} treatment after transport. There were no significant differences between treatments during the recovery period, and the plasma cortisol levels of fish from treatment 350 g L^{-1} returned to initial levels within 24 hours. Fish from the treatments with the three highest loading densities exhibited higher ($P < 0.001$) blood glucose levels when compared to the initial level, recovering within the first 24 hours. After the 4-hour transport, the blood glucose levels of fish submitted to the highest loading density were significantly higher ($P < 0.05$) than that of fish from the lowest loading density (Fig. 1).

Discussion

After four hours of transport, DO levels remained high, even at the highest loading density (350 g L^{-1}). In addition, since the lethal concentration of NH_3 (96 hours) for jundiá juveniles exposed to pH 7.5 is 1450 µg L^{-1} (Miron *et al*., 2008), NH_3 at the end of the transport at the highest loading density (136.82 µg L^{-1}) was much lower than lethal levels. Consequently, jundiá juveniles of the size (around 20 g) used in the present experiment could be transported for a longer period without problems due to NH_3 toxicity. According to Golombieski *et al*. (2003), the transport of smaller (1-2.5 g) jundiá juveniles at a loading density of 168 g L^{-1} and a temperature similar to that used here (25ºC) reduced DO levels to 1.57 mg L^{-1} after six hours. Lower oxygen consumption by 20-g jundiá juveniles (compared to those of 1-2.5 g) throughout the transport was expected. According to Jobling (1994), there is a decline in oxygen consumption per unit of body mass with an increase in body size and age.

According to Emerson *et al*. (1975), ammonia in an aquaculture system exists in equilibrium between two forms: ionized (NH_4^+) in the aqueous form and un-ionized (NH_3) in gaseous form; the ratio between them is mainly a function of pH and temperature. The permeability of plasma membranes to uncharged and lipid-soluble NH_3 is higher than to NH_4^+; therefore NH_3 is considered to be the most toxic form of ammonia

Table 1. Dissolved oxygen (DO), pH, un-ionized ammonia (NH_3) and temperature (Temp) in water before and after transport simulation with jundiá juveniles in plastic bags at different loading densities. Asterisks indicate significant differences ($P < 0.001$) when compared to the initial values before transport. Different lowercase letters in the rows indicate significant differences ($P < 0.05$) among treatments after transport period. [1]Average initial value before the addition of pure oxygen. When plastic bags were locked, dissolved oxygen was over 20.0 mg L^{-1} in all treatments.

Water parameter	Initial value	End of transport simulation (loading densities)			
		75 g L^{-1}	150 g L^{-1}	250 g L^{-1}	350 g L^{-1}
DO (mg L^{-1})	4.9 [1]	> 20.0 ± 0.0 * a	17.4 ± 1.7 * b	13.7 ± 0.8 * c	8.8 ± 2.3 * d
NH_3 (µg L^{-1})	1.06	34.75 ± 6.32 * c	63.47 ± 5.69 * b	110.10 ± 41.36 * a	136.82 ± 58.82 * a
Temp (ºC)	25.2	24.6 ± 0.2 a	24.7 ± 0.2 a	24.6 ± 0.1 a	24.6 ± 0.1 a
pH	7.79	7.24 ± 0.05 * a	7.14 ± 0.05 * a	7.26 ± 0.26 * a	7.20 ± 0.18 * a

Table 2. Plasma ammonia, plasma chloride and hematocrit before and after transport simulation with jundiá juveniles in plastic bags at different loading densities (75, 150, 250 and 350 g L^{-1}). Since there were no significant differences among treatments, lowercase letters in the rows show significant differences ($P < 0.05$) of means (composed by values of all treatments) among sample times.

Blood parameters	Experimental period			
	Before transport (N = 4)	After transport (N = 16)	24 hours (N = 16)	96 hours (N = 16)
Plasma ammonia (µg L^{-1})	450.4 ± 240.3 b	987.0 ± 362.8 a	481.0 ± 177.3 b	400.7 ± 130.4 b
Plasma chloride (mEq L^{-1})	119.7 ± 10.9 a	117.1 ± 5.8 a	126.9 ± 6.4 a	120.0 ± 6.3 a
Hematocrit (%)	28.8 ± 5.1 a	29.9 ± 4.9 a	26.6 ± 3.2 a	30.5 ± 5.4 a

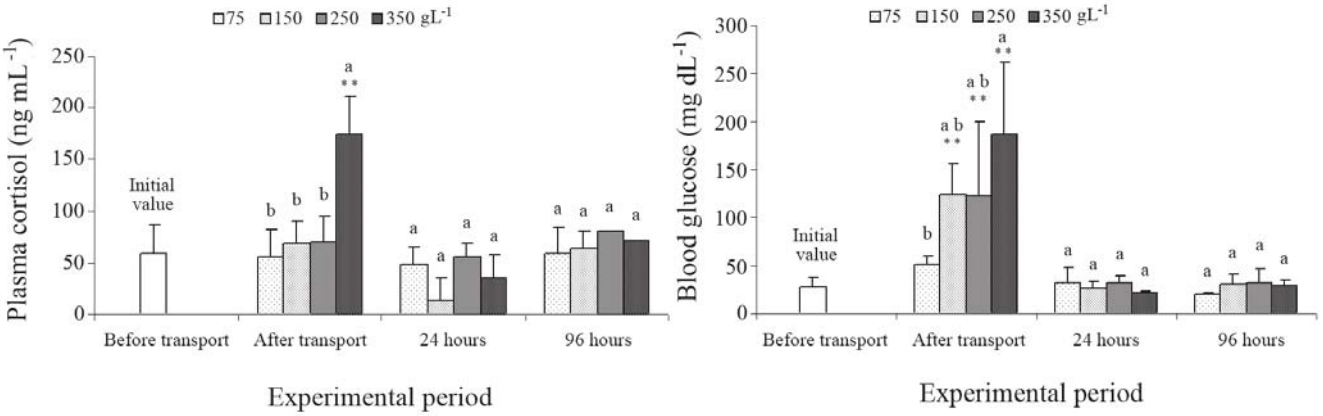

Fig. 1. Plasma cortisol and blood glucose of jundiá juveniles transported at different loading densities. Different letters over the bars indicate significant differences ($P < 0.05$) among treatments within each experimental time. Asterisks show significant differences ($P < 0.001$) when compared to the initial value.

(Das *et al*., 2004). An increase in waterborne NH_3 concentration results in decreased diffusion, causing an increase in internal NH_3 that leads to re-establishment of the NH_4^+/NH_3-equilibrium inside the fish. Das *et al*. (2004) confirmed that a relatively small increase in waterborne NH_3 may cause a large increase in internal ammonia concentration, affecting some physiological functions, for instance diminishing the action of acetylcholinesterase in the brain and liver and reducing levels of hemoglobin and plasma proteins. As a consequence of the cumulative increase in plasma ammonia, some other physiological process could be disturbed, for example pH maintenance, electrolyte balance and general homeostasis (Tomasso, 1994). The increase in waterborne NH_3 was accompanied by a parallel increase in this substance in fish plasma in all treatments after transport, corroborating the relationship presented by Das *et al*. (2004). Fish transported at the two highest loading densities (250 and 350 g L^{-1}) were exposed to more stressful conditions because they were submitted to higher levels of waterborne NH_3. Un-ionized ammonia would be even higher if the pH were higher. However, pH levels were lower after the 4-hour transport, a phenomenon that is probably attributable to the CO_2 excreted by jundiá during transport, as was previously shown by Golombieski *et al*. (2003). CO_2 is recognized for its acid characteristic (Emerson *et al*., 1975).

Plasma chloride and hematocrit did not exhibit significant differences, either among the treatments or during the experimental period. Plasma chloride levels were within the range normally found for this species under minimized stress conditions (118-132 mEq L^{-1}); whereas hematocrit exhibited lower values (range for jundiá under minimum stress: 37-51%), according to Borges *et al*. (2004). In contrast to what was observed by Gomes *et al*. (2006) with pirarucu (*Arapaima gigas*), by Barton *et al*. (2003) with walleye (*Stizostedion vitreum*) and by Carneiro & Urbinati (2001) and Urbinati *et al*. (2004) with matrinxã (*Brycon cephalus*), the plasma chloride and hematocrit analyses did not show results that permitted evaluation of the physiological state of jundiá under the acute stress caused by loading density or transport itself.

The elevation of cortisol concentrations in plasma is recognized as the main hormonal response to stressors, and it is widely used as a stress response indicator (Barton & Iwama, 1991). The initial cortisol value of 59.75 ng mL^{-1} was higher than that found for the same species by Barcellos *et al*. (2001; 29.6 ng mL^{-1}) and Barcellos *et al*. (2004; 28.89 ng mL^{-1}). It is likely that the higher initial cortisol level found in the present study is due to the fact that before fish were packed into plastic bags they were confined at a relatively high density inside a 2-m^3 concrete tank for 24 hours. This is a common procedure to maintain juveniles ready for sale in southern Brazil, and differs from the practice adopted by Barcellos *et al*. (2001, 2004). The plasma cortisol values obtained after the 4-hour transport were significantly higher in fish transported at the highest loading density, reflecting the primary stress responses described by Iwama *et al*. (2004). According to the cortisol analyses, fish transported at the highest loading density seemed to be more stressed than those transported at lower loading densities; however, they also recovered to the initial condition 24 hours after transport, and no mortality was observed during the experimental period. Davis & Small (2006) stated that rapid cortisol clearance is likely due to the lack of a cortisol-binding globulin to protect it from degradation (most fishes do not possess a specific cortisol-binding globulin). Jundiá could be included among those fish species, simply because withdrawing the stressor (*e.g*., lowering the loading density after transport) seemed to help it recover its initial cortisol

level in a short period of time.

Only in fish transported at the lowest loading density were blood glucose levels not significantly elevated after transport, suggesting that secondary stress responses could have occurred only in the fish at the three higher loading densities. The high glucose level characteristic of the secondary response is caused by high catecholamine and cortisol levels and provides energy for the fish to respond to the demand generated by the behavioral response to stress stimuli (Iwama *et al*., 2004). In the present study, fish of all treatments recovered their initial blood glucose values during the first 24 hours after transport, showing that jundiá can reestablish their normal condition relatively rapidly once acute stress is removed. In addition, blood glucose is a simple and inexpensive analysis that, together with plasma cortisol, allows a relatively good understanding of the physiological condition of fish facing a stressful situation.

The costs involved in fish culture, as with other animal production systems, must be minimized, and fish producers depend on optimized techniques to ensure better profit. Therefore, based on the physiological indicators, especially during the recovery period, and the observed fish survival in the present study, the best density at which to transport jundiá in plastic bags for 4 hours is around 350 g/L.

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