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Waterborne ammonia and silver catfish, *Rhamdia quelen*: survival and growth

Sobrevivência e crescimento de jundiá, *Rhamdia quelen*, exposto à amônia

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ABSTRACT

*The aim of the present study was to determine the effects of waterborne un-ionized ammonia (NH_3) on the survival and growth of silver catfish (**Rhamdia quelen**). Juveniles were exposed to 0.10, 0.22, and 0.42mg L^{-1} NH_3 at pH 8.2 for 45 days. After 15 days, a significant mortality rate (33%) was observed in the fish exposed to 0.42mg L^{-1} NH_3. After 20 days, 23% and 43% mortality (both significant) was observed in fish exposed to 0.22 and 0.42mg L^{-1} NH_3, respectively. A significant negative relationship between survival, length, daily weight gain, standard growth rate, and biomass of juveniles of silver catfish per tank with waterborne NH_3 levels was found at the end of the experiment. The resulting survival and waterborne NH_3 relationship indicated that, to avoid any mortality of silver catfish, the maximum level of chronic NH_3 exposure at pH 8.2 would be 0.01mg L^{-1}.*

Key words: *fish culture, nitrogenous compounds, weight gain.*

RESUMO

*O objetivo do presente estudo foi determinar o efeito da amônia não ionizada (NH_3) da água na sobrevivência e no crescimento de jundiá, **Rhamdia quelen**. Os juvenis foram expostos a 0,10; 0,22 e 0,42mg L^{-1} NH_3 em pH 8,2 durante 45 dias. Após 15 dias, uma mortalidade significativa (33%) foi observada nos exemplares expostos a 0,42mg L^{-1} NH_3. Depois de 20 dias, 23% e 43% de mortalidade (ambas significativas) foram registradas nos jundiás mantidos em 0,22 e 0,42mg L^{-1} NH_3, respectivamente. Uma relação negativa significativa entre sobrevivência, comprimento, ganho de peso diário, taxa de crescimento padrão e biomassa por tanque de juvenis de jundiá, com níveis de NH_3 da água, foi encontrada ao final do experimento. A relação entre a sobrevivência e a amônia da água indicou que o nível máximo de NH_3 em pH 8,2 para evitar qualquer mortalidade de jundiás em uma exposição crônica deve ser 0,01mg L^{-1}.*

Palavras-chave: *piscicultura, compostos nitrogenados, ganho de peso.*

INTRODUCTION

Ammonia is the main end product from nitrogen metabolism in most teleosts (ISMIÑO-ORBE et al., 2003). This substance is toxic at low concentrations, especially in the NH_3 (unionized ammonia) form (FELIPO & BUTTERWORTH, 2002; MIRON et al., 2008). Reduced growth rates due to NH_3 exposure have been reported in several studies involving freshwater fish, but safe levels vary for different species (THURSTON et al., 1981, 1986; ATWOOD et al., 2000; TOMASSO, 1994; EL-SHAFAI et al., 2004; FRANCES et al., 2000).

The culture of silver catfish (***Rhamdia quelen***) (Quoy & Gaimard, 1824, Heptapteridae, Siluriformes) occurs mainly in Brazil, and this species was the most commonly raised native species in Rio Grande do Sul state (RS; southern Brazil) in 2001-2005 (BALDISSEROTTO, 2009). Due to its importance for Brazilian fish culture, several studies were performed to determine the best water conditions for improving

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the growth of juveniles of this species (COPATTI et al., 2005; BRAUN et al., 2006; ANDRADE et al., 2007). Biochemical and morphological changes due to short-term exposure of silver catfish to waterborne NH_3 were demonstrated (MIRON et al., 2008; BECKER et al., 2009; CARNEIRO et al., 2009), but no studies assessing chronic exposure have been performed. Thus, the aim of the present study was to determine the effects of waterborne ammonia on survival and growth of this species.

MATERIAL AND METHODS

Silver catfish juveniles (11.04±0.18g, 11.07±0.07cm) were obtained from a commercial fish culture near Santa Maria, RS, Brazil. The juveniles were placed in continuously aerated 250-L tanks at a stocking density of 100 juveniles tank^{-1}. Temperature was maintained at 23-25ºC, pH at 7.4, dissolved oxygen at 7.2mg L^{-1}, water hardness at 20mg $CaCO_3$ L^{-1}, maximum unionized ammonia level at 0.007mg L^{-1}, and maximum nitrite level at 0.04mg L^{-1}. The photoperiod was 12 h light-12 h dark. Fish were fed (5% total biomass) with commercial food (Purina: 45% crude protein) twice a day (8:30 a.m. and 5:30 p.m.) during the acclimation period (seven days).

The NH_3 levels were 0.10±0.02mg L^{-1}, 0.22±0.02mg L^{-1}, and 0.42±0.02mg L^{-1}, (i.e., 5, 10, and 20% of the NH_3 lethal concentration for 96h at pH 8.2, according to MIRON et al., 2008) and 0.005±0.002mg L^{-1} NH_3 for the control group. The ammonia levels were reached by adding concentrated NH_4Cl (ammonium chloride) solution according to BOYD & TUCKER (1992). Juveniles were placed in continuously aerated 40-L freshwater polyethylene boxes with a stocking density of 10 juveniles box^{-1} (three replicates per treatment) and exposed for 45 days to these different ammonia concentrations.

Temperature (25.0±0.6ºC) and dissolved oxygen levels (7.3±0.1mg L^{-1}) were measured with an oxygen meter (Y5512, YSI Inc., Yellow Springs, USA), and pH was measured with a DMPH-2 pH meter (Digimed, São Paulo, Brazil). Nitrite (maximum level was 0.3±0.1mg L^{-1}), alkalinity (87.0±0.1mg $CaCO_3 L^{-1}$) and total ammonia (NH_3+NH_4^+) levels were determined according to BOYD & TUCKER (1992) and NH_3 levels were calculated as described by PIPER et al. (1982). Water hardness (22.0±0.1mg $CaCO_3$ L^{-1}) was analyzed by the EDTA titrimetric method. All feces and residues were removed daily by suction. Consequently, approximately 20% of the water in the boxes was replaced by water with previously adjusted pH and ammonia concentrations.

Forty-five days after the beginning of the experiment, all juveniles were collected for length and weight measurement. The methodology of this experiment was approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria. Standard growth rates (SGR) were calculated according to JØRGENSEN & JOBLING (1993) and total biomass was calculated as mean weight x number of surviving juveniles. The coefficients of length and weight variation were calculated by the following equation: CV=(SD/M)x100, where SD is the standard deviation and M is the length or weight mean.

The parameters of the different groups were compared by two-way analysis of variance and the Tukey test, with the aid of software Statistica (1997 version). The relationships between the parameters and waterborne NH_3 levels were made with the software Sigma Plot 11.0. The minimum significance level was set at $P<0.05$ and all data are expressed as mean ± SEM.

RESULTS AND DISCUSSION

No mortality was observed up to the 15th day. After 15 days, a significant mortality rate (33%) was observed in the fish exposed to 0.42mg L^{-1} NH_3. After 20 days, 23% and 43% mortality (both significantly different from control) was observed in fish exposed to 0.22 and 0.42mg L^{-1} NH_3, respectively. Survival and growth parameters of silver catfish decreased with the increase of waterborne NH_3. A significant positive relationship between waterborne NH_3 levels and silver catfish mortality was observed at the end of 45 days (Figure 1). The relationship between mortality and waterborne NH_3 indicates that the maximum levels to avoid any mortality of silver catfish chronically exposed to NH_3 at pH 8.2 would be 0.01mg L^{-1}. This value is close to the safe NH_3 level proposed (0.02mg L^{-1}) by the European Inland Fisheries Advisory Commission (EIFAC, 1973) and the United States Environmental Protection Agency (U.S. EPA, 1977) for freshwater fishes.

Moreover, significant negative relationships between length, daily weight gain, standard growth rate, and biomass of silver catfish juveniles per tank with waterborne NH_3 levels were found at the end of the experiment (Figures 1 and 2). The coefficients of variation of length and weight did not present any significant differences between treatments (mean range 3.50-6.15 and 9.81-13.69, respectively). The same negative relationship between waterborne NH_3 and weight and length gain after 63 days (pH 7.6-7.8) was found in channel catfish (***Ictalurus punctatus***) and this

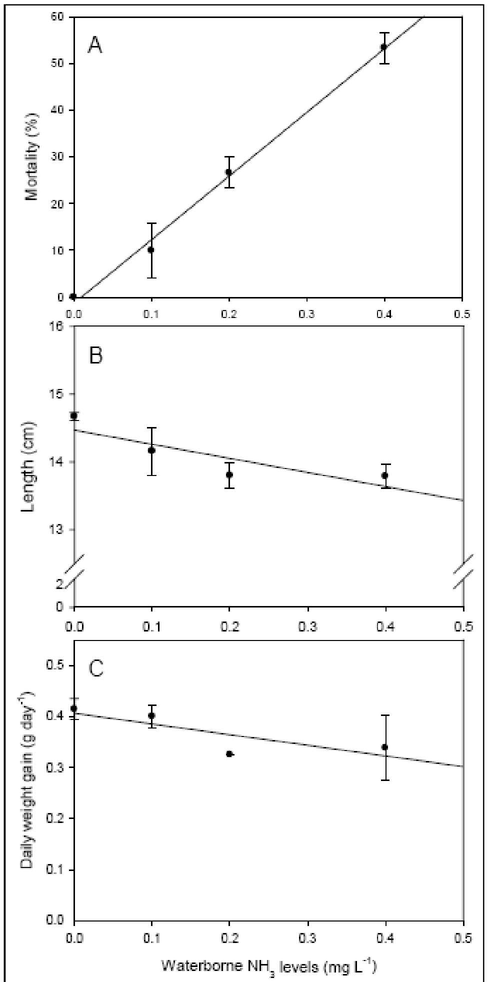

Figure 1 - Mortality (A), length (B), and daily weight gain (C) of ***Rhamdia quelen*** juveniles exposed for 45 days to different NH_3 levels. The relationships are fitted to the following equations: (A) y=-1.34+128.77x (r^2=0.997), (B) y=14.47-1.98x (r^2=0.746), and (C) y=0.41-0.20x (r^2=0.679), where y = (A) survival (%), (B) length (cm) and (C) daily weight gain (g day^{-1}), and x = waterborne NH_3 level (mg L^{-1}).

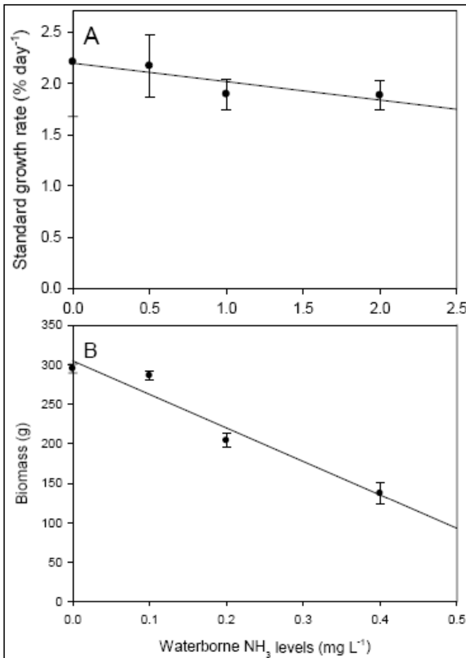

Figure 2 - Standard growth rate (A) and biomass per tank (B) of ***Rhamdia quelen*** juveniles exposed for 45 days to different NH_3 levels. The relationships are fitted to the following equations: (A) y=2.20-0.86x (r^2=0.767) and (B) y=305.54-02.9x (r^2=0.957), where y = (A) standard growth rate (% day^{-1}), (B) biomass (g), and x = waterborne NH_3 level (mg L^{-1}).

study did not present any safe level for this species (ATWOOD et al., 2000). The safe level for growth of Nile tilapia (***Oreochromis niloticus***) and silver perch (***Bidyanus bidyanus***) is 0.060-0.068mg L^{-1} NH_3, pH 7.3-8.1 (FRANCES et al., 2000; EL-SHAFFAI et al., 2004). Fathead minnows (***Pimephales promelas***) can be raised in up to 0.44mg L^{-1} NH_3 (pH around 8.0) without significant mortality and growth reduction (THURSTON et al., 1986). Likely, the differences in the values of these NH_3 safe levels are related to the protective effect of higher water hardness levels against NH_3 toxicity (TOMASSO, 1994). In the experiments with Nile tilapia and silver perch, water hardness was 75-120mg $CaCO_3$ L^{-1} (FRANCES et al., 2000; EL-SHAFFAI et al., 2004) and in the experiment with fathead minnows it was 200mg $CaCO_3$ L^{-1} (THURSTON et al., 1986).

Additional experiments exposing silver catfish to the 0.01-0.1 mg L^{-1} NH_3 range would be interesting, as rainbow trout maintained for 70 days at 0.013, but not at 0.041mg L^{-1} NH_3 (pH 7.6), showed higher weight gain and food conversion rates than those kept in NH_3-free water (WOOD, 2004). This effect was not observed in channel catfish exposed to the same waterborne NH_3 levels (ATWOOD et al., 2000).

CONCLUSION

The obtained results indicate that the safe NH_3 level for silver catfish growth and survival is around 0.01mg L^{-1} NH_3. However, growth experiments exposing juveniles of this species to the 0.01-0.1mg L^{-1} NH_3 range might yield good survival results.

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