Studies on the viability of silverside *Odontesthes argentinensis* cultivations: Acute toxicity of ammonia

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The toxicity of ammonia was estimated in a bioassay using 2-month-old juveniles of *Odontesthes argentinensis* in a semistatic system. The organisms with an average length of 2.94 cm were obtained from laboratory incubated eggs. The values calculated for LC_{50} were 1.48, 1.30, 0.80 and 0.80 mg/L NH_3-N at 24, 48, 72, and 96 h, respectively. These findings, when compared with literature data indicate that *O. argentinensis* is a very resistant species when exposed to ammonia and, from this point of view, may be used for breeding activities.

*Através de bioensaio em sistema semi-estático estimou-se a toxicidade aguda da amônia para juvenis de **Odontesthes argentinensis** com dois meses de idade (comprimento médio de 3,0 cm), obtidos a partir de ovos incubados em laboratório. Os valores calculados para as CL_{50} foram de 1,48; 1,30; 0,80 e 0,80 mg/L de N-NH_3 em 24, 48, 72 e 96 h, respectivamente. Esses resultados, se comparados com os existentes na literatura, indicam que **O. argentinensis** é uma espécie muito resistente a exposição a amônia, o que viabilizaria seu aproveitamento em atividades de cultivo.*

Ammonia is a nitrogen compound that is very toxic to the marine fauna (1,2,3). It can accumulate in cultivation systems by three main pathways: *a)* excretions of animals being bred; *b)* mineralization of organic substances by heterotrophic bacteria (4); and *c)* the use of eutrophic water in these cultivations (5).

The toxicity of ammonia can be considered as one of the most common causes of larval culture mortality (6). It is the object of a large number of studies with the purpose of determining the median lethal concentrations (LC_{50}) for different species at relatively short exposure periods (acute testing) (7,8,9). In general, the main objective of these studies is to comprehend the chemical biological interrelations involved in maintaining aquatic organisms in highly stressing environments, principally in intensive cultivations.

The present study focuses on the toxicity of ammonia to silverside juveniles of *Odontesthes argentinensis*, a fish belonging to the Atherinidae family, of small and medium size, inhabiting seawater, freshwater and brackish water systems (10). The *O. argentinensis* is a fish that inhabits both marine and estuarine waters.

The members of Atherinidae family form a group of important commercial interests. Because of their alimentary nature, they have been increasingly used in pisciculture activities, called Atheriniculture (11).

The basic proposal for generating data about the toxicity of ammonia to *O. argentinensis* is to meet the increasing requirements of aquaculture techniques and cultivation of potentially breedable natural species.

The specimens were 2-month-old juveniles obtained from eggs of *O. argentinensis* collected in the Cassino beach, on the State of Rio Grande do Sul, Brazil, and incubated in the laboratory.

The tests included total-ammonia concentrations of 1, 5, 10, 20, 40, 80, 160 and 200 mg/L (NH_4^+, NH_3)-N, the solution prepared in duplicate from ammonium chloride PA., and two control solutions containing no inorganic ammonia source. The water for the experiments was also collected in the Cassino beach and filtered through a 1 mm sieve.

The fish were previously submitted to an adaptation period of 48 h in an 80 L glass aquarium; all fish that showed any abnormal signs were immediately removed. The experiments were done in 3 L Erlenmeyer flasks containing 10 fish in a 2.5 L solution. The flasks were constantly aerated, in a D.B.O.-type incubator, with photoperiods of 12 h light and 12 h darkness, salinity of 30%, and temperature of 25(±1)°C. To provide *ad libitum* feeding during the bioassays, nauplii of artemia were supplied daily.

The test solutions were completely renewed after 24 h to avoid significant variations in the initial concentrations of ammonia. During renewals, food continued to be supplied, the dead organisms were removed and the pH of the solutions was determined using a Digimed DMPH-PA potentiometer.

The specimens used had an average length of 2.94 cm and weight of 0.141 g in the beginning of the tests. The pH range during the tests was 7.8 and 8.1 (mean of 7.7).

The median lethal concentrations were calculated using the Trimmed

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Table 1 — Acute toxicity of ammonia, in the form of total ammonia, (NH_4^+, NH_3)-N, and gaseous ammonia, NH_3-N, to juveniles of *Odontesthes argentinensis*.

Time	LC_{50} (limits at 95% confidence levels) mg/L ($NH_4^+ + NH_3$)-N	mg/L (NH_3)-N
24 h	53.59 (34.53-77.07)	1.48 (0.96-2.13)
48 h	47.04 (30.36-72.89)	1.30 (0.84-2.02)
72 h	28.96 (19.63-42.74)	0.80 (0.54-1.18)
96 h	28.96 (19.63-42.74)	0.80 (0.54-1.18)

Spearman-Karber software developed by Hamilton et al (12).

The proportion of gaseous ammonia (NH_3) in relation to the concentrations of total ammonia was calculated based on the formulas suggested by Whitfield (13) and adapted by Ostrensky (14) for the average values of salinity, temperature and pH, determined through out the experiments.

As pointed out by Spotte (4), the cellular membranes can obstruct the passage of ammonia in its ionized form but not in its gaseous form. The latter diffuses freely through the membranes until it reaches an equilibrium between the internal and external environments.

For this reason, in the studies that evaluate the toxicity of ammonia to aquatic organisms, there is an almost unanimous affirmation by the authors that the most toxic form is that of ammonium gas and not the ionized form of ammonia (2,6). Therefore, the results (Table 1) were calculated both for total ammonia [(NH_4^+, NH_3)-N] which is the form determined by analytical methods, as well as for the gaseous phase (NH_3-N), the absolute concentration of which define the toxicity of ammonia to the species or populations being considered.

The LC_{50} value at 24 h was 1.48 mg/L NH_3-N. All fish tested in this solution containing 200 and 160 mg/L (NH_4^+, NH_3)-N died within the first 24 h; at 10 mg/L (NH_4^+, NH_3)-N none of the organisms were affected in the initial period. By the end of the tests, the maximum concentration that did not cause death of any organism was 1.0 mg (NH_4^+, NH_3)-N.

The more sensitive fish died within 48 h and none of them endured concentrations as high as 80 mg/L after 96 h of exposure to ammonia. It was noted that between 5 and 40 mg/L (NH_4^+, NH_3)-N, there was a progressive increase in the juveniles mortality, without however, affecting all fish tested.

The LC_{50} values at 72 h and 96 h were the same for total ammonia (28.96 mg/L) and for gaseous ammonia (0.80 mg/L), because no further deaths were observed after the first 48 h of experiment.

The LC_{50} values at 24 h found in this experiment compared with those reported by Brownell (9) for several marine fishes (*Gaidropsaurus capensis* — 0.46 mg/L NH_3-N, *Lithognathus mormyrus* — 0.38 mg/L NH_3-N, *Diplodus sargus* — 0.34 mg/L NH_3-N and 4 other species of the Sparidae family — 0.40 mg/L NH_3-N) show that the silverside is considerably more tolerant towards ammonia.

The tolerance presented by *O. argentinensis* can even be compared to that of freshwater fishes that usually are more resistant to ammonia than seawater fishes. In accordance with McCormick et al (8), the LC_{50} values of NH_3-N for *Lepomis cyanellus* oscillate between 1.57 mg/L at 24 h to 1.06 mg/L at 96 h. Thurston & Russo (15) estimated that the LC_{50} value at 96 h for *Salmo clarki* is 0.80 mg/L NH_3-N and about 0.95 mg/L NH_3-N for *Pimephales promelas* (16).

In any ammonia toxicity test, the results obtained are directly dependent on the variables that act on the ionization of this compound. That is, they depend on the pH, temperature, and salinity in this order. In the present case, the variables were established at most favorable conditions to cultivate *O. argentinensis* along the coast of Rio Grande do Sul State, Brazil. Under natural conditions and those of cultivation (photoperiod, aeration and feeding during the test), the species showed a very low sensitivity when exposed to ammonia, thus indicating, its breeding potential for repopulating ecosystems or commercial exploitation. ■

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