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AQUICULTURE

Water quality and survival rate of *Rhamdia quelen* fry subjected to simulated transportation at different stock densities and temperatures

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ABSTRACT. Transportation of live fish is routinely done in aquaculture; therefore, the aim of this study was to analyze the water quality and survival rate of *Rhamdia quelen* fry (2.55±0.44 g) following 4, 8 and 12h of simulated transportation, at stock densities of 30 and 60 fry 5 L^{-1} and temperatures 15, 20 and 25ºC. Dissolved oxygen, pH, electrical conductivity, total ammonia, nitrite, nitrate, turbidity, total alkalinity and survival were analyzed. Temperature and time had a significant influence on ammonia, pH, electrical conductivity, and dissolved oxygen levels, and fish died after 12 hours of transportation simulation when kept at 20 and 25ºC. The density directly affected the water quality parameters, such as the increase in electrical conductivity and turbidity, and decrease in dissolved oxygen and pH at the density of 60 fish 5 L^{-1}. In conclusion, the transportation of *R. quelen* fry should be carried out at temperatures between 15 and 25°C, for periods of less than 12 hours, and at stock density of up to 6 fish per liter of water so as not to impair the homeostasis of the fry, and consequently, their survival.

Keywords: aquaculture; ammonia; loading densities; management; mortality.

Qualidade de água e sobrevivência de alevinos de *Rhamdia quelen* submetidos a transporte simulado em diferentes densidades e temperaturas

RESUMO. O transporte de peixes vivos é uma prática comum na aquicultura. Por isso, o objetivo do presente trabalho foi analisar a qualidade de água e sobrevivência de alevinos *Rhamdia quelen* (2,55±0,44 g) seguindo 4, 8 e 12h de transporte simulado, em densidades de 30 e 60 peixes 5 L^{-1} e em temperaturas de 15, 20 e 25ºC. Foram analisados: oxigênio dissolvido, pH, condutividade elétrica, amônia, nitrito, nitrato, turbidez, alcalinidade e sobrevivência. A temperatura e o tempo tiveram influência significativa nos níveis de amônia, pH, condutividade elétrica e oxigênio, e verificou-se mortalidades após 12 horas de simulação de transporte nos peixes mantidos a 20 e 25ºC. A densidade influenciou diretamente nos parâmetros de qualidade de água, tais como o aumento da condutividade elétrica e turbidez e a diminuição de oxigênio dissolvido e pH na densidade de 60 peixes 5 L^{-1}. Em conclusão, recomenda-se o transporte de alevinos de *R. quelen* em temperaturas entre 15 e 25°C, em períodos inferiores a 12 horas. E a densidade de seis peixes por litro de água não compromete a homeostase dos alevinos, e consequentemente sua sobrevivência.

Palavras-chave: aquicultura; amônia; densidade; manejo; mortalidade.

Introduction

Rhamdia quelen, commonly known as jundiá, is a fish greatly accepted by the consumer market due to its flavorsome meat and lack of intramuscular spines (Carneiro & Mikos, 2005). Its productive potential makes it an important species for the Brazilian aquaculture, which is well represented by the South region.

The transportation of live fish, from tanks and ponds to other places is routinely done in aquiculture (Braun & Nuñer, 2014); however, it can result in great economic losses, especially as it takes place during the early stages of life and could potentially lead to high mortality (Okamura et al., 2007). High mortality rates often result from the exposure of fish to several stimuli, which in turn triggers a series of physiological responses (Urbinati, Abreu, Silva Camargo, & Parra, 2004), making the identification of a single stressful element difficult in routine fishes activities (Carneiro, Kaiseler, Swarofsky, & Baldisserotto, 2009).

Although adequate farming is essential for fish survival, the environment in which the fry and juveniles are kept is of great importance and thus, water quality deterioration during the period of exposure must be considered. The alterations in several parameters inside bags and boxes used for transportation are complex, and worsen when stock density is excessive (Paterson, Rimmer, Meikle, & Semmens, 2003). Therefore, it is necessary to adjust the number of animals per volume or area unit so that the cost/benefit is satisfactory and so physiological responses due to stress can be mitigated (Carneiro & Urbinati, 2002.

Successful transportation consists of having the highest stock density with the lowest volume of water possible without increasing mortality, water quality deterioration or stress (Grøttum, Staurnes, & Sigholt, 1997).

Thus, the aim of this study was to analyze water quality and the survival rate of *R. quelen* fry subjected to three times of simulated transportation at different stock densities and temperatures.

Materials and methods

This study was carried out at the Laboratory of Aquiculture of GEMAq (Grupo de Estudos de Manejo na Aquicultura) of the Universidade Estadual do Oeste do Paraná, Campus Toledo – PR, Brazil. The study followed a 2 x 3 x 3 factorial randomized block design, where 2,430 *R. quelen* fry were distributed into 20 L plastic bags containing 5 liters of water. The treatments consisted of two stock densities (30 and 60 fry 5 L^{-1}), three water temperatures (15, 20 and 25° C), and three simulated transportation periods (4, 8 and 12 hours). Each treatment was repeated thrice. The water used in the study was obtained from an artesian well.

The fry, weighing (mean ± standard deviation, SD) 2.55 ± 0.44 g and measuring (mean ± SD) 6.19 ± 0.45 cm, were obtained from a commercial fish farm in Toledo – PR, Brazil. Upon arrival at the laboratory, fish were kept in 500 L tanks for 24 hours for acclimatization and depuration. Subsequently, fry was transferred to 20 L plastic bags containing 5 liters of water. These bags were inflated with oxygen, sealed and placed inside 500 L circular polyethylene boxes. A layer of water and ice at the bottom of these boxes was used to regulate the different water temperatures inside the bags, which were constantly monitored with a thermometer so that the temperatures stipulated were maintained.

At the end of the established transportation periods, bags were opened and the water temperature (°C), dissolved oxygen (mg L^{-1}), electric conductivity ($\mu S.cm^{-2}$) and turbidity (NTU) recorded *in situ* using a handheld digital multiparameter probe (YSI Pro 10102030). The remaining parameters were recorded from water samples taken from the bags and kept refrigerated until analysis. Total ammonia, total alkalinity, total nitrite and total nitrate were determined using Alpha Tecnoquímica® analytical equipment (Florianópolis, Santa Catarina, Brazil).

Following the aforementioned times of each treatment, fish were removed from the plastic bags and transferred to 500 L circular polyethylene boxes. Each box received the fry from one bag and the fish remained in the boxes for 48h to determine the survival rate.

Data was analyzed by factorial ANOVA. Whenever significant, Tukey's test was used to compare the means. Additionally, the parameters analyzed were subjected to principal component analysis (PCA) through the use of a multivariate exploratory analysis. Significance was considered at 5%.

Results

At 4 hours of transportation, no mortality was observed. In general, the variables analyzed in this study had a significant ($p < 0.05$) influence on the factors tested. After 8 hours of transportation, mortality was observed in all groups but no significant ($p < 0.05$) difference was observed between them. After 12 hours of transportation, the survival rate was significantly ($p < 0.05$) lower in bags at 20 and 25°C (Figure 1). However, after 48 hours from the end of transportation, no mortality was observed in any of the treatments.

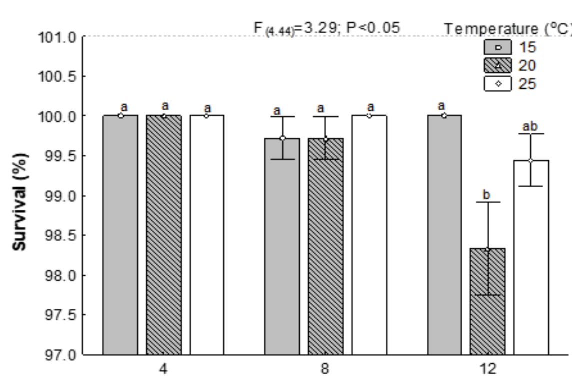

Figure 1. Survival rate of *Rhamdia quelen* fry subjected to 4, 8 and 12 hours of simulated transportation at 15, 20 and 25°C.

Dissolved oxygen (DO) levels were reduced after 4 hours of transportation due to consumption by the fish (Figures 2A and 2B). In high-density bags (60 fry bag^{-1}), the DO was consumed in the first hours ($p < 0.05$). DO levels were significantly ($p < 0.05$) greater when the water temperature was kept close to 15°C.

Electric conductivity was higher after 8 hours of transportation, especially in high-density bags at 25°C ($p < 0.05$); and inversely proportional to DO (Figures 2C and 2D). The pH remained close to alkaline, varying between 6.9 and 7.5. Its lowest level was found in high-density bags (60 fry bag^{-1}) at 25°C (Figure 2E). With regard to transportation time, the pH of high-density bags was ($p < 0.05$) lower than that of low-density bags, remaining constant (±7.05) throughout the period analyzed (Figure 2F).

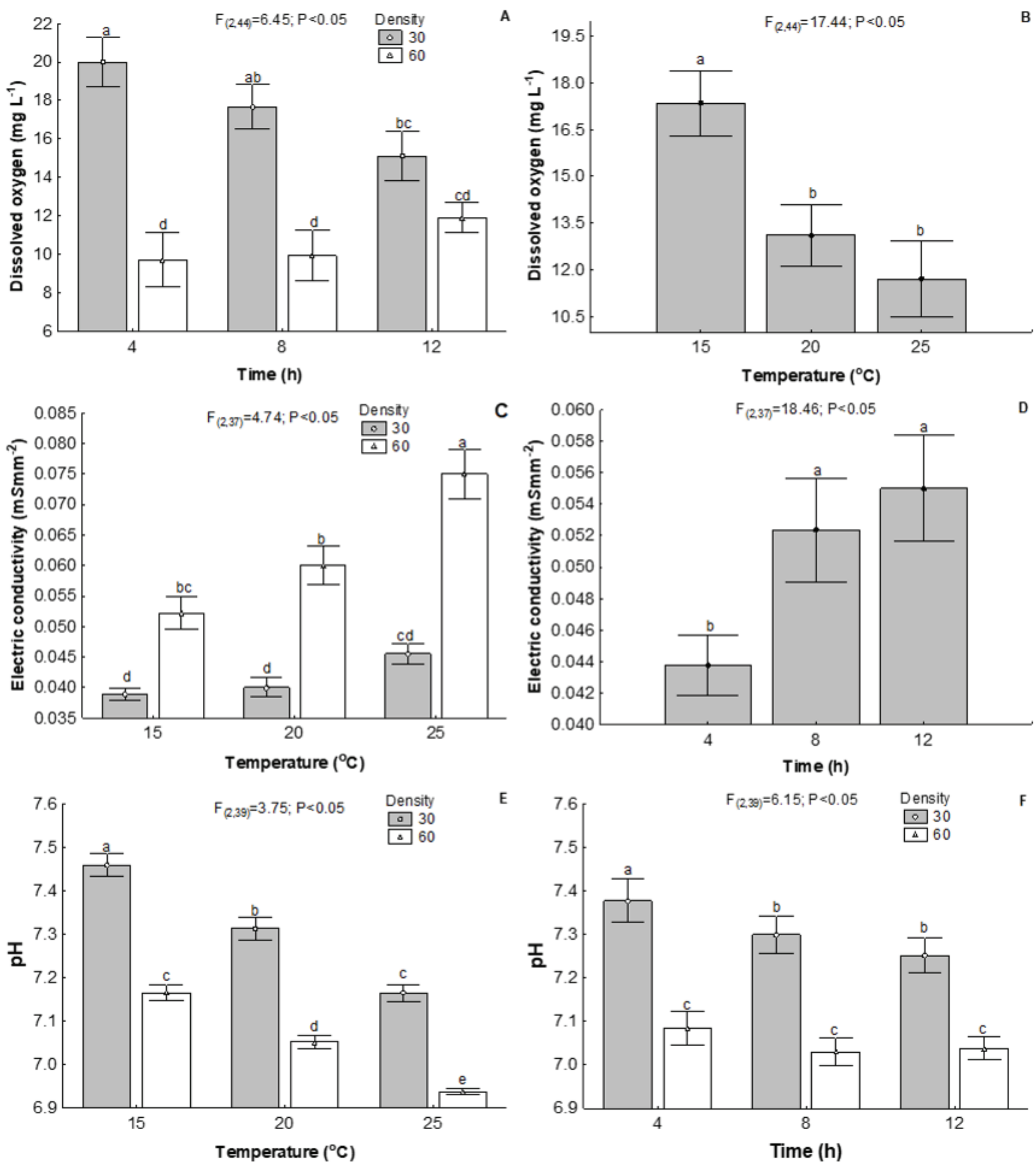

Figure 2. Water dissolved oxygen concentration (A and B), electric conductivity (C and D), and pH levels (E and F) after 4, 8 and 12 hours of simulated transportation of *Rhamdia quelen* fry at two stock densities (30 and 60 fry 5 L^{-1}) at 15, 20 and 25°C.

Total ammonia levels were inversely proportional to pH, with the highest levels being observed in high-density bags at 20°C ($p < 0.05$) (Figure 3A). In relation to transportation time, the periods of 4 and 12 hours showed lower levels of ammonia in low-density bags ($p < 0.05$) (Figure 3B).

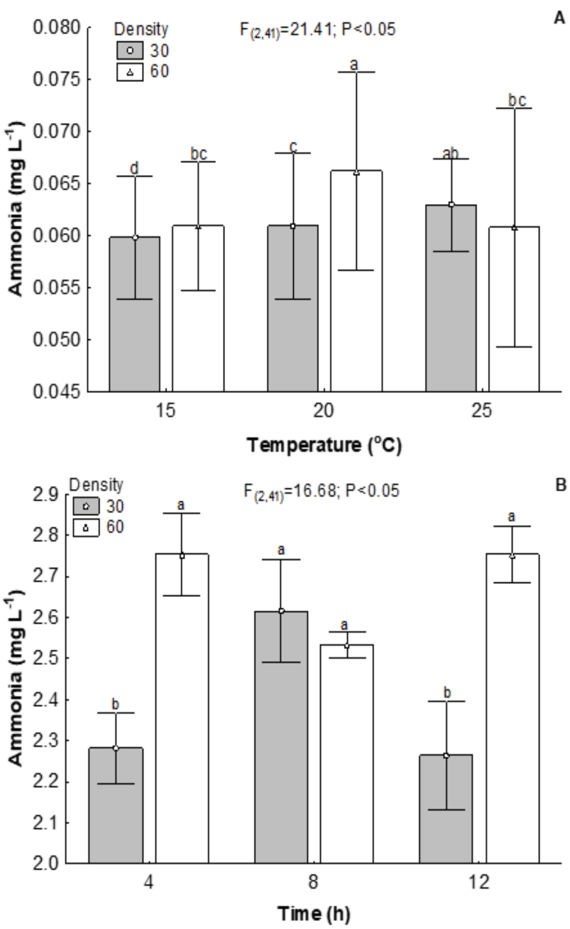

Figure 3. Water total ammonia levels after 4, 8 and 12 hours of simulated transportation of *Rhamdia quelen* fry at two stock densities (30 and 60 fry 5 L^{-1}) at 15, 20 and 25°C.

Nitrate levels gradually reduced over transportation time, with the lowest levels (0.034 mg L^{-1}) recorded in high-density bags after 12 hours of transportation ($p < 0.05$) (Figure 4). Nitrite levels were higher in high-density bags, and its highest concentration was recorded after 12 hours of transportation ($p < 0.05$) (Figure 5).

Mean (± SD) turbidity was 3.61±1.77 NTU; however, after 12 hours of transportation at high-density at 25°C, it significantly ($p < 0.05$) increased to 5.16 ± 1.14 NTU (Figures 6A, 6B and 6C). Mean (± SD) alkalinity was 77.08 ± 30.74 and it also increased over time, with all high-density bags showing mean values greater than 100.00 mg L^{-1} ($p < 0.05$) (Figure 6D).

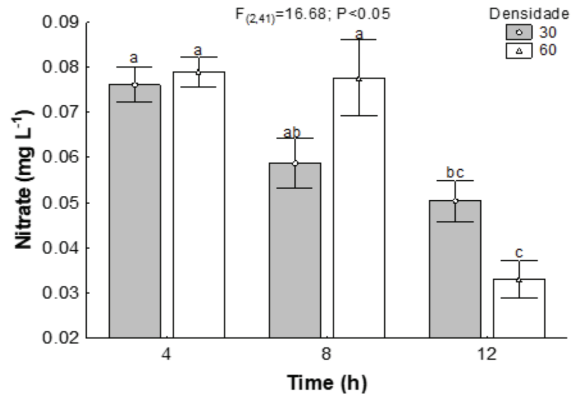

Figure 4. Water nitrate levels after 4, 8 and 12 hours of simulated transportation of *Rhamdia quelen* fry at two stock densities (30 and 60 fry 5 L^{-1}).

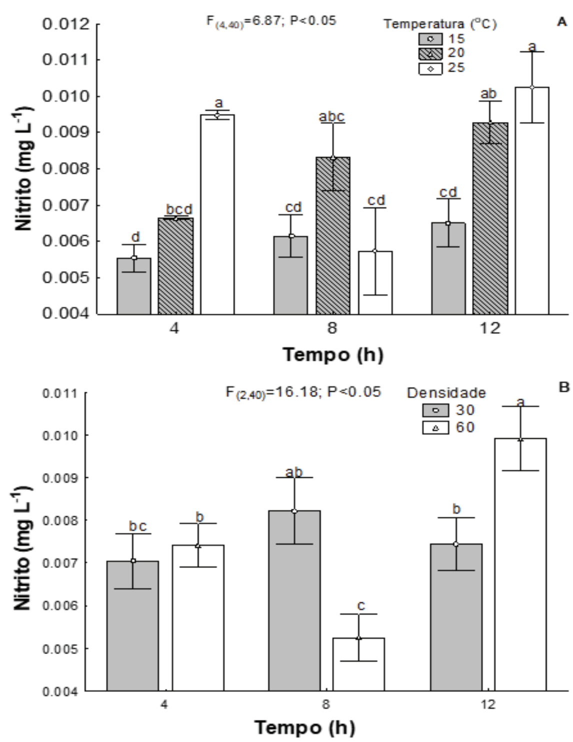

Figure 5. Water nitrite levels after 4, 8 and 12 hours of simulated transportation of *Rhamdia quelen* fry at two stock densities (30 and 60 fry 5 L^{-1}) at 15, 20 and 25°C.

After 12 hours of transportation, high levels of ammonia, nitrate, and nitrite were recorded and high mortality was observed. There was a strong positive correlation between dissolved oxygen and pH levels; and a strong negative correlation between DO and conductivity, conductivity and ammonia, and pH and turbidity (Table 1; Figure 7). The two factors (Factor 1 and Factor 2) obtained from the principal component analysis represented together a percentage accumulation of 62.5%; in other words, these two factors corresponded to 62.5% of the data.

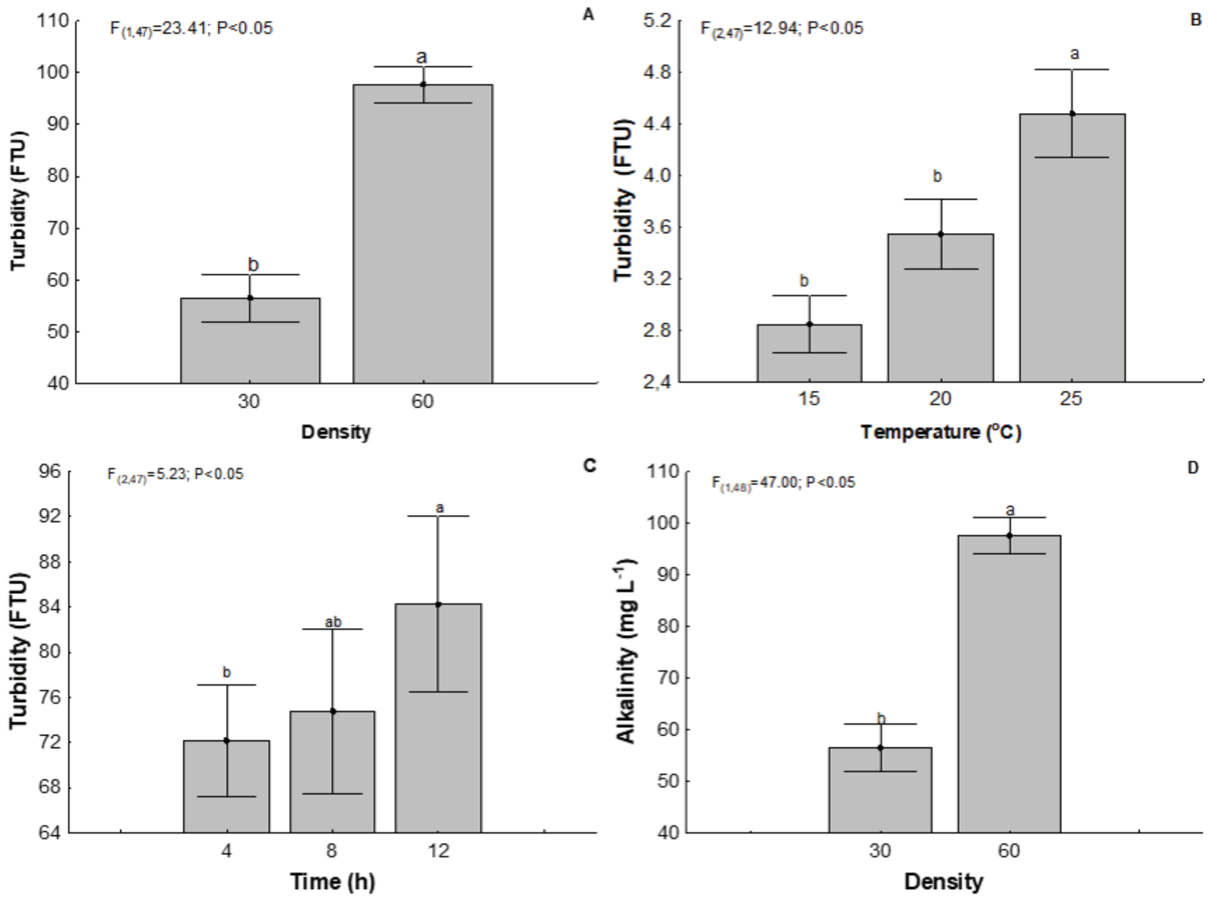

Figure 6. Water turbidity (A, B and C) and alkalinity (D) after 4, 8 and 12 hours of simulated transportation of *Rhamdia quelen* fry at two stock densities (30 and 60 fry 5 L^{-1}) at 15, 20 and 25°C.

Table 1. Pearson's linear correlation between water quality parameters and survival of *Rhamdia quelen* fry.

Parameters	Survival	DO	Cond	pH	Ammonia	Nitrate	Turbidity	Alkalinity	Nitrite
Survival	1.00								
DO	0.16	1.00							
Cond	-0.04	-0.70	1.00						
pH	0.05	0.91	-0.76	1.00					
Ammonia	0.05	-0.48	0.35	-0.58	1.00				
Nitrate	0.22	-0.22	0.21	-0.23	0.25	1.00			
Turbidity	0.03	-0.60	0.64	-0.71	0.48	0.06	1.00		
Alkalinity	-0.34	-0.49	0.51	-0.44	0.08	0.25	0.18	1.00	
Nitrite	-0.02	-0.08	-0.10	-0.13	0.25	-0.08	0.18	-0.18	1.00

*Bold values indicate significant Pearson's correlation (p < 0.05). DO – Dissolved Oxygen, Cond – Electric Conductivity.

Discussion

The transportation of each fish species must be studied so that strict management criteria can be set to ensure water quality and avoid mortality and stress. Fish survival during transportation relies on changes and interactions of several water parameters, which will affect fish physiology (Das, Mishra, Pati, & Mishra, 2015; Refaey, Tian, Tang, & Li, 2017). *Rhamdia quelen* seems to have great resistance to transportation. In this study, mortality was only observed at 12 hours of simulated transported, corroborating with the findings by Golombieski, Silva, Baldisserotto, and Silva (2003). Another limiting factor for fish survival during transportation is stock density, which can cause immediate death, death at the end of management, and losses due to physiological disorders caused by stress (Urbinati et al., 2004).

Water temperature is amongst the factors involved in transport efficiency. Pavlidis, Angellotti, Papandroulakis, and Divanach (2003) reported that water quality and temperature are the most critical factors in the transportation of *Pagrus pagrus*, as similarly observed by Golombieski et al. (2003) for *R. quelen*. In the present study, water temperature above 20°C resulted in a decrease ($p < 0.05$) in oxygen levels, which remained below 13 mg L^{-1}, in contrast to the 17 mg L^{-1} observed in the bags at 15°C. High temperatures tend to speed up the metabolism of fish and, thus, result in greater release of solutes and nitrogen compounds in the water, reducing its quality. High concentration of ions in the water can cause stress (Golombieski et al., 2013) and lead to high mortality.

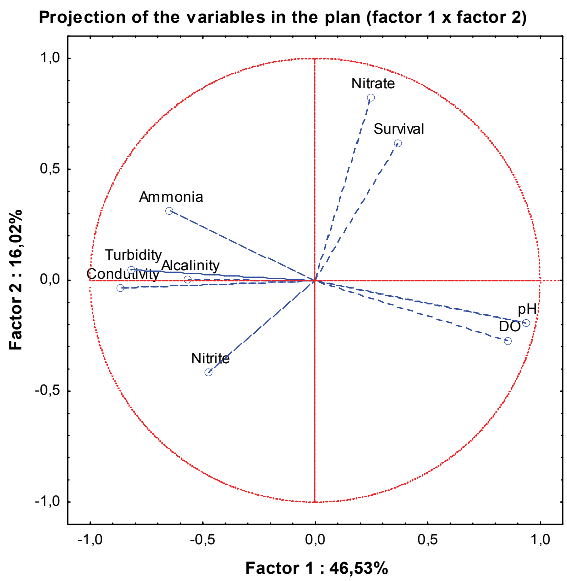

Figure 7. Projection of water quality parameters and survival of *Rhamdia quelen* fry analyzed in relation to the axis Factor1 and Factor2 generated from the principal component analysis.

Aside from temperature, other factors also affect dissolved oxygen levels. High stock density can compromise transportation success, as DO is more rapidly consumed and could lead to mass mortality. This was observed by Carneiro et al. (2009) in a study on *R. quelen*, in which the authors verified that bags containing high biomass (350 g L^{-1}) showed fast consumption of DO.

At high densities, fish are exposed to contact abrasions during transportation, which is an important stress factor. According to Barcellos, Kreutz, and Quevedo (2006), high stock density is an events that cause chronic stress and that, in conjunction with the subsequent acute stress by transportation, can have deleterious effects on fish health and survival. However, the two stock densities tested in this study did not cause damage to the fry, however, if associated with other parameters could result in significant economic losses.

The pH was reduced ($p < 0.05$) after 8 hours of transportation, as well as being slightly acidic in high-density bags above 20°C. These findings are similar to those reported by Das et al. (2015) in *Labeo rohita*, in which pH decline was observed after 9 hours of transportation in spite of the increase in oxygen in the water. This reduction in pH may be due to carbonic gas production by the fish's breathing, which leads to an increase in CO_2 levels and subsequent dissociation of a large quantity of hydrogen ions, making the water acidic (Wurts, 2003). However, the pH levels observed at the end of the experiment are within the normal range for the majority of fish species, including *R. quelen* at the initial phases of development.

Ammonia toxicity can be influenced by temperature and pH (El-Greisy, Elgamal, & Ahmed, 2016) the later acting as an important parameter in fish homeostasis, development, and survival (Bolner, Copatti, Rosso, Loro, & Baldisserotto, 2014). The acidity of the water from transportation tanks is determined by the concentration of CO_2 released by the fish (Colt & Orwicz, 1991). The accumulation of CO_2 reduces the water pH, decreasing the proportion of NH_3 in relation to NH_4^+ (Grøttum et al., 1997). In *R. quelen,* ammonia toxicity is pH-dependent (Miron et al., 2008); however, the pH levels observed in the present study (6.3 to 7.53) had no significant ($p > 0.05$) influence on ammonia toxicity and, therefore, did not have a negative impact on fish survival.

Ammonia is the main product excreted by fish (Bolner et al., 2014), including *R. quelen* (Golombieski et al., 2013), and at high concentration compromises the metabolism, alters growth, and can lead to death (Cavero et al., 2004). Recently, Pottinger (2017) raised an issue that possibly ammonia, nitrate and nitrite influence the function of the stress axis in fish, alone or in combination. Similar to reports of Zeppenfeld et al. (2014), the ammonia excretion in this study increased after 4 hours of transportation. According to Treasurer (2010) the density resulted in greater ammonia concentration. Even though the fish from this study showed good adaptation to the transportation times, studies have shown that exposure to ammonia can lead to histopathological changes of gills, liver, and kidney (Bolner et al., 2014). Ammonia is a stress agent and thus promotes the release of corticosteroid hormones into the blood stream, which triggers metabolic, ionic, and hematological responses, characteristic of stress.

Gonçalves, Takahashi, Urbinati, and Fernandes (2010) observed that ammonia concentration after 6 hours of transportation of *Prochilodus lineatus* was high in all treatment groups, with the highest level observed in high-density containers (0.33 mg L^{-1}). In the present study, ammonia excretion increased during transportation due to fish metabolism and its accumulation in the water exceeded the critical level, surpassing 2.0 mg L^{-1}.

Electric conductivity significantly ($p > 0.05$) increased after 8 hours of transportation, especially in high-density bags. These findings corroborate with Seidelin, Madsen, Byrialsen, and Kristiansen (1999), who reported that increased electric conductivity is due to loss of ions from the fish to the water during transportation in response to stressing factors. In a study on the transportation of *Salminus brasiliensis*, Adamante, Nuñer, Barcellos, Soso, and Finco (2008) reported an increase in the electric conductivity during the first hours of transportation at high stock density. Even with the possible loss of ions, the values observed in this study are probably within the normal range for this species, as high mortality rates were not found even though electric conductivity was increased.

Nitrite is a nitrogen residue that in excess can have several toxic effects, including hemoglobin oxidation, which can lead to hypoxia and death. Nitrite concentration in tropical fish farming systems at low stock density is usually low (0.03 - 0.1 mg L^{-1}) (Andrade et al., 2007). Some metabolic responses are similar to those observed after acute exposure to nitrite in other neotropical species (Moraes, Avilez, & Hori, 2006). However, it is not yet known whether this response is species-specific or dependent on exposure time (Lima, Alencar, Freitas, Packer, & Mourão, 2011). The highest nitrite value observed in this study was 0.010 mg L^{-1}, which was observed after 12 hours of transportation at high-density. *R. quelen* is comparatively resistant to nitrite and can safely withstand levels up to 1.19 mg L^{-1} (Lima et al., 2011). Thus, the nitrite levels observed in this study were well below the safety threshold for this species.

Alkalinity showed a positive correlation with electric conductivity. In the present study, alkalinity was greater in high-density bags and had a negative correlation with pH. The lowest level was observed at low stock density (56.48 mg L^{-1}) and the highest, at high density (97.98 mg L^{-1}). Despite the significant difference between these values ($p < 0.05$), alkalinity remained within the range recommended for fish welfare.

It can be concluded that ammonia, pH, nitrate, nitrite, and temperature did not affect the welfare of *R. quelen* fry and remained within the range tolerated by this species. On the other hand, time and stock density had a negative impact on fry survival rates. Thus, it is recommended to perform the transportation of *R. quelen* between 15°C and 25°C, for periods shorter than 12 hours, and at stock density of up to 6 fish per liter of water so as not to impair the homeostasis of the fry, and consequently, their survival.

Conclusion

We conclude that the transportation of *R. quelen* fry should be carried out at temperatures between 15 and 25°C, for periods of less than 12 hours. The stock density should be of up to 6 fish per liter of water so as not to impair the homeostasis of the fry, and consequently, their survival.

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