Sensitivity of the Sea Urchin *Lytechinus variegatus* Embryos to Unionized Ammonia: Implications to Brazilian Legislation

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Abstract **— The toxicity of unionized ammonia was evaluated by the toxicity test with embryos of the sea urchin *Lytechinus variegatus*. The mean 24h EC50 was 0.53 ± 0.22 mg/l NH_3. The lower effective concentration to the embryos (LOECs) ranged from 0.24 to 0.48 mg/l, whereas the higher non toxic concentrations (NOECs) ranged between 0.10 to 0.24 mg/l. The results showed that the maximum concentration of unionized ammonia permitted by the Brazilian legislation for protecting the aquatic communities is not appropriate and needs an urgent review.**

Key words — **Unionized ammonia, Toxicity tests, *Lytechinus variegatus*, pollution.**

I. INTRODUCTION

Ammonia is commonly introduced into water bodies as a component of industrial and domestic effluents. This compound may also occur naturally in the aquatic environment as a product of biological activity. However, episodes of water contamination by ammonia are generally related to the discharge of anthropic residues.

When dissolved in water, ammonia can be present in two forms: the ionized one (NH_4^+), which is less toxic and may be incorporated by the primary producers; and the unionized form (NH_3), that is usually toxic to the aquatic biota. There is a dynamic equilibrium between them and both occur together in the nature. The equilibrium moves toward one or another form according to physical-chemical conditions of water [1].

The relative concentration of unionized ammonia in salt water is strongly influenced by the pH, but can be also dependent of temperature, salinity and pressure [2]. Increasing pH and/or temperature causes the equilibrium moving toward NH_3, whereas salinity increasing moves it towards NH_4^+ [3].

Due to its electrical charge, ionized ammonia presents low affinity to the biological membranes [1]. Unionized ammonia is highly lipo-soluble, and is easily adsorbed to the biological membranes, modifying their permeability and altering some of their properties. Moreover, due to its molecular characteristics, unionized ammonia can substitute the ion Na^+ in some cellular processes, changing the ionic equilibrium within the cells. In high concentrations, this compound is harmful to the aquatic biota, producing several effects to the organisms, including even lethality [4].

The knowledge of the unionized ammonia toxicity has major importance in Brazil, once the Brazilian legislation has established discussable maximum permitted concentrations in water for this substance, without appropriate scientific background [4]. Thus, the objective of this study was to evaluate the toxicity of unionized ammonia on the embryolarval development of the sea urchin *L. variegatus*, in order to obtain preliminary information that can be used as a basis for further studies aiming to the protection of the marine ecosystems, and also providing initial data for a review of the maximum NH_3 levels permitted by the Brazilian legislation for waters.

II. MATERIAL AND METHODS

Three toxicity tests were conducted, following the procedure described in the standard protocol L5.250 for embryos of the sea urchin *L. variegatus* [5]. Embryos of this species are sensitive to a wide range of substances, can be easily obtained all over the year and their handling is simple [6]. This organisms have been extensively used in ecotoxicological studies conducted in Brazil, allowing also comparisons to data produced by other authors. Adult individuals were collected from a rocky reef situated at Ubatuba, SP, Brazil (23°31'S, 45°07'25"W) and transported to the laboratory. At the end of the experiments, they were returned to their collection site.

The dilution water was collected off Anchieta Island Marine State Park, in Ubatuba, São Paulo, SP, and filtered in Millipore membrane HA 0,45 μm.

An ammonia 100 mg/l stock solution was previously prepared by the dilution of dry salts of NH_4Cl into sea water. This solution initially represented the total ammonia (NH_3 + NH_4^+). Unionized ammonia is a fraction of the total ammonia, and its concentration depends of the water physical-chemical conditions. Thus, pH, salinity and temperature of the dilution water were measured and the correct amounts of NH_4Cl necessary to obtain each

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concentration of unionized ammonia were calculated using the equations proposed by [2]. Immediately, the test concentrations were prepared by diluting the stock solution in dilution water. In each experiment, seven concentrations of unionized ammonia were prepared: 0.05; 0.10; 0.24; 0.48; 1.18; 3.31 and 5.90 mg/l NH_3. Glass tubes were used as test chambers, filled up to 10 ml with test solution. Four replicates were used per concentration. Temperature, pH and salinity were maintained constant during the tests in order to avoid variations in the NH_3 concentrations.

The sea urchin gametes were obtained by osmotic induction, by the injection of 2-4 ml KCl 0.5M into the celomatic cavity of each adult. The sperm was collected by a droplet and stored in a small beaker kept in box with ice. The ovules were collected and stored in 400 ml beakers containing filtered sea water. After gentle washing, the ovules were fecundated by the addition of 0,5 ml sperm solution to the ovules solution. The fecundation success was checked by observing the presence of fertilization membrane in at least 90% eggs. The tests were then initiated by introducing the eggs into the test chambers. After about 24 hours, the experiments were finished by fixing the larvae with 0.5 ml formaldeid 4%. Then, the first 100 larvae were observed and counted under microscope. Larvae developed to echinopluteus stage were considered normal, whereas those presenting morphologic deformations and/or retarded development were considered affected.

For each test, the respective effective concentration to 50% organisms after 24h exposition (24h EC50) were estimated by the Trimmed Spearman-Karber method with Abbot's correction [7]. Significant differences between the larval development in the control group and the tested concentrations were estimated by analysis of variance (ANOVA), followed by Dunnett's test. This analysis was used to estimate the "No Observed Effect Concentration" (NOEC) and the "Lower Observed Effect Concentration" (LOEC) for each test.

III. RESULTS

The results of the 3 tests are shown in the Fig. 1. In the first test, embryos exposed to 0.24; 0.48; 1.18; 3.31 and 5.90 mg/l NH_3 presented development significantly lower than the observed in the control. In the second test, the development was significantly affected in concentrations equal or higher than 0.48 mg/l. The result of the third test was similar to that observed in the first experiment.

The NOECs, LOECs and EC50s are presented in the Table I. The 24h EC50 values ranged between 0.29 and 0.73 mg/l, and the mean EC50 was 0.53 ± 0.22 mg/l NH_3. NOECs ranged between 0.10 and 0.24 mg/l whereas LOECs ranged between 0.24 to 0.48 mg/l.

According to the principle of precaution, the lower values should be considered when the protection of aquatic life is aimed. Thus, the results obtained in the present study allow to estimate the NH_3 threshold effect level as a concentration between the lower LOEC and NOEC (i.e., 0.10 to 0.24 mg/l).

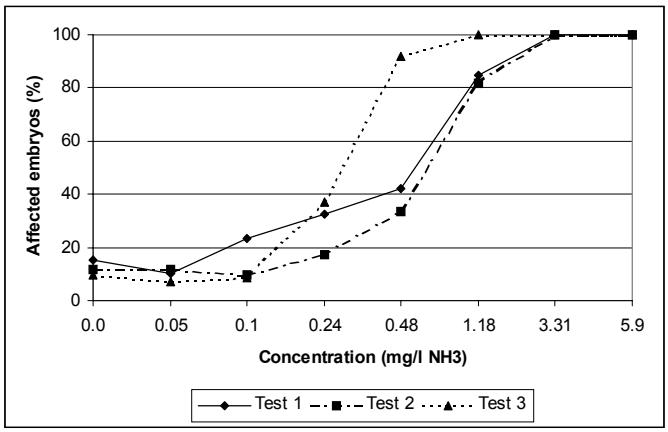

Figure 1. Results of the three toxicity tests with unionized ammonia.

TABLE I

TOXICITY VALUES ESTIMATED FOR UNIONIZED AMMONIA IN EACH TEST.

Test	Estimated values (mg/l)		
	24h EC_{50}	NOEC	LOEC
1	0.57 (0.53 – 0.61)	0.10	0.24
2	0.73 (0.69 – 0.77)	0.24	0.48
3	0.29 (0.27 – 0.30)	0.10	0.24

IV. DISCUSSION

The establishment of maximum acceptable concentrations in the environment for any substance is a difficult matter that requires studies with the widest number of species as possible, in order to provide an effective protection to the whole biota. However, studies based on only one species can be useful and are necessary, at least as an initial investigation, specially when available knowledge is lacking.

The present study, despite its preliminary character, evidenced that the Brazilian legislation concerned to water quality is inappropriate for unionized ammonia. For waters with purpose of protection of marine communities (Class 5 waters), the maximum permitted concentration of this compound is 0.40 mg/l [8]. Our study showed that in this concentration the aquatic biota is not protected, because the embryolarval development of *L. variegatus* was significantly affected from 0.24 mg/l. Thus, according to the results obtained in this study, for class 5 waters the concentration of unionized ammonia should be at least lower than 0.24 mg/l.

Other studies suggest even values lower than 0.24 mg/l. Effects on the growing of juveniles of the pink shrimp *Penaeus paulensis* exposed to the maximum permitted concentration for brackish water were observed [4]. In that study, 0.10 mg/l NH_3 was enough to produce effects on the animals. Moreover, [6] observed a EC50 of 0.15 mg/l for *L. variegatus* embryos exposed to NH_3, which differed from the result obtained in our study. In his study, the author used a brood stock collected in São Sebastião, and this suggests that the sensitivity of the populations from Ubatuba and São

Sebastião may be different for unionized ammonia. However, the author conducted only one experiment, making any comparison to his data to be not conclusive. So, this requires further studies in the future. Another study conducted *with L. variegatus* fro São Sebastião suggests that effects of unionized ammonia may start from 0.05 mg/l [9]. Table 2 shows a comparison among the NH_3 threshold effect levels estimated in different studies.

TABLE II

COMPARISON BETWEEN THE THRESHOLD EFFECT LEVELS OF UNIONIZED AMMONIA ESTIMATED IN DIFFERENT STUDIES AND THE MAXIMUM PERMITTED CONCENTRATION.

Reference	**NH_3 effect level (mg/l)**	**Test organism**
Present study	0.10 – 0.24	*L. variegatus* embryos
[9]	0.05	*L. variegatus* embryos
[6]	0.10	*L. variegatus* embryos
[4]	0.10	*Penaeus paulensis*
Maximum permitted concentration [8]	0.40	-

Despite differences observed in the values by the available studies, all they are in agree with the present study, evidencing that the maximum concentration of NH_3 for class 5 waters is not suitable for marine life and must be reviewed. Further investigation must be done in order to establish appropriate maximum NH_3 permitted levels to Class 5 waters.

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