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Physiological responses of piau (*Leporinus friderici*, Bloch 1794) to transportation

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ABSTRACT - This study evaluated stress indicators of juvenile piau (*Leporinus friderici*) during and after a 4-hour transportation in order to establish an appropriate transportation protocol for this type of fish. Fish were transported in plastic bags (133.1 g/L) and sampled before loading, during 1, 2, 3 and 4 h and after transportation (2, 6, 12 and 24 h). Blood samples were analyzed for cortisol and glucose levels, hematocrit, hemoglobin level, number and mean corpuscular volume of erythrocytes. Water pH, dissolved oxygen, temperature and ammonia were monitored before, during and after transportation. No mortality was observed through the experiment. Ammonia levels increased throughout transportation, but the low pH values kept NH_3 in safe levels for fish. Cortisol levels increased within 4 h of transportation, and returned to control condition 2 h after arrival. Plasma glucose increased within one hour of transportation, reaching peak value within 4 h and returning to initial condition 2 h after arrival. Erythrocyte number and hemoglobin levels showed the lowest levels 2 h after arrival, and mean corpuscular volume increased during transportation, decreasing at 12 and 24 h after arrival. Transporting piau is stressful, but fish recover the initial condition in short time, showing tolerance to the changes in the water quality parameters.

Key Words: cortisol, fish transport, stress

Introduction

One of the most critical determinants of the success of fish farming is the delivery of a quality product to final destination. Studies have shown that, during transportation, fish are exposed to different stressful stimuli that may cause adverse physiological responses affecting their welfare (Schreck et al., 1989; Erikson et al., 1997; Iversen et al., 1998; Urbinati et al., 2004; Sulikowski et al., 2006; Fagundes & Urbinati, 2008).

Transporting live fish in closed systems results in degradation of water quality, because of excretory products, mucus and regurgitated food. Also, several factors can become deleterious agents during transportation, including dissolved oxygen, carbon dioxide, water temperature, pH and ammonia level (Berka, 1986; Erikson et al., 1997). In aqueous solution, ammonia exists as two chemical species, un-ionized ammonia gas (NH_3), the most toxic, and the ammonium ion (NH_4+) (Berka, 1986; Paterson et al., 2003). The toxicity of ammonia is affected by water temperature and pH, with un-ionized ammonia increasing as water temperatures and pH rise (Emerson et al., 1975; Berka, 1986).

The adverse stimuli produced by the transportation operation have shown to cause increased secretion of catecholamines and cortisol in fish (Robertson et al., 1987; Barton & Iwama, 1991). This primary stress response can induce a cascade of secondary effects, including metabolic, osmoregulatory and immune disturbances (Barton & Iwama, 1991; Urbinati et al., 2004). Although mortality as a direct consequence of transport is low, the secondary stress effects are responsible for delayed mortality, caused by osmoregulatory dysfunction and disease (Berka, 1986).

The genus *Leporinus* has several species of commercial value, and comprises the species piau (*Leporinus friderici*, Bloch 1794, cited by Garavello & Britski, 2003), also named *piau-três-pintas*, whose excellent flesh makes it attractive for consumption despite the numerous spines found in meat (Nomura, 1984).

To evaluate the suitability of any species for farming, stress indicators should be measured during common management practices such as transportation. However, studies to evaluate transportation stress response of *L. friderici* do not exist. This study investigated the transport-induced physiological responses on juveniles of piau during a 4-h procedure and a 24-h period of recovery.

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Material and Methods

The study utilized 106 juveniles (33.2± 10.4 g; 13.4± 1.2 cm) obtained from a commercial supplier. Ten fish were sampled before loading into the polyethylene bags and were considered the control group. Blood was drawn from the caudal vasculature in anesthetized fish (benzocaine, 66 mg/L) and dispensed in microtubes with or without anticoagulant (potassium fluoride) to separate plasma and serum.

The packing system involved 10-L polyethylene bags, containing 2 L of fresh water. Eight fish (133.1 g/L) were introduced in each plastic bag in the same density previously used for *Brycon cephalus* (Urbinati et al., 2004). The polyethylene bags were then squeezed above the water level to expel the air, inflated with oxygen gas, tied with rubber strings and packed in cardboard boxes. Transportation proceeded through paved roads for 4 h, which represents the average transportation time in most parts of the São Paulo State. Hourly, three bags were opened for fish samplings, when four fish of each bag were anesthetized and bled (n = 12 per hour). For one time, sampled bags were closed again, but they were not considered in further samplings. After transportation, fish were transferred to 12 recovery tanks (100 L) with a continuous flow water system. The water was continuously aerated with compressed air diffused through air stones. Fish were sampled again at 2, 6, 12 and 24 h in the recovery tanks. Blood samples were obtained from 4 fish of each tank, with 3 tanks sampled each time (n = 12 per hour).

The plasma glucose concentration was measured using an enzymatic glucose assay (kit Labtest, São Paulo, Brazil) and serum cortisol, with radioimmunoassay technique (Coat-A-Count kit, DPC, California, USA). Hematocrit, hemoglobin, number and mean corpuscular volume of erythrocytes were measured in a sample of heparinized blood (Celm, Model CC550, automatic blood cell counter).

The water quality parameters were monitored in the stocking tanks in the commercial supplier, immediately after the opening of the bags (n = 3 per hour) and in the recovery tanks (n = 3 per hour). Water temperature and dissolved oxygen concentration (DO) were measured using a YSI 55 oxygen meter, and the water pH, using a YSI 63 pH meter (Yellow Springs Instruments, Yellow Springs, OH, USA). Total ammonia was determined (Nessler reactive), and unionized ammonia was calculated according to Emerson et al. (1975).

Differences among treatments were assessed using one-way ANOVA. Means were compared by Tukey test ($P<0.05$). Results are presented as means ± standard deviation.

Results and Discussion

During transportation, DO was around 20 mg/L in the first 3 hours and 12 mg/L at the end of transportation. Either in stocking tanks or in recovery tanks, DO was above 6.5 mg/L. No mortality was observed through the experiment.

The pH ranged from 6.76 to 6.93 in stocking tanks and through the transportation process, differing significantly from the values registered in the recovery tanks where pH ranged from 7.8 to 8 ($P<0.0001$) (Table 1). Water temperature increased throughout the transportation (from 20.8±0.34 to 24.2±0.12 °C; $P<0.0001$) (Table 1). The values in the recovery tanks were higher (27.8 - 28.0 °C), and did not change until the end of the recovery period. Total ammonia in the stocking tanks was 6.23 mg/L, and increased gradually throughout the transportation, from 2.73 to 7.23 mg/L ($P<0.0001$) (Table 1). During the recovery, with water flowing in the tanks, ammonia levels varied between 0.4 and 0.79 mg/L ($P<0.1558$). NH_3 levels did not alter throughout the experiment and the levels registered were below the LC_{50} reported for other fish species (from 0.6 mg/L, for rainbow trout *Oncorhynchus mykiss*, to 2.9 mg/L, for channel catfish *Ictalurus punctatus*; Tomasso 1994).

Table 1 - Water quality parameters during the transportation of *L. friderici* in the stocking tanks (control), in the bags (1, 2, 3 and 4 h of transportation) and in the recovery tanks (2, 6, 12 and 24 h)

Sampling		Water quality parameters			
		pH	Temperature (°C)	Total ammonia (mg l^{-1})	NH_3 (mg l^{-1})
Control		6.81±0.08b	19.6±0.32c	6.63±0.59a	0.016±0.003
Transportation	1 h	6.85±0.08b	20.8±0.34c	2.73±0.24cd	0.008±0.002
	2 h	6.93±0.03b	21.4±0.35c	3.78±0.68bc	0.018±0.004
	3 h	6.76±0.02b	23.7±0.63b	5.27±0.46ab	0.016±0.002
	4 h	6.93±0.11b	24.2±0.12b	7.22±0.02a	0.033±0.009
Recovery	2 h	8.06±0.02a	27.9±0.22a	0.59±0.20d	0.042±0.013
	6 h	7.99±0.07a	28.0±0.19a	0.44±0.02d	0.028±0.004
	12 h	7.80±0.08a	28.0±0.23a	0.40±0.07d	0.018±0.006
	24 h	7.92±0.05a	27.8±0.32a	0.79±0.22d	0.044±0.013

Results are given as mean ± S.E.M. (n = 3). Means with different letters denote significant differences at $P<0.05$ (ANOVA).

In spite of the elevated levels of total ammonia during the transportation, the pH values contributed to keeping NH_3 in acceptable levels for *L. friderici*. The water pH can influence ammonia toxicity. Low pH decreases the proportion of the toxic form of ammonia (NH_3) (Emerson et al., 1975; Berka, 1986). With increasing CO_2 production throughout the transportation, fish respiration shifted water pH towards acidity. The pH registered during the transportation allowed for the production of small levels of NH_3 (0.008 - 0.033 mg/L) in a range around the levels recommended by Wedemeyer (1996) to guarantee good health condition for fish (below 0.02 mg/L).

The total ammonia levels verified in this study are in a range related in other studies on fish transport. During the 4-h transportation of juvenile matrinxã (*Brycon cephalus*), in a density of 125 g/L, ammonia levels were 5.7 mg/L and unionized ammonia levels were around 0.006 mg/L (Urbinati et al., 2004). Fingerlings of silver catfish (*Rhamdia quelen*) were transported for up to 24 h at densities of up to 168 g/L and the levels of ammonia in the water reached 7.5 mg/L,but the low pH and temperature kept the NH_3 levels in tolerable range for the species (below 0.02 mg/L) (Golombieski et al., 2003).

In the present study, levels of cortisol prior to handling and transportation (control) were 108.1±75.2 ng/mL, and at the end of the operation the fish experienced a significant increase in the average plasma cortisol levels compared with the previous samplings including the control (78.7±33.4; 83.4±28.7; 76.1±21.6 and 239.7±24.5 ng/mL at 1, 2, 3 and 4 h, respectively ($P<0.05$, Figure 1A). Within 2 h after transportation, cortisol levels were shown to return to the initial levels (104.3±32.3 ng/mL). The use of cortisol to evaluate stress levels associated with transportation has been used in many fish species. On the other hand, the short recovery time observed in this study was not described in other species. The cortisol profile of fish subjected to transportation varies among fishes. The recovery of resting plasma cortisol levels after an acute stressor of moderate intensity is normally found within 6 h (Barton et al., 1980; Pickering & Pottinger, 1989). However, hauling and transportation seem to result in a longer recovery, more than 24 h in several species (Nikinmaa et al., 1983; Robertson et al., 1987; Schreck et al., 1989). In Atlantic salmon smolts commercially transported for 4.5 h, plasma cortisol concentrations increased from resting values, with a peak 1 h after transportation and no recovery within 48 h (Iversen et al., 1998). Eurasian perch (*Perca fluviatilis*, L) were subjected to stress by transportation and their cortisol levels increased after the operation, and returned to basal levels between the 7th and the 14th days. The maximum

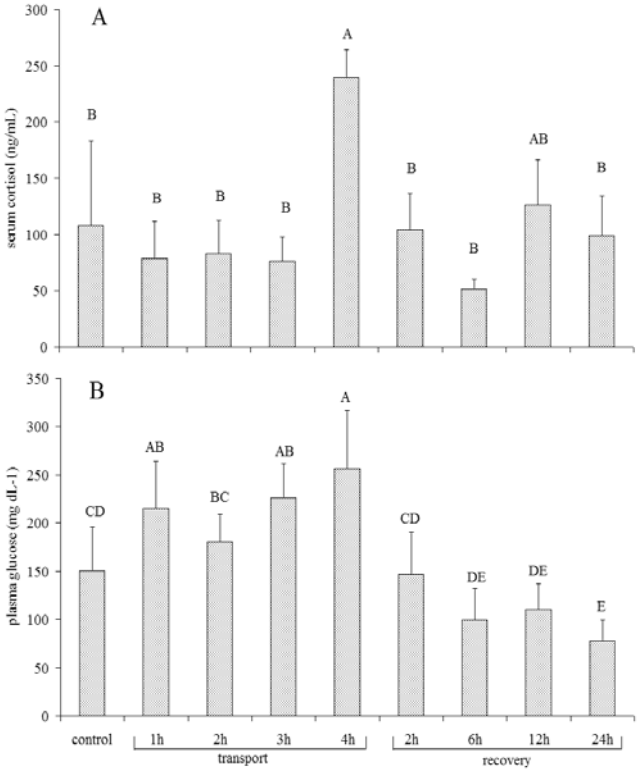

Figure 1 - Serum cortisol (A) and plasma glucose (B) levels during the transportation of *L. friderici* in the stocking tanks (control), in the bags (1, 2, 3 and 4 h of transportation) and in the recovery tanks (2, 6, 12 and 24 h). The results are given as the mean ± S.E.M. (n = 12). Bars with different letters denote significant differences at $P<0.05$ (ANOVA).

cortisol was measured on the 2nd day (Acerete et al., 2004). Adult and juvenile of *Brycon cephalus* transported for 4 h showed increased cortisol levels immediately after transport and recovered pre-transport levels within 24 h, although no measurement of the hormone concentrations has been done before that sampling time (Carneiro & Urbinati, 2001; Urbinati et al., 2004).

During the transportation of *L. friderici*, cortisol levels had a latency period of 4 h for the increased response, which may be associated with the highest levels of total ammonia (7.23 mg/L) and NH_3 (0.033 mg/L) observed in the water transportation at that sampling time, although those levels of un-ionized ammonia are around the levels recommended by Wedemeyer (1996) as safe for fish health. An increase in juvenile pirarucu (*Arapaima gigas*) cortisol was found 24 h after transportation, showing a long latency period in their response (Gomes et al., 2006). Contrarily, after 45 and 90 min of transportation, increased cortisol levels for juvenile winter flounder (*Pseudopleuronectes americanus*) were observed (Sulikowski et al., 2006).

Regarding secondary responses, plasma glucose increased significantly in the first hour of transportation compared with the control levels (214.8±48.8 mg/dL and 150.3±45.5 mg/dL respectively; P<0.0001; Figure 1B) and the highest mean value was observed at fish arrival (256.8±59.8 mg/dL) returning to pre- transportation levels (146.9±43.5 mg/dL) within two hours, following the same cortisol profile. Plasma glucose showed the typical catabolic response in *L. friderici* after transportation stress, thus providing extra energy resources, enabling the animal to overcome the disturbance. Alterations in glucose metabolism are a common response to stress in fish (Barton & Iwama, 1991) also during transportation. The results observed in this study corroborate studies on *Brycon cephalus* (Urbinati et al., 2004) and *Arapaima gigas* (Gomes et al., 2006), transported for similar periods of time.

Regarding hematology (Table 2), hematocrit, hemoglobin levels and erythrocyte number did not show important variations through transportation and decreased within two hours of recovery. The mean corpuscular volume (MCV) values showed significant differences from control fish at one and four hours of transportation and the values significantly decreased at 12 and 24 hours of recovery (P<0.0001). The reduction in hemoglobin levels (P<0.0091) and erythrocyte number (P<0.0006) may be attributed to the erythrocytic cell lysis caused by the sudden change of the water pH when fish were transferred to the recovery tanks after the transport (from 6.93 to 8.06). According to Das et al. (2006), a change in water pH either due to acidic or alkaline conditions exerted stress in three species of Indian major carps characterized by swelling of erythrocytes, production of immature erythrocytes, and reductions in the total erythrocyte counts and hemoglobin content. The increase in the volume of red blood cells occurs due to the regulatory volume increase mechanism of the cell, which requires an activation of the Na^+/H^+ with accompanying Cl^-/HCO_3^- exchanger at the membrane (Weaver et al., 1999).

In the present study, reduction in the erythrocyte number and hemoglobin content indicated a reduced blood oxygen carrying capacity (Jensen, 2003) and also indicated that there was a possibility of respiratory stress in transported *L. friderici*, although no additional biological changes besides cortisol and glucose were observed.

Table 2 - Hematology during the transportation of *L. friderici* in the stocking tanks (control), in the bags (1, 2, 3 and 4 h of transportation) and in the recovery tanks (2, 6, 12 and 24 h)

Sampling		Hematology			
		Ht (%)	RBC (10^6 mm^{-3})	MCV (μm^3)	Hb (g dL^{-1})
Control		41.5±1.96	2.50±0.13abc	167.1±3.93b	14.2±0.60ab
Transportation	1 h	48.7±1.62	2.62±0.07abc	186.2±3.48a	13.6±0.39ab
	2 h	45.5±1.80	2.56±0.07abc	177.6±3.95ab	12.9±0.38ab
	3 h	47.8±3.40	2.70±0.19abc	177.1±3.12ab	13.9±0.93ab
	4 h	50.6±0.97	2.74±0.05ab	188.3±2.73a	14.0±0.82ab
Recovery	2 h	38.9±4.30	2.09±0.21c	173.2±4.53ab	11.7±1.14b
	6 h	42.0±3.24	2.28±0.15bc	175.0±2.12ab	12.5±0.89ab
	12 h	47.7±2.11	2.93±0.13a	162.7±2.77bc	16.0±0.85a
	24 h	45.2±2.62	2.97±0.18a	148.8±4.73c	15.8±1.12a

Ht – hematocrit; RBC – red blood cells; MCV – mean corpuscular volume; Hb – hemoglobin. Results are given as mean ± S.E.M. (n = 12). Means with different letters denote significant differences at P<0.05 (ANOVA).

Conclusions

Juvenile *L. friderici* have a good physiological tolerance and can cope with the stressors imposed by a 4-hour long transportation.

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