Salaro, Ana Lucia; Vasconcelos Campelo, Daniel Abreu; Moraes Tavares, Mateus; Tavares Braga, Luiz Gustavo; Duarte Pontes, Marcelo; Sampaio Zuanon, Jener Alexandre

Transport of Astyanax altiparanae Garutti and Britski, 2000 in saline water
Acta Scientiarum. Biological Sciences, vol. 37, núm. 2, abril-junio, 2015, pp. 137-142
Universidade Estadual de Maringá
Maringá, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=187141162001
Transport of *Astyanax altiparanae* Garutti and Britski, 2000 in saline water

Ana Lucia Salaro1*, Daniel Abreu Vasconcelos Campelo2, Mateus Moraes Tavares1, Luiz Gustavo Tavares Braga2, Marcelo Duarte Pontes3 and Jener Alexandre Sampaio Zuanon1

1Universidade Federal de Viçosa, Av. Peter Henry Rolfs, s/n, 36570-000, Viçosa, Minas Gerais, Brazil. 2Universidade Estadual de Maringá, Maringá, Paraná, Brazil. 3Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil. *Fundação Instituto de Pesca do Estado do Rio de Janeiro, Rio de Janeiro. *Author for correspondence. E-mail: salaro@ufv.com

ABSTRACT. Two experiments were performed. The first aimed to assess the tolerance of fingerlings *Astyanax altiparanae* to water salinity. Fish were exposed to salinity of 0, 3, 6, 9, 12 or 15 g NaCl L⁻¹ for 96 hours. The fish mortality was 0%, in the levels of 0, 3 and 6 g L⁻¹; 75% in the level of 9 g L⁻¹ and 100% at 12 and 15 g L⁻¹ of common salt. The second experiment aimed to assess the parameters of water quality, mortality and blood glucose during transport. For this, *A. altiparanae* were stored in plastic bags at 22, 30 and 37 g of fish L⁻¹ stocking densities and salinity of 0, 3, 6 and 9 g L⁻¹, for. Fish showed similar mortality levels in the different salinities and stocking densities. The increase in fish density reduced the dissolved oxygen levels and salinity decreased the pH. The blood glucose levels were higher in those fish with 0 g L⁻¹ salinity and higher stocking densities. The addition of salt to the water reduces the stress responses of *A. altiparanae* during transport.

**Keyword:** density, stress, glucose, oxygen, common salt.

Introduction

Commercial fish farming increases the quest for efficient handling practices to minimize the losses during the reproductive period (GROTTUM et al., 1997). Transport can trigger physiological responses in these animals due to stress, therefore carefully monitoring of such responses is essential to help the fish adapt to the handling operations (GOMES et al., 2006b).

Conditioning fish in plastic bags, despite the lack of a steady supply of dissolved oxygen is a transportation method for these animals, particularly in Brazil (GOLOMBIESKI et al., 2003; AZAMBUJA et al., 2011). However, the transportation time, temperature, fish density and the high concentrations of ammonia and other metabolites excreted into the water can induce stress in the fish during transport in closed systems (GOMES et al., 2006a).

The stress caused to the fish during transport includes physiological changes in the animals in order to adapt to the new conditions. These responses are categorized into three groups: primary; corresponding to the endocrinal changes, such as increased plasma catecholamines and corticosteroids (MAZEAUD et al., 1977; CARNEIRO et al., 2009). These changes trigger secondary responses such as spikes in the blood glucose levels, changes in the hematological parameters and increased membrane permeability (MAZEAUD et al., 1977; BARTON, 2002) which increases the
water uptake and blood electrolyte losses in freshwater fish (MAZEAUD et al., 1977; CECH et al., 1996). The tertiary response reduces growth, causes behavioral changes and increases the susceptibility to disease due to the depression of the immune system, which in turn may increase the mortality (WENDELAAR BONGA, 1997). The plasma cortisol or glucose, which varies with the type and duration of animal exposure to stressors, is a good indicator to assess the stress levels in fish (HASAN; BART, 2007; OBA et al., 2009).

Storage density can also cause stress in fish, especially during transport (TANG et al., 2009). Decreased density during transport reduces the stress as it minimizes the abrasive contact between the fish (PICKERING, 1993) and reduces the drop in water quality (GOLOMBIESKI et al., 2003). Common salt (NaCl) can attenuate the responses to stress during transport as reported for Colossoma macropomum (GOMES et al., 2003) as it reduces the osmotic gradient between the fish plasma and the water and also reduces the ion transport through the cell membranes by increasing the mucous production in the gill epithelia.

Astyanax altiparanae, one of the most common among the freshwater fish, is reared in various regions of Brazil. This fish is attractive for commercial production because of its rapid growth and easy handling (GONÇALVES et al., 2014), early sexual maturity and excellent meat for the consumer. However, knowledge regarding the responses of A. altiparanae to stress during transport is still meager.

The aim of this study was to evaluate the responses of the A. altiparanae fingerlings to stress during transport, using different stocking densities and common salt (NaCl) levels.

Material and methods

This experiment was approved by the Ethic Commission in Use of Production Animals of the Federal University of Viçosa (process number 28/2013). This certifies that the experiment is in agreement with the Ethical Principles for Animal Research established by the National Council of Animal Experimentation Control (CONCEA) and with present Brazilian legislation.

The experiment was conducted in the Laboratory of Fish Nutrition, Department of Animal Biology (DAB) of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil.

Common salt (NaCl) was the agent used to mitigate stress in the Astyanax altiparanae fingerlings, in two consecutive experiments (salinity tolerance and transport simulation). In the first, the acute tolerance of the fish to the salinity in the water was evaluated. In the second, the A. altiparanae responses to stress during transport were evaluated, using different stocking densities and salt levels. The final salt concentrations were those that provided the best survival rates of this fish in the salt tolerance test.

Salinity tolerance:

This experiment was performed by conducting six treatments (0, 3, 6, 9, 12 and 15 g L⁻¹ of salt) and four replications in a randomized design.

Eight Astyanax altiparanae fingerlings (mean weight 0.37 g ± 0.05) were placed per aquarium (30 x 35 x 14 cm) with 8 liters of water each, constant aeration and temperature of 24ºC controlled by a thermostat and heater (10 watts) with a 12-hour photoperiod. Twenty-four aquariums with 3mm screens to prevent the fish from escaping were used, each of which was considered as an experimental unit.

The A. altiparanae fingerlings were maintained without food for 96 hours and their mortality evaluated every three hours. Individuals were considered dead when spontaneous movements or responses to mechanical stimuli were lacking. The dead fish were removed from the aquarium, counted and discarded. The total number of dead fish per treatment was recorded at the end of this experiment and the averages evaluated by descriptive analysis.

Transport simulation:

This experiment was conducted in a completely randomized design in a factorial layout (4 x 3) with three replications. The treatments included concentrations of 0, 3, 6 and 9 g L⁻¹ of salt and stocking densities of 22, 30 and 37 g fish L⁻¹.

The Astyanax altiparanae fingerlings were transferred to a tank in the Fish Farming Sector, DAB/UFV for depuration and starved for 24 hours to empty their digestive tracts. Following this period, they were distributed in densities of 22, 30 and 37 g fish L⁻¹ per plastic bag (45 x 27 cm) with a quarter of its water capacity and two quarters of oxygen, introduced via a hose attached to an oxygen cylinder, in salinity concentrations of 0, 3, 6 and 9 g L⁻¹ of salt. Plastic bags were sealed to prevent the escape of O₂ and placed in styrofoam boxes. Each plastic bag was considered an experimental unit.

The styrofoam boxes with plastic bags containing fish, were shaken manually every ten minutes for eight hours, to simulate the conditions of transport. Immediately after transport simulation, the fish were transferred from the plastic bags to the aquarium (30 x 35 x 14 cm) with 8 L of freshwater
and controlled temperature (24°C) thermostat and heater (10 watts), constant aeration (7.0 to 7.5 mg L⁻¹) and 12-hour photoperiod. Fish mortality was observed after 24 hours.

For blood collection, the fish were anesthetized in benzocaine solution (50 mg L⁻¹), the caudal peduncle was cut with a scalpel and the blood was directly deposited on test strips of glucose. Blood samples of 10 fish per treatment were collected, prior to the commencement of the experiment and eight hours post transport simulation. Blood glucose was measured using a digital monitor Optium Xceed™.

Temperature, pH, dissolved oxygen and ammonia dissolved in water were evaluated during the experimental period utilizing a common alcohol thermometer, potentiometer, portable oximeter and digital photometer, respectively.

Data were subjected to analysis of variance (ANOVA) and to regression at 5% probability (p < 0.05) for means with significant differences.

Results and discussion

Salinity tolerance

The concentrations of 0, 3 and 6 g L⁻¹ of salt were found to induce no mortality in the Astyanax altiparanae. However, fish mortality began only after 12 hours and reached up to 75% after 96 hours in saline 9 g L⁻¹, and 100% at 12 and 15 g L⁻¹ (Table 1).

Table 1. Astyanax altiparanae mortality (Mort.) of 3-96 hours in water with 0 to 15 g L⁻¹.

| Hour | Salinity (g L⁻¹) | 0  | 3  | 6  | 9  | 12 | 15 |
|------|-----------------|----|----|----|----|----|----|
| 3    |                 | 0  | 0  | 0  | 0  | 50 | 100|
| 6    |                 | 0  | 0  | 0  | 0  | 9.4| 100|
| 12   |                 | 0  | 0  | 0  | 0  | 18.8|100|
| 15   |                 | 0  | 0  | 0  | 0  | 21.9|100|
| 18   |                 | 0  | 0  | 0  | 0  | 25.0|100|
| 30   |                 | 0  | 0  | 0  | 0  | 34.4|100|
| 33   |                 | 0  | 0  | 0  | 0  | 40.6|100|
| 42   |                 | 0  | 0  | 0  | 0  | 46.9|100|
| 54   |                 | 0  | 0  | 0  | 0  | 50.0|100|
| 57   |                 | 0  | 0  | 0  | 0  | 53.1|100|
| 69   |                 | 0  | 0  | 0  | 0  | 56.3|100|
| 75   |                 | 0  | 0  | 0  | 0  | 59.4|100|
| 87   |                 | 0  | 0  | 0  | 0  | 65.6|100|
| 90   |                 | 0  | 0  | 0  | 0  | 71.9|100|
| 93   |                 | 0  | 0  | 0  | 0  | 75.0|100|
| 96   |                 | 0  | 0  | 0  | 0  | 75.0|100|

Mort. (%)* 0 0 75 100 100 100

*Descriptive analysis

The survival values after placing Astyanax altiparanae individuals in concentrations of 0, 3 and 6 g L⁻¹ of salt for 96 hours concur with the reports that a salinity of 6 g L⁻¹ does not affect the survival of the freshwater fishes (ALTINOK; GRIZZLE, 2001; LUZ et al., 2008; LUZ; SANTOS, 2010). Moreover, the survival of all the A. altiparanae individuals for the first 12 hours of exposure to a concentration of 9 g L⁻¹ of salt showed that this fish can be transported to short distances. Water salinity is used extensively to reduce stress in the fish during transport (CARNEIRO; URBINATI, 2001), with a positive or negative effect, depending on the salt concentration. The adaptation of any species to salinity changes is related to its ability to alter its hormone secretions and mechanisms to adjust the ion transport and permeability of the gills, kidneys and intestines to water, minimizing the changes in the plasma ion concentration (BALDISSEROTTO, 2009). Acipenser medirostris exhibited changes in the cellular structures of the gills and kidneys, the osmoregulatory organs, when placed in water with high salinity (POLETTO et al., 2013).

The high mortality of A. altiparanae with 9, 12 and 15 g L⁻¹ of salt in the water can be explained by the osmoregulatory disorders due to the dissolved ions. Freshwater fish maintain a hyperosmotic condition, reducing the ion losses and eliminating the excess body water, water salinity reduces the osmotic and ionic differences between the fish and the water (RILEY et al., 2003). However, excess salt in the water induces difficulty in fish osmoregulation (JOMORI et al., 2012). Hypo and hyperosmotic treatment may or may not cause stress and/or affect the metabolic rate of the animals (STIEGLITZ et al., 2012), although the concentrations of 9, 12 and 15 g L⁻¹ of salt were observed to increase the osmotic imbalance in the fingerlings A. altiparanae. Tolerance to salinity varies with the species, developmental stage (FASHINA-BOMBATA; BUSARI, 2003) and fish exposure time. The Lophiosilurus alexandri fry, Ctenopharyngodon idella fingerlings and Pseudoplatystoma corruscans fry tolerate salinities of 2, 10 and 2 g L⁻¹, respectively (YILDIZ; UZBILEK, 2001; SANTOS; LUZ, 2009). This reveals the importance of evaluating tolerance to salinity of the fish based on its species and developmental stage.

Transport simulation

The salinity and stocking density were found to have no effect upon the mortality of Astyanax altiparanae during transport (Table 2). The stocking density reduced the dissolved oxygen level in the water \( (y = -0.1397x + 24.018, R^2 = 0.9969) \), especially with the 37 g fish L⁻¹ (Table 3). The salinity of 8.14 g L⁻¹ showed the lowest pH content of the water, based on the regression equation \( (y = 0.0062x^2 - 0.1009x + 6.8482, R^2 = 0.9742) \). The temperature and ammonia level did not differ among treatments (Table 3).
The osmotic pressure gradient between the plasma and water enabling the fish to maintain a closer isosmotic state (RILEY et al., 2003). This reduces the energy expenditure with the osmoregulation processes (STIEGLITZ et al., 2012) and stress, which is advantageous to the live fish during the transport. The positive impact of salinity was also revealed by the low mortality of Arapaima gigas during transport in 3 and 6 g L\(^{-1}\) of salt (BRANDÃO et al., 2008).

A similar mortality rate of the A. altiparanae with different stocking densities shows that the reduction of dissolved oxygen in the water transport did not harm this species. The water quality varies with the density and depends on the time duration of transportation (PENG et al., 2011). The addition of pure oxygen into the bags raises the dissolved gas levels to very high magnitudes (CARNEIRO et al., 2009). This is desirable because, in addition to consuming oxygen, breathing produces carbon dioxide (CO\(_2\)), which leads to the formation of carbonic acid, which can be dissociated into H\(^+\) and HCO\(_3^-\), lowering the pH and reducing the NH\(_3\) formation, which is toxic ammonia (TOMASSO et al., 1980; TANG et al., 2009; SINK, 2010). The pH of the water decreased after 30 to 90 minutes of transporting Salmo salar (FARRELL, 2010). Similar pH levels with different stocking density helped in keeping the ammonia toxicity at low levels. Dissolved oxygen levels also decreased as the stocking density of Pampus argenteus increased (PENG et al., 2011). The pH range best suited for A. altiparanae, hovers between 6.5 and 8.0 (PORTO-FORESTI et al., 2010) and despite the fact that the pH levels varied with the saline water, they continued to remain within this range.

The higher glucose levels in the A. altiparanae blood at the higher densities (30 and 37 g fish L\(^{-1}\)) of this fish are related to the increase in the stress intensity without common salt dissolved during the water transport (0 g L\(^{-1}\)). The blood glucose levels did not vary when 3, 6 or 9 g L\(^{-1}\) of salt were added to the water transport, independent of the density used. Exposure to stressors such as transport increases the plasma glucose levels of the fishes depending on the catabolic responses after the stress (BARTON; IWAMA, 1991) and provides additional energy resources for the animal to overcome the disturbance. This increase is related to the higher blood cortisol levels (GOMES et al., 2006b), which reduces the growth and increases the susceptibility to disease, besides increasing fish mortality (OBA et al., 2009). The plasma glucose levels of the Arapaima gigas (GOMES et al., 2006b) were higher at the end of the transport, although the differences in salinities did not affect this fish. In this study, the

### Table 2. Astyanax altiparanae mortality (mean ± standard deviation) after eight, 24 and 96 hours of transport, depending on water salinity and density storage.

| Density (g fish L\(^{-1}\)) | 8h | 24h | 96h |
|-----------------------------|----|-----|-----|
|                             | Mean ± Standard Deviation | Mean ± Standard Deviation | Mean ± Standard Deviation |
| 0                           | 0.30 ± 0.53 | 4.94 ± 4.79 | 7.14 ± 6.82 |
| 3                           | 0.00 ± 0.00 | 2.33 ± 1.57 | 4.34 ± 3.04 |
| 6                           | 0.00 ± 0.00 | 4.04 ± 4.04 | 5.56 ± 5.12 |
| 9                           | 2.39 ± 3.72 | 7.78 ± 3.49 | 12.04 ± 7.88 |

### Table 3. Levels of dissolved oxygen (DO) and pH (mean ± standard deviation) depending on stocking density and salinity in transport of Astyanax altiparanae.

| Salinity (g L\(^{-1}\)) | Density (g fish L\(^{-1}\)) | pH | DO\(^1\) |
|-------------------------|-----------------------------|----|---------|
| 22                      | 0                           | 6.66 ± 0.20 | 6.57 ± 0.18 |
| 30                      | 20.98 ± 1.34 | 19.76 ± 0.80 | 18.88 ± 1.53 |
| 37                      | 22.67 ± 1.84 | 20.98 ± 1.34 | 19.76 ± 0.80 |

### Table 4. Blood glucose levels (mean ± standard deviation) of Astyanax altiparanae depending on water salinity and stocking density (salinity x density) eight hours after transport simulation.

| Salinity (g L\(^{-1}\)) X Density (g fish L\(^{-1}\)) | 0h | 24h | 96h |
|---------------------------------------------------|----|-----|-----|
| 22 0                                             | 6.67 ± 2.22 | 36.67 ± 6.44 |
| 30 0                                             | 6.67 ± 2.22 | 36.67 ± 6.44 |
| 37 0                                             | 6.67 ± 2.22 | 36.67 ± 6.44 |

ns = not significant by analysis of variance, F test (p > 0.05).
interaction between the stocking densities and different salinities clearly reveals the role of salt in the mitigation of stress during the transport of the fish.

Conclusion

*Astyanax altiparanae* fingerlings tolerate water salinity up to 6 g L⁻¹. High densities increase the stress response and the addition of salt to the water reduces the stress responses of *A. altiparanae* during transport.

Acknowledgements

We would like to thank the National Council of Technological and Scientific Development (CNPq) for the fellowships (Research Productivity, Master's Degree and Scientific Initiation), and the Foundation for Support of Research of the State of Minas Gerais (FAPEMIG) for the financial support on the presentation of this work.

References

ALTINOK, I.; GRIZZLE, J. M. Effects of brackish water on growth, feed conversion and energy absorption efficiency by juveniles euryhaline and freshwater stenohaline fishes. *Journal of Fish Biology*, v. 59, n. 5, p. 1142-1152, 2001.

AZAMBÚJA, C. R.; MATTIAZZI, J.; RIFFEL, A. P. K.; FINAMOR, I. A.; GARCIA, L. O.; HELDWEIN, C. G.; HEINZMANN, B. M.; BALDISSEROTTO, B.; PAVANATO, M. A.; LLÉSUY, S. F. Effect of the essential oil of *Lippia alba* on oxidative stress parameters in silver catfish (*Rhamdia quelen*) subjected to transport. *Aquaculture*, v. 319, n. 1-2, p. 156-161, 2011. BALDISSEROTTO, B. *Fisiologia de peixes aplicada à piscicultura*. Santa Maria: Editora UFSM, 2009.

BARTON, B. A. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, v. 42, n. 3, p. 517-525, 2002. BARTON, B. A.; IWAMA G. K. Physiological changes in fish stress in aquaculture with emphasis on the response and effects of corticosteroids. *Review of Fish Diseases*, v. 1, n. 1, p. 3-26, 1991.

BRANDÃO, F. R.; GOMES, L. C.; CRESCIÉNCIO, R.; CARVALHO, E. S. Uso de sal durante o transporte de juvenis (1 kg) de pirarucu (*Arapaima gigas*). *Acta Amazonica*, v. 38, n. 4, p. 767-772, 2008. CARNEIRO, P. C. F.; URBINATI, E. C. Salt as a stress response mitigator of *matrinxã* (*Astyanax altiparanae*) fingerlings at different salinities clearly reveals the role of salt in the mitigation of stress during the transport of the fish. *Transport in saline water* 141

HASAN, M.; BÄRT, A. N. Improved survival of rohu, *Labeorohita rohita* (Hamilton-Buchanan) and silver carp, *Hypophthalmichthys molitrix* (Valenciennes) fingerlings using low-dose quinaldine and benzocaine during transport. *Transport in saline water* 141

HASAN, M.; BÄRT, A. N. Improved survival of rohu, *Labeorohita rohita* (Hamilton-Buchanan) and silver carp, *Hypophthalmichthys molitrix* (Valenciennes) fingerlings using low-dose quinaldine and benzocaine during transport. *Transport in saline water* 141
aspera (Pisces: Loricariidae) larviculture. *Journal of Applied Ichthyology*, v. 26, n. 3, p. 453–455, 2010.

LUZ, R. K.; MARTÍNEZ-ÁLVAREZ, R. M.; DE PEDRO, N.; DELGADO, M. J. Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. *Aquaculture*, v. 276, n. 1-4, p. 171-178, 2008.

MAZEAUD M. M.; MAZEAUD, F.; DONALDSON, E. M. Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society*, v. 106, n. 3, p. 201-212, 1977.

OBA, Y.; MIYAUCHI, N.; HIDAKA, H.; CHIGAI, T.; WATANABE, N.; KOUCHI, A. Formation of compact amorphous H2O ice by codeposition of hydrogen atoms with oxygen molecules on grain surfaces. *The Astrophysical Journal*, v. 701, n. 1, p. 464-470, 2009.

PENG, S.; CHEN, X.; SHI, Z.; YIN, F.; SUN, P. Survival of juvenile silver pomfret, *Pampus argenteus*, kept in transport conditions in different densities and temperatures. *Israeli Journal of Aquaculture – Bamidgeh*, v. 64, n. 1, p. 2-6, 2011.

PICKERING, A. D. Growth and stress in fish production. *Aquaculture*, v. 111, n. 1-4, p. 51-63, 1993.

POLETTI, J. B.; COCHERELL, D. E.; KLIMLEY, A. P.; CECH, JR. J. J.; FANGUE, N. A. Behavioral salinity preferences of juvenile green sturgeon *Aipenser medirostris* acclimated to fresh water and full-strength salt water. *Journal of Fish Biology*, v. 82, n. 2, p. 671-685, 2013.

PORTO-FORESTI, F.; CASTILHO-ALMEIDA, R. B.; SENHORINI, J. A.; FORESTI, F. Biologia e criação do lambari-do-rabo-amarelo (*Astyanax altiparanae*). In: BALDISSEROTTO, B.; GOMES, L. C. (Ed.). *Espécies nativas para piscicultura no Brasil*. Santa Maria: UFSM, 2010. p. 101-105.

RILEY, L. G.; HIRANO, T.; GRAU, E. G. Effects of transfer from seawater to fresh water on the growth hormone/insulin-like growth factor-I axis in the tilapia, *Oreochromis mossambicus*. *Comparative Biochemistry and Physiology*, v. 136, n. 4, p. 647-655, 2003.

SANTOS, J. C. E.; LUZ, R. K. Effect of salinity and prey concentrations on *Pseudoplatystoma corruscans*, *Prochilodus costatus* and *Lophiosilurus alexandri* larviculture. *Aquaculture*, v. 287, n. 3-4, p. 324-328, 2009.

SINK, T. D. Influence of pH, salinity, calcium, and ammonia source on acute ammonia toxicity to golden shiners, *Notemigonus crysoleucas*. *Journal of the World Aquaculture Society*, v. 41, n. 3, p. 411-420, 2010.

STIEGLITZ, J. D.; BENETTI, D. D.; SERAFY, J. E. Optimizing transport of live juvenile cobia (*Rachycentron canadum*): Effects of salinity and shipping biomass. *Aquaculture*, v. 364-365, n. 1, p. 293-297, 2012.

TANG, S.; THORARENSEN, H.; BRAUNER, C. J.; WOOD, C. M.; FARRELL, A. P. Modelling the accumulation of CO2 during high-density, re-circulating transport of adult Atlantic salmon, *Salmo salar*, from observations aboard a sea-going commercial live-haul vessel. *Aquaculture*, v. 296, n. 1-2, p. 102-109, 2009.

TOMASSO, J. R.; GOUDIE, C. A.; SIMCO, B. A.; DAVIS, K. B. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. *Transactions of the American Fisheries Society*, v. 109, n. 2, p. 229-234, 1980.

WENDELAAR BONGA, S. E. The stress response in fish. *Physiological Reviews*, v. 77, n. 3, p. 591-625, 1997.

YILDIZ, H. Y.; UZBILEK, M. K. The evaluation of secondary stress response of grass carp (*Ctenopharyngodon idella*; Val. 1844) after exposing to the saline water. *Fish Physiology and Biochemistry*, v. 25, n. 4, p. 287-290, 2001.