Toxic Effects of Ammonia on Survival and Behavioural Responses in Labeo Rohita as a Function of Body Size

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Abstract

The 96 hr LC_{50} value of ammonia for small and large size groups of *L. rohita* were 1.47 and 1.63 mg l^{-1} respectively. The high 'b' value obtained in small fish indicates that, the toxic impact of ammonia was more in smaller fish than larger fish. The 95% confidence limits were 1.45 (lower limit) and 1.48 (upper limit) mg NH_3 l^{-1} in small size group of *L. rohita* and it increased to 1.43 and 1.76 mg NH_3 l^{-1} in large size group of *L. rohita* respectively. The slope function calculated for small and large size groups of test animal was 1.02 and 1.13 respectively. A positive correlation co-efficient was obtained for the relationship between the ammonia concentrations and per cent mortality and it was statistically significant for small ($r = 0.965$; $P < 0.01$) and large ($r = 0.952$; $P < 0.01$) size groups of *L. rohita* exposed to toxic levels of ammonia . The relationship between the toxic concentrations of ammonia and per cent mortality of small and large size groups of *L.rohita* expressed by the regression lines ($Y = a + bX$) were $Y = -1238.60 + 879.77X$ and $Y = -327.57 + 232.06X$ respectively. The 'b' value obtained for small size group was 880 and it significantly declined to 232 in large size group of *L. rohita* exposed to ammonia levels.

Keywords: Ammonia, LC50, Large size, Small size, *L.rohita*

Introduction

Environmental pollutants such as heavy metals, pesticides, gases like NH_3, H_2S and other organics pose serious risks to many aquatic organisms including fish. Fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic system (Farkas *et al.,* 2002). Besides the extraneous sources of pollution, water masses are contaminated by metabolic products produced by fish themselves. Protein metabolism results in the production of various metabolites which are quite harmful to fish. Ammonia is the major nitrogenous waste product produced by fish (Colt and Tchobanoglous, 1976). Ammonia has been found to be toxic even in very trace quantities. Moreover, water bodies adjacent to paddy fields are directly exposed to the fertilizers contamination and hence a study on the survival of *Labeo rohita* with reference to ammonia is much required. Obviously, finding the safe level for ammonia in the water medium will be much useful in water quality management as well. Hence, in the present study, an attempt was made to investigate the effect of ammonia on survival and behaviour of *L. rohita* as a function of body size.

Materials and methods

L. rohita (3.35 ± 0.15g) was collected from the Sabari Fish Farm, Vellanguli, Tirunelveli, Tamil Nadu, India. They were acclimated to the laboratory conditions for 3 weeks, during which they were fed with 35% protein diet. The fish were starved one day prior to the experiment and throughout the bioassay test. After acclimation, two size groups of *L. rohita* were separated from the stock. The small size groups (2.55 ± 0.2 g) were separately exposed to 1.40, 1.43, 1.45, 1.47, 1.49 and 1.51 mg l^{-1} (ppm) in plastic troughs containing 10 *l* of test media and mortality was recorded for 96 hr. Similarly, large size (7.53 ± 0.5 g) *L. rohita* groups were exposed to 1.40, 1.47, 1.59, 1.68, 1.76 and 1.80 mg l^{-1}. A control was also maintained simultaneously. Experiment was conducted in the water temperature of 27 + 1^0 C, DO of 4.2 ml O_2 l^{-1} and pH of 7.7. A static renewable bioassay method was adopted to determine the 96 hr LC_{50} (Sprague, 1973) and juveniles were not fed during the bioassay test. The test media were changed daily at 0800 hr to maintain the constant toxic concentrations (Sprague, 1971) during the bioassay test. The 96 hr LC_{50}, its 95% confidence limits and the slope function were determined following the method of Finney (1971). The behavioural changes of small and large sized *L. rohita* were observed in lethal concentrations of ammonia during the bioassay test.

Results and discussion

The behavioural changes of small and large size groups of *L. rohita* were found to be different when they exposed to toxic concentrations of ammonia. At lethal concentrations (1.47 – 1.80 mg^{-1}), they tried to avoid the toxicant (ammonia) by irregular erratic swimming, jerky movements, rapid opercular movements, restlessness, frequent surfacing, gulping of air, upside down surface movement and extension of fins. An important local effect was the abundant discharge of mucus at the gills and on the skin. Secretion of mucus was regarded as a defense and excretory response (Bennet and Dooley, 1982) which might help in protecting gills and skin from ammonia toxicity. Suffocation of fish exposed to ammonia was discernible in the form of air bubbles on the water surface when the fish had been directed towards the water surface. Finally they lost their equilibrium and settled at the bottom before death. The dead animals showed blood clots on the gill surface and widely opened mouth and gills. Eventhough, the same behavioural changes have been observed in small and large size groups of *L. rohita* but these strange phenomena were greatly exhibited in the smaller size group of *L. rohita* exposed to toxic concentrations of ammonia than the larger size.

Karasu Benli and Koksal (2005) observed that, the larvae of *Oreochromis niloticus* exposed to different concentrations of ammonia moved very rapidly, lost the equilibrium and showed sideways swimmings. In the case of fingerlings, an increase in their movements, ventilations, convulsion, engulf the atmospheric air, mucous secretion from the gills and on the body surface, haemorrhage in the gills etc., were observed which supports the findings of present study. Acute ammonia toxicity can cause an assortment of clinical signs in fish, the most severe of which include convulsion, coma and death (Randall and Tsui, 2002) as well as behavioural changes such as hyper excitability and appetite suppression (Ortega *et al.,* 2005) in various fish species. Bhakta (2006) reported the reduction of opercular movement and lethargic movements with frequent surfacing were noticed in four fresh water fish species viz., *Catla catla, Labeo bata, Cyprinus carpio* and *Oreochromis mossambicus* exposed to different concentrations of ammonia, supports the present investigation.

The 96 hr LC_{50} value of ammonia for small and large size groups of *L. rohita* were 1.47 and 1.63 mg l^{-1} respectively. No mortality was observed below the ammonia concentration of 1.40 mg l^{-1} in both size groups. However, the concentrations of 1.47 mg l^{-1} and above were observed to be toxic (Table 4.1 and 4.2). The 95% confidence limits were 1.45 (lower limit) and 1.48 (upper limit) mg NH_3 l^{-1} in small size group of *L. rohita* and it increased to 1.43 and 1.76 mg NH_3 l^{-1} in large size group of *L. rohita* respectively. The slope function calculated for small and large size groups of test animal was 1.02 and 1.13 respectively. A positive correlation co-efficient was obtained for the relationship between the ammonia concentrations and per cent mortality and it was statistically significant for small ($r = 0.965$; $P < 0.01$) and large ($r = 0.952$; $P < 0.01$) size groups of *L. rohita* exposed to toxic levels of ammonia (Fig. 4.1 and 4.2). The relationship between the toxic concentrations of ammonia and per cent mortality of small and large size groups of *L. rohita* expressed by the regression lines ($Y = a + bX$) were $Y = -1238.60 + 879.77X$ and $Y = -327.57 + 232.06X$ respectively. The 'b' value obtained for small size group was 880 and it significantly declined to 232 in large size group of *L. rohita* exposed to ammonia levels (Fig. 4.1 and 4.2). It manifests that, small size group was 4 times more sensitive to ammonia toxicity which affected the survival of small fish as compared to large size group. Karasu Benli and Koksal (2005) observed that, the 48 hr LC_{50} value of larvae and fingerlings of Nile tilapia, *Oreochromis niloticus* were 1.01 and 7.40 mg NH_3 l^{-1} respectively and it revealed that small size larvae was 7 times more sensitive to large size fingerlings against ammonia toxicity, which supports the present investigation.

The results of the current study indicates that, smaller size group of *L. rohita* was less tolerant or more sensitive to ammonia toxicity than the larger fish. Ammonia exposed small fish affected by their toxicity in two ways: (i) higher effective dosage of ammonia per unit body weight and (ii) higher metabolic rate resulted the more mortality even in low level of ammonia as compared to large *L. rohita.* The above two strange factors elicited the more abnormal behaviour in small fish as compared to large fish. Hence the 'b' value was more in smaller fish (880) than the larger fish (232) of *L. rohita* (Fig. 4.1 and 4.2) which evidently confirmed the more erratic behaviour in ammonia exposed small fish. The differences in sensitivity to ammonia based on the body weight and life stage have been already observed in other species. For instance, Meade (1985) found that fry was more sensitive to ammonia than the larger rainbow trout, *Salmo gairdneri.* In addition, Salin and Williot (1991) observed that the youngest fish showed the highest sensitivity to ammonia with a 24 hr LC_{50} value of 1 mg NH_3 l^{-1}, whereas for the large fish, the LC_{50} value increased to 2.5 mg NH_3 l^{-1} and supports the findings of *L. rohita* in the present study.

Similar results were reported by many other researchers in the different species such as rainbow trout (Smart, 1978), sunfish (McCormick *et al.,* 1984) and common carp (Mallet and Eddy, 1995). On contrary, the size of fish could not influence the mortality of certain fish species exposed to toxicants (Bradley and Sprague, 1985; Chapman, 1978). The LC_{50} values from this study are comparable to those derived from other types of cultured fish (Table 4.3).

The present chapter concludes that, the 96 hr LC_{50} value of ammonia for small and large size groups of *L. rohita* were 1.47 and 1.63 mg l^{-1} respectively. The high 'b' value obtained in small fish indicates that, the toxic impact of ammonia was more in smaller fish than larger fish.

Table 4.1. Effect of ammonia concentrations on per cent mortality in small size (2.55 g) *Labeo rohita* exposed for 96 hr. Lethal concentration, slope function and 95% confidence limits are expressed in mg l^{-1}.

Concentrations of NH_3 (mg l^{-1})	Dead / Tested	Mortality (%)	Lethal concentration (mg l^{-1})			Slope function (S)	95% confidence limit	
			16%	50%	84%		Lower	Upper
1.40	0/6	0						
1.43	1/6	16.7						
1.45	2/6	33.3						
1.47	3/6	50.0	1.43	1.47	1.49	1.02	1.45	1.48
1.49	4/6	66.7						
1.51	6/6	100.0						

Table 4.2. Effect of ammonia concentrations on per cent mortality in large size (7.53 g) *Labeo rohita* exposed for 96 hr. Lethal concentration, slope function and 95% confidence limits are expressed in mg l^{-1}.

Concentrations of NH_3 (mg l^{-1})	Dead / Tested	Mortality (%)	Lethal concentration (mg l^{-1})			Slope function (S)	95% confidence limit	
			16%	50%	84%		Lower	Upper
1.40	0/6	0						
1.47	1/6	16.7						
1.59	2/6	33.3						
1.68	3/6	50.0	1.36	1.63	1.74	1.13	1.43	1.76
1.76	5/6	83.3						
1.80	6/6	100.0						

Table 4.3. The LC_{50} values of different types of cultured fishes exposed to ammonia.

	LC_{50} level (mg l^{-1})	**hr**	**References**
Oreochromis niloticus fry	1.01	48	Karasu Benli (2005)
Oreochromis niloticus fingerlings	7.40	48	Karasu Benli (2005)
Oreochromis niloticus	0.98	96	Evans *et al.* (2006)
Tilapia aurea	2.40	48	Redner and Stickney (1979)
Cirrhinus mrigala fingerlings	0.055	24	Zafar Iqbal *et al.* (2013)
Ictalurus punctatus	0.50	96	Tomasso *et al.* (1980)
Hybrid striped bass Morone chrysops X *M. saxatilis*	0.40	96	Harcke and Daniels (1999)

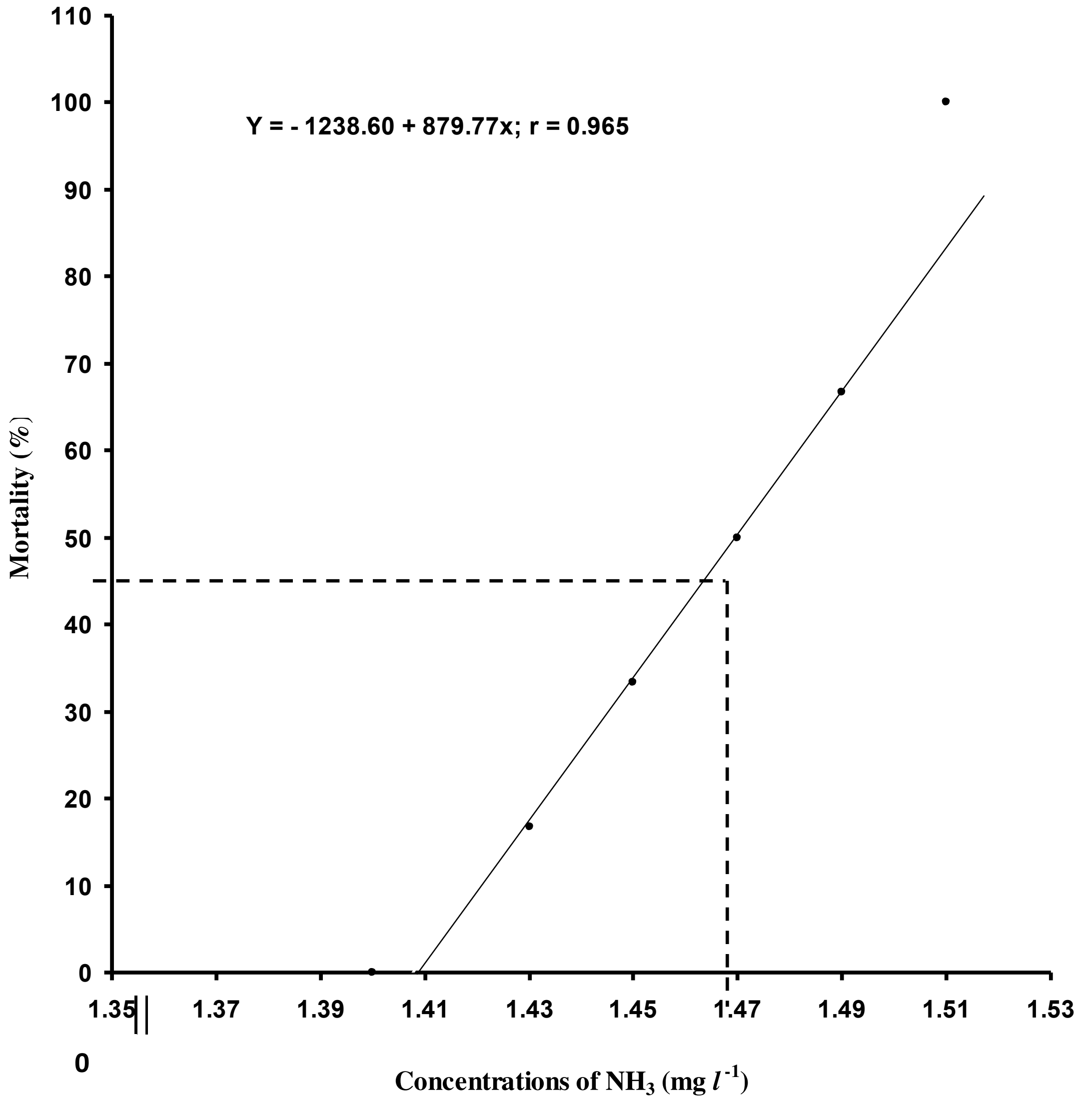

Fig. 4.1. Effect of ammonia concentrations on mortality (%) in small size *Labeo rohita* exposed for 96 hr.

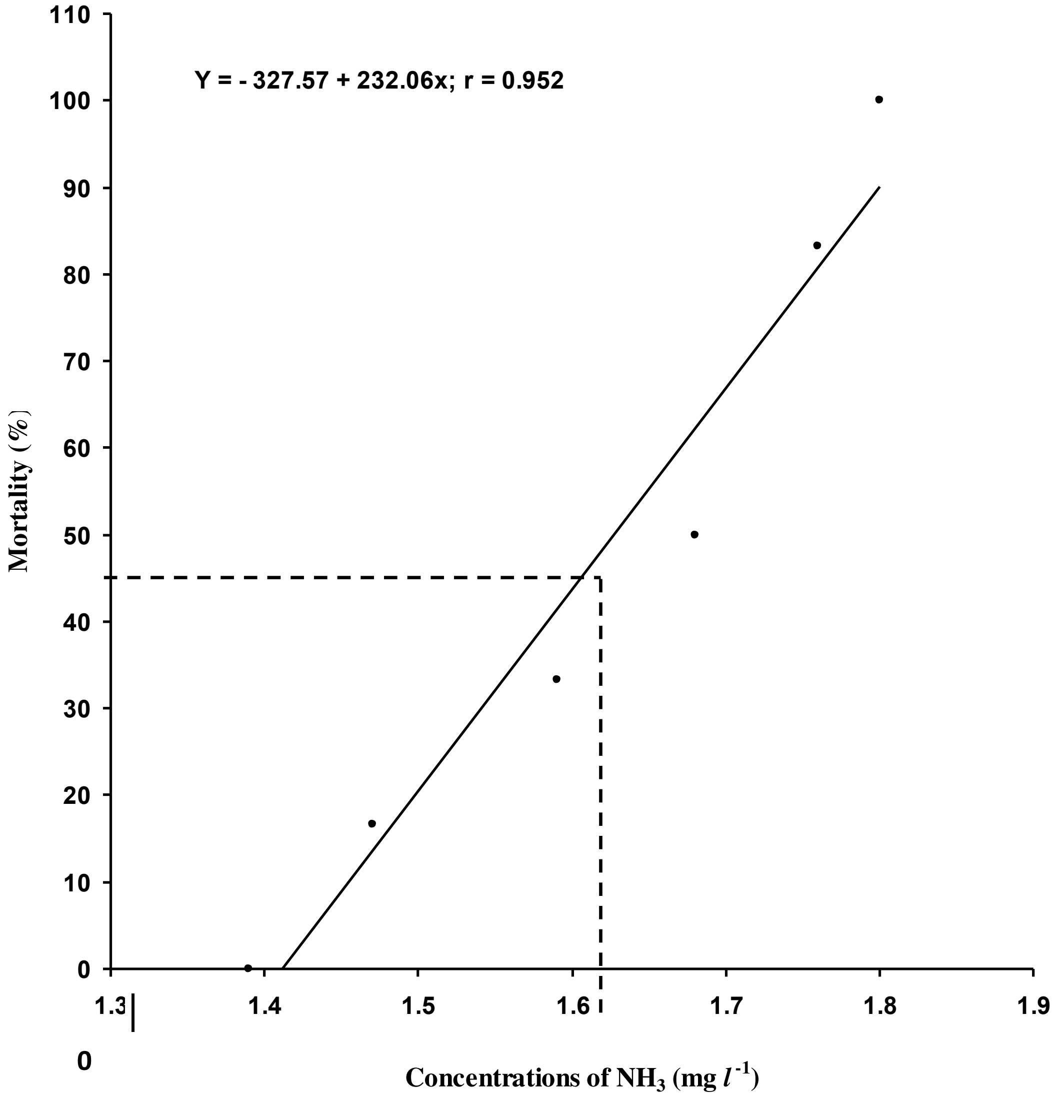

Fig. 4.2. Effect of ammonia concentrations on mortality (%) in large size *Labeo rohita* exposed for 96 hr.

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