TRANSPORT OF PIRARUCU *Arapaima gigas* JUVENILES IN PLASTIC BAG

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ABSTRACT - This study examined the stress response of pirarucu juveniles in a closed system transport. Pirarucu (*Arapaima gigas*) is a native Amazonian fish species from the Osteoglossidae family and an obligated air breather. A short duration transport trial (6h) was undertaken comparing closed polyethylene bags filled with atmospheric air (Air group) and bags filled with pure oxygen (Oxi group). Dissolved oxygen was the only water parameter that presented a difference between fish groups, and was saturated in the oxi group as expected. There was no mortality in either group after transport. Fish feeding was observed 36 h after transport for all fish, and normal feeding consumption was observed at 72 h. In both groups physiological responses were similar. Cortisol did not show any significant alteration during the sampled period. Unlike most fish species, cortisol values were unaltered in both groups during sampling, while glucose presented a significant change up to 12 h after transport. The results showed that pirarucu transport in plastic bags could be made with either atmospheric air or pure oxygen, since physiological response to stress, water quality and feeding behavior after 36 h were similar in both groups.

Key-words: aquaculture, *Arapaima gigas*, stress, cortisol, glucose

Transporte de juvenis de pirarucu *Arapaima gigas* em sacos plásticos

RESUMO - Este estudo examinou as respostas de estresse de juvenis de pirarucu transportados em sistema fechado. Pirarucu (*Arapaima gigas*) é um peixe nativo da bacia Amazônica, da família Osteoglossidae que possui respiração aérea obrigatória. Foi realizado um transporte de curta duração (6 h) em sacos de polietileno inflados com ar atmosférico (grupo ar) ou com oxigênio puro (grupo oxi). O oxigênio dissolvido foi o único parâmetro de qualidade da água que apresentou diferença estatística entre os grupos, e como esperado, o oxigênio estava supersaturado para o grupo oxi. Não houve mortalidade após o transporte em ambos os grupos. Os peixes se alimentaram 36 h após o transporte e apresentaram um consumo de ração habitual após 72 h. As respostas fisiológicas foram semelhantes nos dois grupos. O cortisol não apresentou mudança significativa durante o período de amostragem. Ao contrário da maioria das espécies, os valores de cortisol se apresentaram inalterados nos dois grupos durante a amostragem, enquanto a glicose teve um aumento significativo até 12 h após o transporte. Os resultados mostram que o transporte de pirarucu em sacos de polietileno pode ser realizado com ar atmosférico ou oxigênio puro, uma vez que as respostas de estresse, a qualidade da água e o comportamento alimentar após 36 h foram similar entre os grupos.

Palavras-chave: aqüicultura, *Arapaima gigas*, estresse, cortisol, glicose

INTRODUCTION

Pirarucu (*Arapaima gigas*) is an exclusively air breather and carnivorous fish species. It is a highly prized native species from the Amazon region and has experienced a huge increase in its demand due to its growth rate in intensive and semi-intensive systems reaching up to 10 kg in a twelve-month cycle (Saint-Paul, 1986; Imbiriba, 2001; Cavero, 2002). Broodstock recruitment and reproduction for seed mass production are still the main issue for further research efforts.

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There is still a lack of information available towards a standard and an efficient transportation method for pirarucu, mainly to 1 to 2 kg fish. Since most fish transportation uses the closed system within polyethylene bags filled with oxygen (Piper, 1989; Gomes *et al.*, 1999; Ross & Ross, 1999), this method should be a starting point with pirarucu. With the increasing demand for pirarucu of various sizes for the grow-out operations and broodstock formation, the determination of an appropriate, simple, reliable, and inexpensive method for efficient transportation without stress is required. Pirarucu is an obligated air breathing fish, therefore the use of a closed system with polyethylene bags without the use of pure oxygen can be a feasible option for its transportation. For this transportation trial with a short duration (6 h), we compared the closed system (polyethylene bags) filled with atmospheric air versus pure oxygen. The intensity and duration of fish stress through physiological responses (cortisol and glucose) were measured along with the water quality after the operation, fish survival and feeding response within 96 h.

MATERIALS AND METHODS

Captive reared pirarucu juveniles were obtained from a commercial fish farm from Coari (Amazonas, Brazil) transported to the Aquaculture Department at Instituto Nacional de Pesquisas da Amazonia/INPA, where it were reared for 6 months in a 75-m^2 pond.

Ten fish (weight 1,123 ± 322.5 g and total length 51.3 ± 3.5 cm; mean ± SEM) were transferred to a 1-m^3 fish cage in the same pond and held off feeding for 24 h to gastrointestinal emptying (Grottum *et al.*, 1997). Fish were individually placed in ten 30-L polyethylene bags with 10 L of water, in two different groups (5 bags per group): one group with bags inflated with pure oxygen (commercial grade oxygen, White Martins S.A., Manaus, Brazil) (Oxi group) and the other group with bags inflated with atmospheric air (Air group). Bags were tied with rubber strings and packed in styrofoam boxes, according to Gomes *et al.* (2002). Simulated transport took 6 h and during this period, fish were periodically disturbed by shaking the boxes. After transportation, each group of fish was transferred to two 1-m^3 cages for subsequent monitoring. Commercial extruded feed was offered to the fish 36 h after transportation to observe fish feeding behavior.

Water parameters were measured before transportation, and at the opening of the bags (after 6 h). Water temperature and DO were measured using a YSI 55 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA), and the water pH using a digital pH meter (Digimed model DMPH-2, São Paulo, SP, Brazil). Determination of total ammonia was made according to Verdow *et al.* (1978). Concentration of un-ionized ammonia (NH_3) was calculated according to Boyd (1982). Levels of CO_2 concentration were determined by titration (APHA, 1992).

Fish stress responses were evaluated in fish from the cage (control; before transportation); immediately after transportation (AT) and at 6 h AT, 12 h AT and 24 h AT intervals, respectively. All fish were sampled in each sampling time, similar proceedings as used by Barnett & Pankhurst (1998). Blood was withdrawn from the fish caudal vein with heparinized syringes. Blood glucose concentration was measured using the Advantage™ blood glucose system (Boehringer Mannheim Gmbh, Mannheim D-68298, Germany). Blood plasma was separated by centrifugation (3,000 *g*, 10 min) and stored at –20 C for further analysis of cortisol using radiommunoassay technique (Coat-A-Count® kit, DPC®, CA, USA).

Water quality after transportation and stress parameters of control group were compared by t-test ($P < 0.05$). Stress parameters of both groups at different samplings times were compared against the control group by ANOVA and Dunnett's test ($P < 0.05$).

RESULTS

There was no mortality after transportation in both groups. Fish feeding was observed 36 h after transportation for all fish and, the normal feeding consumption was observed at 72 h.

In both groups fish physiological responses were similar. Comparison of the control groups between both treatments with a t test did not show any difference for plasma cortisol (df = 8, P=0.292) or for glucose (df = 7, P=0.528).

Cortisol did not show any significant alteration through the sampled period (Fig. 1A). Immediately after transport there was significant increase on plasma glucose concentrations, which were stable up to 12 h AT. At 24 h AT glucose level recovered the control group values (Fig. 1B).

Water quality parameters after transport are presented on Table 1. As expected, DO concentration was significantly higher for the Oxi group. All other water parameters did not change significantly in both groups.

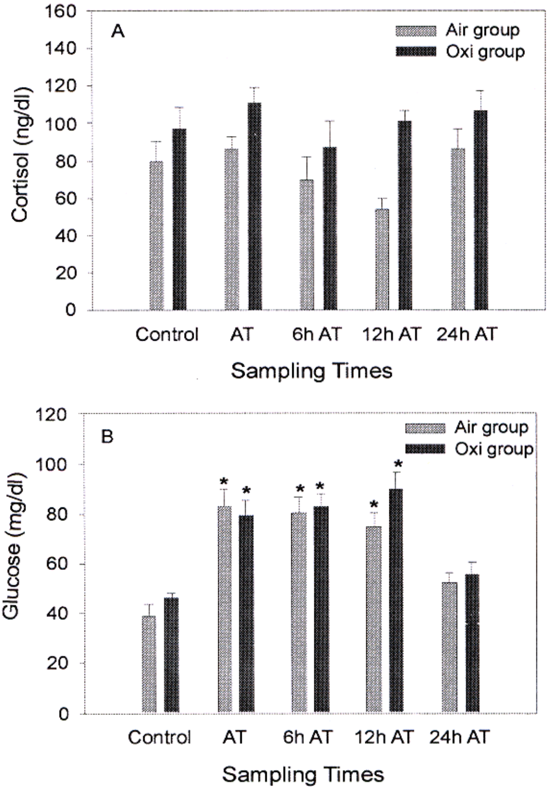

Figure 1 - Cortisol (A) and glucose (B) concentrations during pirarucu transport procedures. Data are means ± SEM of 5 fish for each group. control - before transport; AT - immediately after transport; 6h AT, 12h AT and 24h AT - 6, 12 and 24 h after transport. Columns marked with * are significantly different from the control (Dunnett's test; $P < 0.05$).

Table 1 - Water quality from the control and immediately after 6 h of transporting two groups pirarucu in a closed system: (1) in plastic bags inflated with air (air group) and (2) in plastic bags inflated with oxygen (oxi group). Data are means ± SEM of five replicates for each group. Means within a row followed by different letters are significantly different ($P < 0.05$), as determined by t-test.

Parameters	Control	Groups	
		Air	Oxy
Temperature (°C)	27.1	27.1 ± 0.1a	27.2 ± 0.1^a
DO (mg/L)	4.5	4.6 ± 0.6^a	>20^b
pH (units)	5.54	5.63 ± 0.10^a	5.81 ± 0.10^a
CO_2 (mg/L)	13.2	44.88 ± 5.80^a	46.64 ± 7.31^a
NH_3 (µmol/L)	1.67	14.70 ± 0.31^a	14.44 ± 0.07^a

DISCUSSION

Monitoring physiological parameters during stressing operations, like fish transportation, can provide us important data for the establishment of adequate management practices, even for situations where we are not able to observe any fish mortality. For a successful fish handling and transporting a stronger effort towards animal well being is more desirable than surveying for fish mortality. Usually a severe stress condition could compromise fish adaptive capacity to a new environment and even result in a temporary growth interruption, along with a higher susceptibility to parasite infections due to lower feeding activity (Wedemeyer, 1996). Plasma glucose is a useful stress indicator, besides being easy to monitor (Morgan & Iwama, 1997; Wells & Pankhurst, 1999; Gomes *et al.*, 2001). Cortisol is also a good indicator, and one of the most studied hormones for stress monitoring (Mommsen *et al.*, 1999; Barton, 1997).

The present results are important for future studies towards the stress amelioration in pirarucu husbandry and management as it does give some baseline values for plasma cortisol and glucose, in a comfort zone (control) and in stressful situations (transportation). Pirarucu values for plasma cortisol and glucose in the control group (80-97 ng/ml and 40-45 mg/dl, respectively) were similar to values found for other Amazonian species, as for tambaqui (*Colossoma macropomum*) (80 ng/ml and 45-70 mg/dl for plasma cortisol and glucose, respectively) (Wood *et al.*, 1998; Gomes *et al.*, 2001) and for matrinxã (*Brycon cephalus*) (100 ng/ml and 40 mg/dl for plasma cortisol and glucose, respectively) (Carneiro & Urbinati, 2001).

For most fish species, cortisol is the main hormone that activates glucose, which provides an increase in the energy supply, so fish can withstand the stress situation (Mommsen *et al.*, 1999). This pattern was not observed with pirarucu, where cortisol levels were unaltered at both groups during sampling, while glucose presented a significant increase up to 12 h after transportation. For further studies with pirarucu this is an important aspect to approach, since there are other hormones that could be the precursors of a glycemic state in stressful situations, as the cathecholamines (Mommsen *et al.*, 1999).

Another important result was a not significant change on pirarucu plasma cortisol levels even when fish were submitted to continuous sampling. Observations with the sea raven (*Hemitripterus americanus*), fish with a sedentary and sluggish life-style and a low metabolic activity, presented a higher threshold for the endocrine response to stress (cortisol and catecholamines) and a delayed release of cortisol, which was attributed to an adaptation to prevent the excessive mobilization of energy stores to suit its life-style (Vijayan & Moon, 1994). On the other hand, Barnett & Pankhurst (1998) observed that continuous sampling in greenback flounder (*Rhombosolea tapirina*) caused a progressive increase on fish plasma cortisol values. This shows that, like in the sea raven, pirarucu has a great capacity to keep cortisol at low levels even when successive stress occurs within a short time interval (24 h).

Glucose results can be related to the continuous samplings, mostly during samplings at AT, 6 and 12 h AT. The glycemic rise after transport could be related to the stress during transportation procedures. In addition, the short time intervals of AT, 6 and 12 h AT of fish sampling could have had an effect on the maintenance of high glucose values. Between 12 h AT and 24 h AT sampling procedures there was a bigger time interval, allowing fish to return to normal glycemic levels. In such cases, pirarucu did not present an accumulative stress effect, because even with successive samplings, blood glucose levels kept similar values from AT until 12 h AT sampling.

Barton *et al.* (2000) observed that juvenile pallid sturgeon (*Scaphirhynchus albuns*) and the hybrid of pallid x shovelnose (*S. albuns* x *S. platorynchus*) presented low physiological response to transportation, acute handling and severe confinement, and suggested that it can be related either to the species evolutionary history, neuroendocrine mechanisms involved in the corticosteroid responses, or with the anatomy structure of the intra-renal tissues. Pirarucu is also a fish with a long evolutionary history, and an obligate air breather. Therefore, the low responses observed in pirarucu could also be related to its evolutionary history or different physiologic mechanisms due to its respiratory mode. Besides that, pirarucu is a sluggish type of fish, and as suggested by Vijayan & Moon (1994), it could also explain the delayed release of cortisol as an adaptation to prevent the excessive mobilization of energy stores to suit its life-style.

Water quality is an important stress precursor under poor conditions, like in fish transportation (Berka, 1986; Erikson *et al.*, 1997). In the present study, water quality after transportation, was at acceptable levels in both groups according to Berka (1986). Therefore it was probably not the triggering cause for the plasma glucose increase. DO was the only water parameter that presented a difference between fish groups. As expected in the Oxi group, DO in the water was saturated. Deleterious effects due to oxygen saturated water during transportation have already been described by Wedemeyer (1996), although this probably did not happen with pirarucu, since cortisol and glucose values were similar in both groups. The fact that oxygen saturation does not have an adverse effect on pirarucu could be an advantage for long distance transportation. Even though oxygen consumption was not measured, it was probably low, since fish frequency to breath at the surface followed its normal pattern observed in fish in an earth pond.

The results showed that pirarucu transportation using a closed system with plastic bags can be realized with atmospheric air or with pure oxygen, since fish physiology response to stress, water quality and feeding behavior after 36 h were similar between groups. More studies are needed with pirarucu to evaluate longer duration fish transport and the possibility of utilizing sedative products to help reduce fish glycolytic stress.

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