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TOXICITY OF HYDROGEN SULPHIDE TO JUVENILES OF *MACROBRACHIUM ROSENBERGII*

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Abstract: Static bio-assays were performed for 96 h to determine the mean lethal concentrations (LC_{50}) of hydrogen sulphide for juveniles of *Macrobrachium rosenbergii* De Man. Broods of two size classes were tested and LC_{50} values were determined for 24, 48, 72 and 96 h. The LC_{50} values were 6.36, 4.25, 3.09 and 2.57 mg/l H_2S for brood 1 (7.26 ± 0.85 mm) and 5.57, 4.79, 4.25 and 4.20 for brood 2 (15.08 ± 1.08 mm) respectively, for 24, 48, 72 and 96 h. The safe concentration of hydrogen sulphide for juveniles of *M. rosenbergii* was determined to be 0.26 mg/l.

INTRODUCTION

Hydrogen sulphide is a toxic gas which is harmful to aquatic life, particularly in the un-ionized form. It may occur naturally in water, at levels which can be inimical to fish production and survival.[1] Under natural conditions it is produced by the anaerobic decomposition of organic materials in sediments. It can also be produced either by the decomposition of organic effluents from municipal sewage and many industries[1] or released directly in industrial effluents from pulp mills[2], from oil refineries[3], and from chemical and gas industries.[4] In fish culture ponds, it is liberated as a result of decomposition of food and organic matter.[5]

The toxic effects of hydrogen sulphide to fish have been reviewed by Adelman and Smith.[6] Smith and Oseid[7] reported reduced swimming endurance of bluegill sunfish *(Lepomis macrochirus)* after exposure to 0.04 mg/l H_2S. Even very low concentrations of H_2S have been shown to be detrimental to fish eggs, fry and juveniles.[1,6,7] Long term exposure of fish to sub-lethal levels can slow growth, increase mortality and reduce fecundity. Sub-lethal levels of H_2S also can influence the hatching of fry[1] and cause anatomical malformations.[6] Fish mortality due to H_2S was reported by Colman in *Clarius batrachus* in grow-out ponds.[8] He observed that the mortality in their system depended primarily upon the presence or absence of H_2S. Peturiyawate[9] determined the median lethal concentrations (LC_{50}) for the fingerlings of *Clarius batrachus*.[9] The author estimated 6.32 mg/l and 5.31 mg/l of H_2S as the LC_{50} levels for 24 h and 48 h, respectively.

However, there are very few studies carried out on the effect of H_2S on crustaceans. Smith[11] studied the effects of H_2S on a crustacean *Gammarus*

pseudolimnaeus used as fish food, and indicated safe levels between 0.002 and 0.003 mg/l.[11]

In the present study, experiments were performed to determine the LC_{50} values for juveniles of the giant freshwater prawn *Macrobrachium rosenbergii* De Man. This species was selected for two main reasons, *viz.* 1) its importance as an economically valuable and popularly cultured species in the Indo-Pacific region; 2) unsolved sudden mortalities occurring in grow-out ponds during the latter part of culture.

METHODS AND MATERIALS

Static bio-assays were performed for 96 h on two separate broods of 21-day and 2 month-old juveniles. Body weights of the specimens from the two broods were measured using a Sartorius balance with a precision of four decimal points. The body lengths of the specimens from the two broods were measured under the microscope using a stage micrometer. The mean ± S.E. body weights of the specimens of the 1st and 2nd broods were 0.0028 ± 0.006 g and 0.005 ± 0.002 g while the mean body lengths were 7.25 ± 0.85 mm and 15.08 ± 1.08 mm respectively.

Test solutions were prepared by diluting $Na_2S.9H_2O$ fresh stock solution (1000 mg/l), prepared using deoxygenated distilled water. Dilution water was analysed for dissolved oxygen, pH, total alkalinity, total hardness, temperature and dissolved sulphides, prior to the experiment and once daily after the experiment was started. Temperature was measured using a thermometer, pH by a pH meter (ORION Ltd.). Dissolved oxygen was measured using a DO meter (YSI Model). Total alkalinity, total hardness and total sulphide were measured titrimetrically.[10]

A preliminary test was conducted to find out the critical range prior to the full-scale experiment. For the full-scale experiment concentrations of 1 mg/l, 2 mg/l, 4 mg/l, 6 mg/l and 8.0 mgl were tested along with a control. All test solutions were renewed every 24 h, at which time the H_2S concentration was never less than 88% of the specified levels.

Tests were performed in 2 litre flasks covered with cork lids. Ten juveniles were placed in each flask and each concentration was run in triplicate for a total of 30 animals per concentration. Each experiment was repeated twice. The juveniles were starved for 24 h prior to the commencement of experiment and were not fed during the experimental period.

Dead prawns were counted and recorded at the exposure times of 1,2,3,6,9,12,24,48,72 and 96 h respectively. The death was defined as opaqueness in immobile animals and the dead prawns were removed from the test solutions immediately.

The LC_{50} values and 95% confidence intervals were calculated as described by Finney.[12] The data were analysed statistically by two-way ANOVA, the dependant variable being the time of death for each juvenile. Mortality of the controls, was 0% at the end of 96 h period. The LC_{50} value and 95% confidence limits of 96 h period for brood 2 were calculated according to the Litchfield and Wilcoxon.[13]

RESULTS AND DISCUSSION

The chemical characteristics of dilution water are shown in Table 1. The dilution water used for brood 2 indicated higher pH and DO although the water was obtained from the same tank. Differences in temperature in water used in experiments involving brood 1 and 2 is specially due to the climatic changes. First brood was tested in late December, 1985, when the ambient temperature was low, while brood 2 was tested during early February, 1986, when the ambient temperature was higher.

Table 1. Chemical characteristics of the dilution water used in bioassay experiments.

Parameter	Dilution water used for brood 1	Dilution water used for brood 2
Dissolved oxygen (mg/l)	5.4 ± 1.21	6.1 ± 1.32
pH	8.0 ± 0.20	8.8 ± 0.15
Temperature (°C)	21.6 ± 4.26	26.1 ± 0.50
Alkalinity as $CaCO_3$ (mg/l)	224 ± 4.26	242 ± 5.68
Total hardness (mg/l)	290 ± 2.78	301 ± 3.83

In all the test solutions prawns exhibited erratic movements. In the highest concentration (3.0 mg/l), erratic movement began after 30 min of exposure; while in the lowest concentration (1.0 mg/l) it began only after 80 - 96 h. The prawns swam up and down around the flask at the beginning and later jumped to the sides of the flask. They began to lose balance, swim to the surface and come down rapidly in upside down position and in a zig zag manner. They moved weakly at the bottom and died after a period of time. A similar behaviour of *Macrobrachium rosenbergii* juveniles exposed to lead was documented by Kalayanamitr.[14] He suggested that the erratic movement may be due to the affected central and peripheral nervous system. The major effect of hydrogen sulphide is exerted on the nervous system, the eyes and the respiratory system and the type of poisoning depends on the duration and level of exposure to gas.[15] In the present study it was observed that the prawns lost balance in test solutions indicating that their nervous systems were affected.

Percentage mortality of juvenile prawns in relation to exposure times are given in Table 2. A 100% mortality occurred both in brood 1 and 2 within a 24 h period at the highest concentration (8.0 mg/l). At the 6.0 mg/l concentration 100% mortality

occurred at 96 h period. No mortality was observed in any of the test solutions due to cannibalism although this species is well known for their cannibalistic nature. Some attempts at feeding on dead prawns were observed but this was prevented by removing the dead prawns immediately. The relationship between exposure time and % mortality observed in the present instance is different from that observed for fish. In a study of *Clarius batrachus*, Peturiyawate[9] observed that the fish get adapted to the toxic environment after 24 h of exposure.[9] In the present study the mortality increased with the time of exposure until the end of the experiment.

Table 2. Percent mortality of the *M. rosenbergii* juveniles exposed to different concentrations of hydrogen sulphide for 24h, 48h, 72h and 96 h.

Concentration of H_2S (mg/l)	Number tested	Brood	Exposure time (h)			
			24	48	72	96
0	30	1	0	0	0	0
		2	0	0	0	0
1.0	30	1	0	3.3	3.3	3.3
		2	0	0	0	0
2.0	30	1	0	3.3	10.3	23.3
		2	0	3.3	3.3	13.3
4.0	30	1	3.3	43.3	73.3	86.7
		2	10.3	20.7	26.7	46.7
6.0	30	1	46.7	70.0	96.7	100
		2	60.0	80.0	96.7	100
8.0	30	1	76.7	100	-	-
		2	93.3	100	-	-

Percent mortality of juvenile prawns in relation to concentrations are given in Figure 1 and 2. Percent mortality increased with the increase in concentrations.

The two broods of juveniles were similar in susceptibility to H_2S concentration. At exposure times of 24, 48, 72, and 96 h, no significant difference was found ($p < 0.005$) in the % mortalities between the two broods.

The LC_{50} values, their confidence limits are presented in Table 3. LC_{50} value for 96 h were 2.57 mg/l and 4.2 mg/l respectively for brood 1 and brood 2. The differences in LC_{50} values for specimens of brood 1 and brood 2 may probably be due to either the size of the prawn or the water quality, specially pH, DO and temperature. According to the toxicity curve (Figure 3) the smaller juveniles seem to get adjusted to the toxic environment easily compared to the bigger ones. However, during the tests conducted with brood 2 specimens, pH and DO were higher compared to the

brood 1. Adelman and Smith reported that lower oxygen levels resulted in lower LC_{50} values in finfish.[16] Colby and Smith[1] and Adelman and Smith[6] found the same effect in other finfish species, although the latter authors found no effect of oxygen differences on the toxicity of hydrogen sulphide to northern pike eggs. In the present study, the specimens from brood 2 which were at a lower dissolved oxygen level

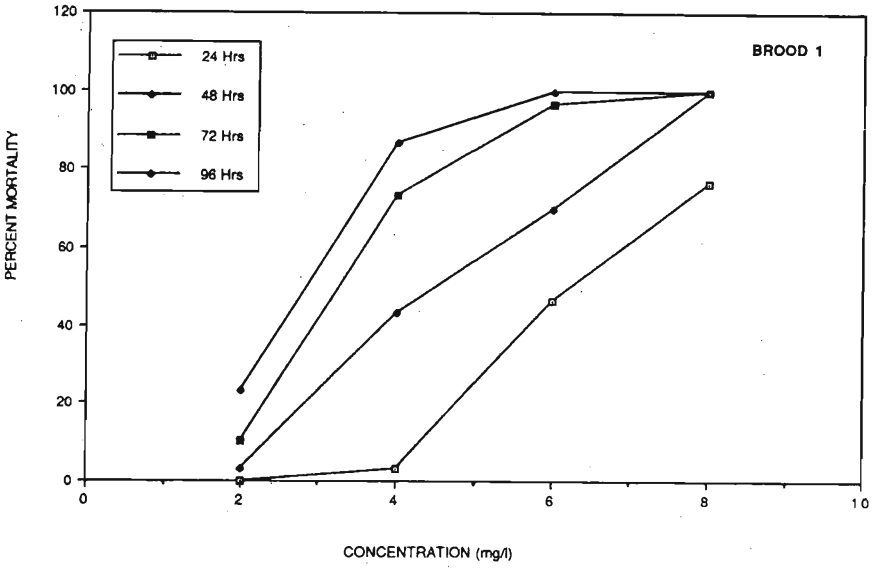

Figure 1: **Per cent mortality of the juveniles of *M. rosenbergii* (brood 1) exposed to different concentrations of hydrogen sulphide for 24, 48, 72 and 96 h.**

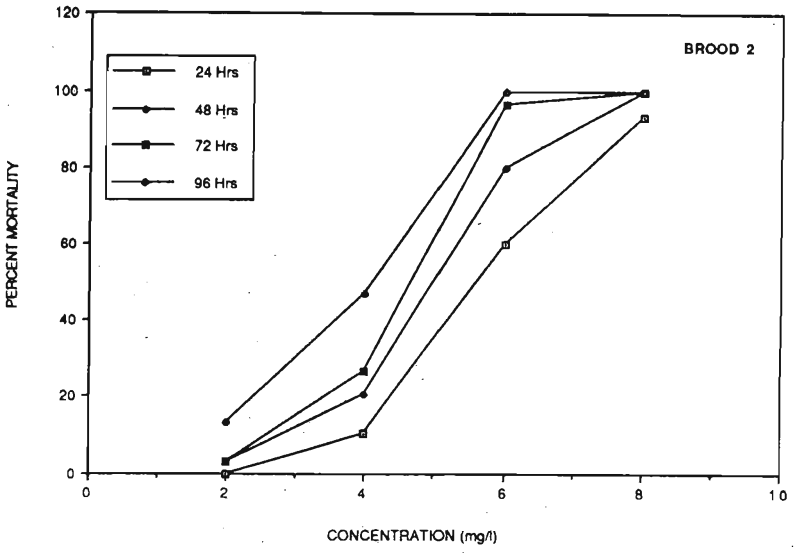

Figure 2: **Per cent mortality of the juveniles of *M. rosenbergii* (brood 2) exposed to different concentrations of hydrogen sulphide for 24, 48, 72 and 96 h.**

showed lower LC_{50} values. Jones[16] and Bonn and Follis[17] showed that raising the pH could in turn lower the toxicity of un-ionized hydrogen sulphide. At higher pH, the greater proportion of hydrogen sulphide is in the form of ionized hydrogen sulphide which is less toxic. It was also observed in the present study that at higher pH juveniles of brood 2 showed less toxicity effects.

Table 3. LC_{50} values and 95% confidence limits at indicated times during 96 h test of the toxicity of hydrogen sulphide.

Time	LC_{50} values mg/l		95% confidence limits mg/l	
	brood 1	brood 2	brood 1	brood 2
24	6.36	5.57	5.81 - 6.95	5.13 - 6.04
48	4.25	4.79	3.36 - 5.39	3.77 - 6.09
72	3.03	4.25	2.65 - 3.46	3.03 - 5.95
96	2.57	4.20	2.21 - 2.98	2.38 - 7.39

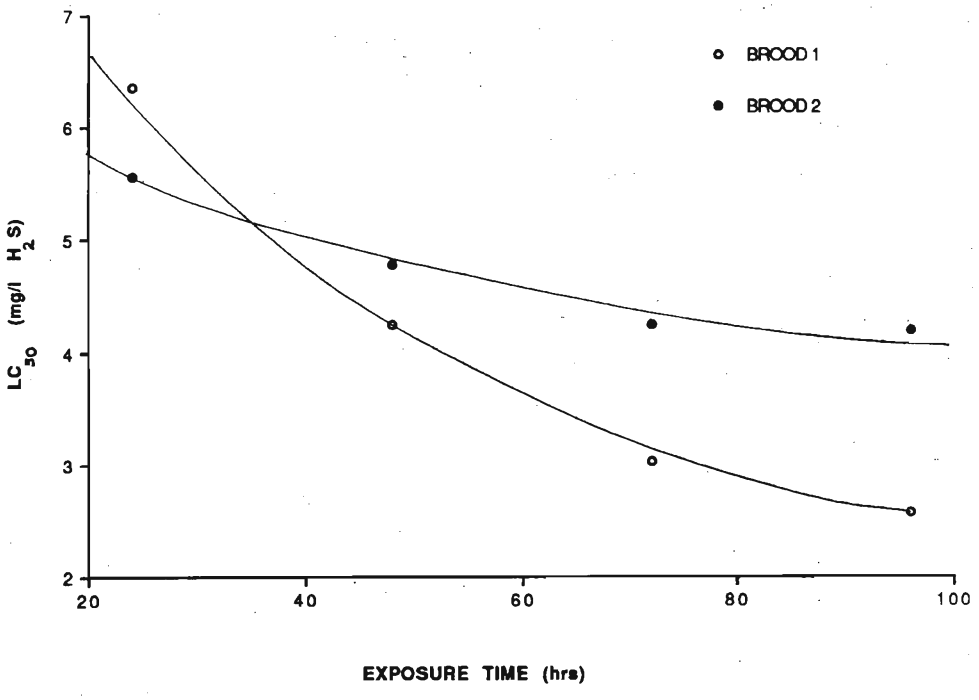

Figure 3: The toxicity curve of hydrogen sulphide for juveniles of *M. rosenbergii*.

The toxicities of hydrogen sulphide to other aquatic organisms have been reported by various authors. Doudoroff and Katz[18] reported that the concentrations below 10.0 mg/l (mostly 1.0 - 6.0 mg/l) were lethal to several fish species such as *Lepomis gibbonus, Salmo gairdneri, Catostomus commersoni, Carassius auratus* and *Cyprinus carpio*.[18] Bonn and Follis reported that fish could survive in H_2S concentrations between 0.3 and 4.0 mg/l.[17] Colby and Smith showed that 0.3 mg/l sulphide were acutely lethal to the amphipod *Gammarus pseudolimnaeus* eggs and fry.[1] Bonn and Follis found that 24 h LC_{50} value for fingerlings of channel cat fish *(Ictalurus punctatus)* ranged from 0.53 - 0.8 mg/l of H_2S.[17] Peturiyawate reported that the 48 h LC_{50} value for *Clarius batrachus* as 6.52 mg/l.[9] The LC_{50} values obtained in this study lie within the range given by Doudoroff and Katz.[18] The value is lower than that observed for *Clarius batrachus* and *Carassius auratus*.[9,10] Unfortunately, no data are available on the H_2S toxicity of other prawn species for comparison. The safe concentration of H_2S which is equivalent to 20% of the LC_{50} was calculated according to Peturiyawate[9] and was found to lie within the range of 0.26 - 0.43 mg/l of hydrogen sulphide.

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