Use of salt during transportation of air breathing pirarucu juveniles (Arapaima gigas) in plastic bags

L.C. Gomes a,⁎, Edsandra Campos Chagas a, Richard Philip Brinn b, Rodrigo Roubach c, Carlos Eduardo Coppati d, Bernardo Baldisserotto d

a Embrapa Amazônia Ocidental, Manaus, AM, Brazil
b Florida International University, Miami, FL, USA
c Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil
d Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

Received 14 October 2005; received in revised form 23 December 2005; accepted 1 February 2006

Abstract

The farming of the Amazonian air breathing fish, Arapaima gigas, has been growing substantially over the last decade in Brazil and other South American countries. Previous study demonstrated that transportation of pirarucu juvenile in plastic bags is a suitable procedure, although it stimulated some stress responses. Therefore the objective of this study was to investigate the addition of salt as a stress mitigator in pirarucu juveniles during transportation in plastic bags. Fish were reared for a month in earthen pond and held in 3 indoor 2000-L depuration tanks for 24h to allow complete gastrointestinal evacuation, then placed in 30-L polyethylene bags with 10L of water at a density of 12 fish/bag (40g/L) with different table salt (NaCl, 97%) concentrations in the water: 0, 1, 3, 5g/L (3 replicates each concentration). Transportation took 3h and afterwards fish were transferred to 1-m³ floating cages installed inside an earth pond for recovery. Fish stress responses were evaluated before, during and after transportation procedure, and the analyses performed were: cortisol, glucose, lactate, haematocrit and waterborne net Na+, Cl−, K+, and Ca²⁺ fluxes. No mortality was recorded in any treatment during transportation and recovery periods. Cortisol exhibited an increase after transport with 1g salt/L and at 24h after transportation for all treatments showing a latency period in their response. Glucose exhibited a similar pattern for all treatments with a significant increase before and after transportation, returning to basal levels in 24h. Lactate concentrations increased before transportation and after transportation, presenting a significant decrease in all treatments. Addition of salt in the transport water increased Na⁺, Cl− and Ca²⁺ net fluxes in pirarucu. Using salt during pirarucu juvenile transportation should be avoided since there is no reduction on stress responses and causes osmoregulatory disturbances. © 2006 Elsevier B.V. All rights reserved.

Keywords: Stress; Net ion fluxes; Cortisol; Glucose; Pirarucu

1. Introduction

Monitoring physiological parameters during stressful operations, like transportation can provide valuable data for the establishment of adequate management practices, even for situations where there is no fish mortality (Sulikowski et al., 2005). For successful fish handling
and transportation, a stronger effort towards the animal well-being is more desirable than surveying for fish mortality (Gomes et al., 2003c). Usually a severe stress condition could compromise fish adaptive capacity to the environment and even result in a temporary growth disruption and higher susceptibility to parasites and infections (Wedemeyer, 1996).

Fish respond to stress in a proportional way that reflects the severity and duration of the stressor (Barton, 1997). These responses prepare the organism to the so-called “fight or flight” response, which is an attempt to escape from the adversity (Morgan and Iwama, 1997). Consequently the fish physiological reactions to that specific type of stressor needs to be analyzed, both in relations to the response, as to its intensity (Kriger-Azolini et al., 1989).

Pirarucu (*Arapaima gigas*) is an exclusively air breathing fish native from the Amazon basin. Pirarucu farming is booming in some South and Central America countries mainly due to its growth rate, reaching from 7 to 10kg in a twelve-month cycle (Imbiriba, 2001; Pereira-Filho et al., 2003). With the increasing demand for pirarucu to grow-out operations the determination of an appropriate, simple, reliable, and inexpensive method for an efficient transportation with the lowest stress possible for the fish is required. Previous results obtained by Gomes et al. (2003c) demonstrated that plastic bag with pure oxygen is a feasible method for pirarucu transportation, although fish exhibited some stress responses.

The use of salt during transportation helps fish to maintain homeostasis by reducing the osmotic gradient between plasma and external environment and by increasing mucus production (Wurts, 1995) which has been demonstrated to reduce stress responses for some fish species like rainbow trout (*Oncorhynchus mykiss*) (Barton and Peter, 1982) and matrixxã (*Brycon cephalus*) (Carneiro and Urbinati, 2001), but has not been tested for pirarucu transportation. Therefore this study analyzed several parameters (blood glucose, lactate, cortisol, haematocrit and ion fluxes) in an attempt to evaluate the effect of salt on physiological homeostasis of pirarucu during transportation of juveniles in plastic bags.

2. Material and methods

2.1. Experimental procedure

Pirarucu juveniles were obtained from a commercial supplier and transferred to a 2000L indoor tank at the Embrapa fish culture sector (Manaus, Amazonas, Brazil) to feed train them to accept a commercial (extruded) fish diet. After 100% acceptance of extruded feed they were reared for 1 month in a 200-m³ earth pond and fed four times a day with a commercial pelleted feed with 45% crude protein (TR 45; Nutron®, São Paulo, Brazil).

Fish (weight 32.79±2.35g, n=162) were caught from the earth pond and held in three indoor 2000-L depuration tanks (one for each replicate) for 24h to allow gastrointestinal emptying. Fish were placed in 30-L polyethylene bags with 10L of water at a density of 12 fish per bag (40g/L). Commercial table salt (NaCl, 97%) was added to the water at: 0, 1, 3, 5 g/L (3 replicates for each concentration). Bags were then inflated with oxygen, tied with rubber strings and packed in styrofoam boxes. Transportation proceeded though paved roads for 3h. After transportation fish were transferred to 1m³ floating cages installed in an earth pond for recovery. Fish from each bag were kept in separate cages for the subsequent monitoring. Fish density (385g/m³) in the recovery cage was in the appropriate range for rearing this species (Cavero et al., 2003).

Fish stress responses were evaluated in the earth pond (control; before disturbance [BD], n=9); before transportation in the depuration tanks ([BT], n=3 for each tank); immediately after transportation ([AT], n=3 for each replicate) and at 24, 48, and 96h after transportation ([24AT], [48AT] and [96AT] n=3 for each replicate). To avoid unaccounted cumulative stress responses occasioned by repeated disturbance during 48 and 96AT, fish were individually captured in the recovery cage with a hand net when they came for surface air. Therefore the fish that remained in the cage were minimally disturbed.

2.2. Water sampling and analyses

During the transportation total ammonia, measured by the endophenol method (APHA, 1992) and water ions levels, were assessed. Rigid cannulas were inserted through the opening of the bags before closing and at the end of these tubes weights were attached to prevent floating. Water was sampled (400mL) through a small valve, every hour during the transport process. Four bags containing the same salt concentrations, temperature and pH as all the treatments were used to replenish the volumes sampled.

2.3. Ion fluxes

Water Na⁺ and K⁺ levels were measured directly in a B462 flame photometer (Micronal, Brazil), Ca²⁺...
with an AA-1475 atomic absorption spectrophotometry (AAS VARIAN, Australia) after dilution in lanthanum chloride and Cl\(^{-}\) levels by the colorimetric assay described by Zall et al. (1956). Net ion fluxes were calculated according to Gonzalez et al. (1998), and the replacement of water every hour (as explained in the previous item) was considered for the final calculation.

2.4. Blood analysis

Blood was drawn from the caudal vasculature with heparinized syringes in less than 1 min without anesthesia since till this date there are no non-lethal anesthetics methods known for this species. Blood glucose was measured using the digital Advantage™ blood glucose system (Roche, Germany). Blood lactate was measured using the digital Lactate-Pro system (Arkray Inc., Japan). Haematocrit values were obtained with a microhaematocrit centrifuge. Plasma was separated by centrifugation (3000 \( \times \) g, 10 min) and stored at \(-20\) °C for further analysis. Cortisol was measured with an enzyme-linked immunoassay method (ELISA, kit 55050, Human, Germany) using a microplate reader, intra-assay coefficient of variation was 8.4%, the analytic sensitivity of the assay was 1.1 ng/dL.

2.5. Statistical analysis

Differences between treatments (salt concentrations) and physiological stress indicators at different samplings were compared against the control group (before disturbance; BD) by one-way ANOVA and Dunnett’s test \((P<0.05)\). Data for Na\(^{+}\) and Cl\(^{-}\) fluxes were submitted to linear regression. Ammonia concentrations and K\(^{+}\) and Ca\(^{2+}\) fluxes were evaluated by two-way ANOVA (time of transport and salt concentration) and Tukey’s test \((P<0.05)\). All statistical analyses were performed with the software Sigma Stat 3.0.1.

**Fig. 1.** Total ammonia during 3h transportation of pirarucu juvenile *Arapaima gigas* in plastic bags with different salt concentrations in the water. Data were analyzed by two-way ANOVA, statistical significances are in the text.

**Fig. 2.** Cortisol, glucose, lactate and haematocrit during transportation procedure of pirarucu juvenile *Arapaima gigas* in plastic bags with different salt concentrations in the water. Data are means±SEM. BD — before disturbance \((n=9)\) at the earth pond; BT — before transportation \((n=9)\) at the depuration tank; AT — immediately after transportation \((n=3\text{ for each tree replicates within each salt concentration}); 24AT, 48AT and 96AT — at 24, 48 and 96h after transportation \((n=3\text{ for each tree replicates within each salt concentration}). Columns marked with * are significantly different from the control (BD) (Dunnett’s test; \(P<0.05)\).
3. Results

3.1. Water quality and mortality

No mortality was recorded in any treatment during transportation and the recovery period. Water quality parameters before transportation were: DO: 5.32 mg/L; temperature: 25 °C, pH 5.8 and total ammonia 0.02 mg/L. Ammonia concentration in the water increased significantly with transportation time (P < 0.05), although with no effect from the different salt concentrations (Fig. 1).

3.2. Metabolic and haematological disturbance

Cortisol exhibited a significant increase AT in the 1 g salt/L treatment (29.66 ± 4.6ng/mL) and at 24 AT for all treatments, returning to basal levels (8.26 ± 3.9ng/mL) and at 24AT for all treatments (Fig. 1). Glucose concentrations exhibited similar levels for all treatments with a significant increase BT and AT, returning to basal levels (34.9 ± 2.8ng/mL) after 24h (Fig. 2). Lactate basal levels were 3.8 ± 0.3mmol/L and their concentrations exhibited similar levels for all treatments that persisted until the last sample (Fig. 2). Haematocrit increased significantly in the water without salt were near zero. The increase of salt in the transport water led to an increase of Na+ influx. The increase of salt in the water led to an increase of Na+ influx and later to an increase of Cl− influx throughout the transport. In addition, there was a significant increase on Na+ and Cl− influx after 2 or 3h of transport compared to after 1h of transport in fish transported in the water with 3 and 5g (Fig. 3). Pirarucu transported in water with 0 and 1g showed low net Ca2+ influx (except a low efflux in fish transported in water with 1g/L salt for 1h) throughout the transport. Fish transported in water with 3 and 5salt/L exhibited a significant increase on Ca2+ influx after 3h of transport compared to Ca2+ fluxes after 1h of transport (Fig. 4A). Net K+ flux was affected significantly by treatments and time of transport. Fish transported in water without salt addition did not show any significant change on K+ flux throughout the time of transport. Addition of any amount of salt in the water increased K+ efflux significantly after 1h of transport, but after 2h of transport only pirarucu exposed to water with 3 and 5g salt/L showed significantly higher K+ efflux compared to those transported in water with 0 and 1g salt/L. After 3h of transport, fish transported in

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 3. Net Na+ (A) and Cl− (B) fluxes during 3h transportation of pirarucu juvenile Arapaima gigas in plastic bags with different salt concentrations in the water. Data are reported as mean±SEM, and values of net fluxes at 2 and 3h from fish exposed to zero salt in the water are superimposed to 1h. Data are fitted to the following equations:

\[
\begin{align*}
A & : \quad y = 30.56 - 72.17x, \quad r^2 = 0.880, \\
2 \text{ h} & : \quad y = -51.54 + 230.63x, \quad r^2 = 0.970, \\
3 \text{ h} & : \quad y = -15.64 + 157.42x, \quad r^2 = 0.998, \\
& \text{ where } y = \text{Na}^+ \text{ net flux (mmol/kg h)} \\
& \text{ and } x = \text{salt addition in the water (g/L)} \\
B & : \quad y = -60.27 + 53.77x, \quad r^2 = 0.440, \\
2 \text{ h} & : \quad y = -88.11 + 198.87x, \quad r^2 = 0.863, \\
3 \text{ h} & : \quad y = 20.15 + 141.79x, \quad r^2 = 0.985, \\
& \text{ where } y = \text{Cl}^- \text{ net flux (mmol/kg h)} \\
& \text{ and } x = \text{salt addition in the water (g/L).}
\end{align*}
\]
4. Discussion

4.1. Water quality

Pirarucu is highly tolerant to ammonia and able to survive in waters with 30mg/L of ammonia (Cavero et al., 2004). Therefore ammonia concentrations did not reach a critical level during this experiment. According to Berka (1986) depuration procedure has a direct relation to ammonia concentration during transportation, with low ammonia concentration when fish are submitted to this procedure. The low values of ammonia obtained at the end of our experiment can be explained by an adequate depuration period to allow gastrointestinal evacuation.

4.2. Metabolic and haematological disturbance

There was a great latency period in pirarucu cortisol responses. After transportation, a period of great disturbances, cortisol levels remained unaltered (except for 1g salt/L treatment) and 24 AT their levels increase significantly in all treatments. Gomes et al. (2003c) also obtained low cortisol responses after transportation of pirarucu juvenile (1kg) in plastic bag without salt. This great latency on cortisol response has also been shown in sea raven (Hemitripterus americanus) (Vijayan and Moon, 1994), pallid sturgeon (Scaphirhynchus alburnus) juvenile and the hybrid of pallid×shovelnose (S. alburnus×S. platotypus) (Barton et al., 2000), and the authors suggested that it can be related with the neuroendocrine mechanisms involved on the corticosteroid responses to prevent the excessive mobilization of energy stores to suit its life-style. Cortisol levels returned to their basal values after disturbance (48 AT), demonstrating that pirarucu is able to return to their basal cortisol levels relatively quickly. This result is similar to the one observed with tambaqui, which exhibited a cortisol increase after transportation in plastic bag and return to basal levels after 24h (Gomes et al., 2003a). On the other hand, some temperate fishes such as salmonids species present a significant increase in cortisol levels after 2h transportation and maintain their cortisol elevated for more than 48h (Barton, 2000). Addition of salt in the water reduced cortisol secretion in tambaqui (Gomes et al., 2003b), matrinxã (Carneiro and Urbinati, 2001) and rainbow trout (Barton and Peter, 1982) during and after transportation. As the treatments with salt and control exhibit the same cortisol pattern, no evidence that salt decreases cortisol production and excretion by pirarucu juveniles was found.

Different from cortisol, glucose levels increased significantly at the moment of greater disturbances (BT and AT) for all treatments. Glucose levels normally increase during acute stress which is intermediated by catecholamines, which stimulates glycogenolysis in the liver (Fabbri et al., 1998; Mommsen et al., 1999) and has already been shown in some fish species, like common dentex (Dentex dentex) (Morales et al., 2005), stellate sturgeon (Acipenser stellatus) (Bayunova et al., 2002), and red drum (Scianops ocellatus) (Robertson et al., 1987). Glucose increase in BT indicates a cumulative stress response to the several different stressors including catching and handling between the pond (BD) and depuration tank, and is a first stress response during the different phases that compose the transportation procedure. This result is contrary to one obtained

Fig. 4. Net Ca2+ (A) and K+ (B) fluxes during 3 h transportation of pirarucu juvenile Arapaima gigas in plastic bags with different salt concentrations in the water. Values are reported as mean ± SEM. Means identified by different capital letters indicate significant difference among treatments in the same time of transport (P < 0.05), while means identified by different small letters indicate significant difference among times of transport in the same treatment as determined by two-way ANOVA and Tukey’s comparison of mean values.
by Gomes et al. (2003a) with tambaqui juveniles. Glucose values remained elevated AT and returned to basal levels at 24 AT, a similar pattern to other Amazon fishes like the matrixá (Carneiro and Urbinati, 2002), that exhibit glucose elevation after 4h transportation and returned to basal values after 24h. Salt in the water is efficient to mitigate glucose responses of matrixá transported at low density (100kg/m³), although not for fish transported at highest densities (200 and 300kg/m³) (Carneiro and Urbinati, 2002). As for cortisol there is no evidence that addition of salt in the water mitigates pirarucu glucose responses.

Lactate was the most pronounced metabolic stress response after pirarucu transportation, and their levels did not reach basal values until the final sampling. The response of lactate levels in stress situations is a rapid and severe rise as a consequence of muscle glycogen hydrolysis (Vijayan et al., 1997). In accordance to previous description lactate levels increase BT, however at all others sampling times lactate levels diminished in all treatments (except for 0g salt/L at 24AT) and the levels maintained lower throughout the experiment. The main explanation for this decrease is the hepatic lactate capacity used as a gluconeogenic precursor in stressed fish with a lactate clearance from the circulation for glycogen replenishment in the liver of stressed fish as suggested by Vijayan et al. (1994). Basal levels of lactate in pirarucu are higher than the majority of known basal lactate in fishes (Barton et al., 2002), suggesting their use as important fuel product to liver metabolism during stressful situations in pirarucu. Another air breathing (facultative) Amazon fish the armored cat fish (Glyptoperichthys gibbiceps) also presents this response pattern with a lactate depression under stressful situation like acute hypoxia (Almeida Val et al., 2006) and common carp (Cyprinus carpio) present the same pattern under prolonged hypoxia (Zhou et al., 2000), the authors suggest that these fish species reduce accumulation of lactate and save on the use of energy reserves in the face of stressor, as probably occurs with pirarucu.

According to Morales et al. (2005) changes in secondary stress responses as haematocrit normally are not displayed in handling stress (acute stressor), being more responsive to chronic stress situations. Our results are in accordance as pirarucu submitted to an acute stress (rapid transportation) presents just a slight alteration in their haematocrit 24 AT.

The low disturbance procedure used to sample fish during recovery period to avoid cumulative stress responses was efficient, as the results of cortisol, glucose and haematocrit during samples at 48 AT and 96 AT show values similar to basal levels.

4.3. Ionic flux disturbance

The stress of handling or confinement led to an increase of ion branchial efflux and high Na⁺ loss in rainbow trout (McDonald et al., 1991). Therefore, the use of adequate amounts of salts (as NaCl) in the water for transport could reduce the plasma–water gradient and consequently the loss of ions from fishes into the environment (Wurts, 1995). However, in the present study pirarucu transported in water without addition of salt did not show any significant loss of ions (except for K⁺ after 3h), because ion net fluxes were close to zero. If the fish would loose ions through transport, there would be a significant net ion efflux. Consequently, the use of salt in the water to avoid Na⁺ or Cl⁻ loss is not necessary. Moreover, there was a significant increase of Na⁺, Cl⁻ and Ca²⁺ influxes in fish transported in the water with higher amounts of salt, and these influxes increased up to 2h of transport. This was expected, since all osmoregulatory effort of freshwater fishes consists of obtaining these ions from the water (or avoids ion loss) (Baldisserotto, 2003), and when transferred to water with higher salt content freshwater fish must adapt to the new conditions, i.e., reduce ion influx.

The use of salt in the transport water for pirarucu juveniles, instead of protecting them from ion body loss, obliged them to provide a fast osmoregulatory adaptation to cope with an environment with higher salt content. The lack of adaptation to this osmoregulatory change may lead to death if the transport lasts for a longer period of time, as was observed in silver catfish (Rhamdia quelen): addition of salt in the transport water (6g/L) increased Na⁺ body levels in this species and led to death after 12h of transport (Gomes et al., 1999).

4.4. Final remarks

Our data suggests that the use of salt as a stress mitigation did not work for this species and should be avoided, since there are no signs of improvement both on the metabolic or hematological parameters analyzed and the increase of salt concentration causes an osmoregulatory disturbance.

Acknowledgements

This study was supported by CNPq grant # 475093/2003-8 and # 506943/2004-6 and Embrapa (MP2). The authors are thankful to students and technical staff of Embrapa Amazônia Ocidental. R. Roubach and B. Baldisserotto are research fellowship recipients from CNPq.
References


