Note

LETHAL CONCENTRATION (CL_{50}) OF UN-IONIZED AMMONIA FOR PEJERREY LARVAE IN ACUTE EXPOSURE

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ABSTRACT: Ammonia results from decomposition of effluents organic matter, e.g. feed wastes and fish faeces. Its un-ionized form can be toxic because diffuses easily through fish respiratory membranes, damaging gill epithelium and impairing gas exchanges. The objective of this work was determining the 96-hour CL_{50} of un-ionized ammonia for newly hatched pejerrey *Odontesthes bonariensis* larvae. Trials were set up completely randomized design, with three different concentration of un-ionized ammonia (0.57, 0.94, and 1.45 mg L^{-1} NH_3-N) and a control treatment (n = 3). Experimental units were 20-L, aerated aquaria stocked with 20 larvae (average weight 3.9 mg). Pejerrey larvae exposed to un-ionized ammonia during 96 hours present 50% mortality at 0.71 mg L^{-1} NH_3-N.
Key words: pejerrey, *Odontesthes bonariensis,* toxicity, nitrogen, larvae

CONCENTRAÇÃO LETAL (CL_{50}) DE AMÔNIA NÃO IONIZADA PARA LARVAS DE PEIXE-REI EM EXPOSIÇÃO AGUDA

RESUMO: A amônia é originada da decomposição da matéria orgânica dos efluentes, como restos de rações e fezes dos peixes. Sua forma não ionizada pode atingir concentrações tóxicas, pois difunde-se facilmente através das membranas respiratórias causando danos ao epitélio branquial, dificultando as trocas gasosas entre o animal e a água. O objetivo deste trabalho foi determinar a CL_{50} para 96 horas de exposição a amônia não ionizada de larvas recém-eclodidas de peixe-rei *Odontesthes bonariensis*. O experimento foi conduzido em delineamento completamente casualizado, usando três diferentes concentrações de amônia não ionizada (0,57, 0,94 e 1,45 mg L^{-1} NH_3-N) e um controle (n = 3). As unidades experimentais constaram de aquários de 20 litros, aerados, povoados com 20 larvas (peso médio 3,9 mg). Larvas de peixe-rei expostas por 96 horas à amônia não ionizada apresentam mortalidade de 50% na concentração de 0,71 mg L^{-1} NH_3-N.
Palavras-chave: peixe-rei, *Odontesthes bonariensis*, toxicidade, nitrogênio, larva

INTRODUCTION

Decomposition of effluents organic matter, e.g. fertilizers, feed wastes and fish feces, yields both ionized (NH_4) and un-ionized (NH_3-N) ammonia forms that, dissolved in the water, remain in ordinary equilibrium, affected by pH, temperature and salinity. Un-ionized ammonia can be toxic to fish because diffuses easily through fish respiratory membranes, damaging gill epithelium and impairing gas exchanges, disrupting the osmoregulatory system (Arana, 1997).

Ammonia concentration in fish plasma and tissues increases in direct proportion to its increase in water. When exposed to un-ionized ammonia, fish show nervous system disturbance, increased gill ventilation, loss of equilibrium, convulsions and high mortality (Foss et al., 2003). Ammonia effects are immediate and linear in relation to the concentration, and can be observed after 15 minutes of exposition through swimming difficulties and increasing oxygen consumption. Increasing ammonia concentration in blood and other tissues (e.g. brain, liver, and muscle) negatively affects cerebral synaptic connections (Lamarié et al., 2004).

Acute exposure to 0.6 mg L^{-1} NH_3-N is lethal to most fish, while chronic exposure to 0.6 mg L^{-1} NH_3-N damages respiratory and hepatic tissue reducing growth, for it causes cerebral dysfunction and reduces blood oxygenation capacity (Duborow et al., 1997). Therefore, it is important to know tolerance limits of NH_3-N of all farmed fish in phases of life.

Pejerrey *Odontesthes bonariensis* (Atheriniformes: Atherinopsidae) are small to medium size, agile fishes. The species good quality, tasty flesh led to the proposition of a special chapter in fish culture: Atheriniculture, which spread from South America to several countries, such as Japan, France, Italy and Israel, among others (FAO, 2004). Atherinopsidae fish, *Odontesthes* species in particular, are endemic of southernmost South America and found especially in small, fresh or brackish water lakes and lagoons, from Itapeva Lake (29º 56' 20''S, 50º 21' 52''W) to Mirim and Mangueira Lakes (32º 40' 01''S, 52º 52' 02''W; 33° 09' 24''S, 52º 47' 52''W), in the state of Rio Grande do Sul, Brazil (Bemvenutti, 1995). This work aims to determine the lethal concentration (CL_{50}) of un-ionized ammonia to pejerrey larvae during 96 hours of exposure, fostering the development of the species rearing techniques.

MATERIAL AND METHODS

Newly-hatched pejerrey larvae (9.0 ± 0.2 mm; 3.9 ± 0.9 mg) were stocked in 20-L, aerated aquaria (20 larvae per aquarium) and exposed to 0, 0.57, 0.94, and 1.45 mg L^{-1} of NH_3-N for 96 hours in a completely randomized experimental design (n = 3). Varying concentrations of NH_3-N were obtained by diluting a stock NH_4Cl solution (APHA, 1998) in organic matter-free water [flocculation with aluminum sulphate and correction of alkalinity with $Ca(HC0_2)_3$]. Water temperature was maintained at 20.51 ± 0.44ºC, and pH was adjusted to 7.5 by adding $Ca(HCO_2)_3$ at 24-hour intervals.

NH_3-N concentration was calculated daily as a percentage of total ammonia, using Nesslerizacion method (APHA, 1998), corrected for temperature and pH. Temperature, dissolved oxigen, O_2 saturation, pH, alkalinity, carbon dioxide – CO_2, and conductivity, were monitored daily (APHA, 1998). Dead larvae were collected and counted every 24 hours, during 96 hours. Mortality rate data were submitted to regression analysis (SAS, 1998).

RESULTS AND DISCUSSION

Water physical and chemical characteristics remained within the species comforr limits along the experimental period (Piedras & Pouey, 2004). Registered variations in CO_2 concentration and conductivity (Table 1) originate from dilution of NH_4Cl solution to yield the desired concentration of un-ionized ammonia.

Un-ionized ammonia concentration [NH_3-N]and mortality (M%) of pejerrey larvae presented linear relationship: M% = 6.3502 + 61.351 [NH_3-N], CL_{50} occurring at 0.71 mg L^{-1} NH_3-N after 96 hours of exposure (Figure 1). Registered values are similar to those reported for *Odontesthes argentinensis* 15 day-old larvae (15 mm; 30 mg) by Sampaio & Minillo (2000) – CL_{50} between 0.73 and 0.96 mg L^{-1} NH_3-N, at 15 ~ 23°C, salinity 28‰. These results match those reported by Wicks & Randall (2002), who registered that 0.8 mg L^{-1} NH_3-N impairs swimming of *Oncorhynchus kisutch* juveniles.

Abdalla & MacNabb (1998) reported that lethal concentration of un-ionized ammonia for fish varies from 0.32 to 3.1 mg L^{-1} for 96-hour acute exposure. However, toxicity level is affected by fish size,

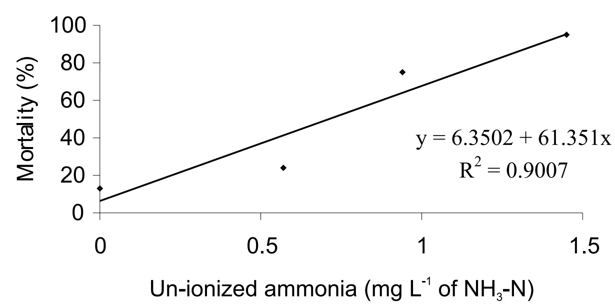

Figure 1 - Mortality of larvae after 96 hours of acute exposure to un-ionized ammonia concentrations.

Table 1 - Physical and chemical characteristics of the water by treatments

Parameter	T0	T1	T2	T3
Temperature (ºC)	20.86 ± 0.23	20.96 ± 0.19	20.76 ± 0.25	20.86 ± 0.20
O2 (mg L^{-1})	6.46 ± 0.17	6.33 ± 0.20	6.70 ± 0.12	6.60 ± 0.19
Saturation O2 (%)	73.00 ± 1.4	68.00 ± 2.1	73.33 ± 1.0	73.66 ± 1.2
pH	7.60 ± 0.05	7.60 ± 0.04	7.53 ± 0.07	7.56 ± 0.06
Alkalinity (mg L^{-1})	25.33 ± 1.45	26.33 ± 1.05	25.33 ± 1.30	27.00 ± 1.41
CO2 (mg L^{-1})	0.86 ± 1.7	5.26 ± 1.3	8.00 ± 0.9	10.00 ± 0.7
Conductivity (μMhos)	154 ± 7.0	494 ± 6.5	652 ± 6.2	840 ± 6.2
NH4-N (mg L^{-1})	0.75 ± 0.01	42.26 ± 0.2	76.13 ± 5.3	117.00 ± 6.2
NH3-N (mg L^{-1})	0.009 ± 0.02	0.57 ± 0.3	0.94 ± 0.026	1.45 ± 0.03

i.e., smaller fishes are exposed to higher dosage per unit of weight than larger fishes, being therefore more susceptible to NH_3-N toxicity. Studying *Arapaiama gigas* juveniles (2.6 kg live weight), Cavero et al. (2004) observed that concentrations up to 2.0 mg L^{-1} of NH_3-N did not cause mortality of impair feeding activity, a phenomenon attributed to the aerial respiration of the species.

Fish respond differently to varying NH_3-N concentrations. When exposed to NH_3-N for long periods, some fish can even adapt to endure low concentrations (0.06 mg L^{-1} of NH_3-N) (Foss et al., 2003). However, Frances et al. (2000) reported that as little as 0.06 mg L^{-1} NH_3-N causes 5% reduction on growth rate of juvenile perch *Bidyanus bidyanus*. Hargreaves & Kucuk (2001) reported that growth of striped bass *Morone saxatilis* juveniles is not affected by concentrations up to 0.37 mg L^{-1} NH_3-N, but concentrations higher than 0.65 mg L^{-1} NH_3-N depress growth. According to El-Shafai et al. (2004), 0.26 mg L^{-1} NH_3-N do not kill but negatively affect the proteic efficiency of tilapia *Oreochromis niloticus* juveniles. Similar results are registered by Lamarié et al. (2004), who consider 0.26 mg L^{-1} NH_3-N the maximum acceptable concentration to maintain weight gain performance of European seabass *Dicentrarchus labrax* juveniles.

Bergmann (1994) emphasizes that NH_3-N toxicity results from its interaction with other water quality parameters, such as pH, ionized ammonia and microbial activity in the sediment, and shows diurnal variation according to intensity of photosynthesis and respiration processes occurring in the environment. The 96-h, LC_{50} (0.71mg L^{-1} NH_3-N) registered for pejerrey larvae in aquaria lies above NH_3-N LC_{50} levels registered in rearing tanks for either *O. bonariensis* (Piedras et al., 2004) or *O. argentinensis* (Sampaio & Minillo, 2000). However, larvae are fed mostly dry diets and high stocking densities are used in the species larviculture, so ammonia concentration may rise above the estimated 96-h CL_{50}.

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Received September 17, 2004
Accepted March 02, 2006