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ACUTE TOXICITY OF AMMONIA ON *Macrobrachium tenellum* (SMITH) LARVAE

Gerardo FIGUEROA-LUCERO[1], María Cecilia HERNÁNDEZ-RUBIO[2]* y
Miguel de Jesús GUTIÉRREZ-LADRÓN DE GUEVARA[2]

[1] Planta Experimental de Producción Acuícola. Departamento de Hidrobiología. DCBS. Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186, Colonia Vicentina. C. P. 09340. México, D. F.
[2] Laboratorio de Hidrobiología Experimental, Depto. de Zoología, Escuela Nacional de Ciencias Biológicas, IPN. Prol. M. Carpio esq. Plan de Ayala s/n. Col. Sto. Tomás. 11340. México, D.F. Apdo. Postal 4-132. 06400. México
*Corresponding author; cecheru@yahoo.com.mx

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ABSTRACT

The prawn shrimp *Macrobrachium tenellum* is a potential species for culture in México. The effect of ammonia on larvae was evaluated to provide basic information on safe levels for larviculture. A 72 h static assay was performed on 5 days old *M. tenellum* larvae. The nominal concentrations tested ranged from 2.89 to 185.48 mg NH_4-N/L which represent 0.103 to 6.585 mg NH_3-N/L at 20 g/L salinity, 28 ºC and pH 7.79. LC_{50} for 12, 24, 48 and 72 h were 2.939 ± 0.505, 0.749 ± 0.301, 0.477 ± 0.163 and 0.409 ± 0.068 mg NH_3-N/L, respectively. These results suggest that *M. tenellum* exhibits a slightly higher tolerance to ammonia in the zoea stage when compared to most of the prawn and shrimp species.

Palabras clave: camarón de río, langostino, zoea, tolerancia

RESUMEN

El langostino *Macrobrachium tenellum* es una especie potencial para cultivo, en México. El efecto del amonio en larvas se evaluó para proveer información básica de los niveles seguros para el cultivo en esta etapa de desarrollo. Un ensayo estático de 72 h se realizó con larvas de *M. tenellum* de cinco días de edad. Las concentraciones nominales probadas fueron desde 2.89 a 185 mg NH_4-N/L, que equivalen a 0.103 hasta 6.585 mg NH_3-N/L a 20 g/L de salinidad, 28 ºC y pH 7.79. Las LC_{50} a 12, 24, 48 y 72 h fueron 2.939 ± 0.505, 0.749 ± 0.301, 0.477 ± 0.163 y 0.409 ± 0.068 mg NH_3-N/L, respectivamente. Estos resultados sugieren que *M. tenellum* presenta una tolerancia ligeramente más alta al amonio en el estado de zoea que otras especies de langostinos y camarones.

INTRODUCTION

Macrobrachium tenellum (Smith) is a freshwater prawn, from the Pacific coast rivers of America. In Mexico, it is a commercially important resource, particularly in the state of Guerrero, and it is considered suitable for mass culture (Guzmán 1987) even through the capture and growth of wild postlarvae (Martínez *et al*. 1980).

Ammonia is the main excretory product in crustaceans (Hartenstein 1980, Cavalli *et al*. 2000). This is an end product of amino acid catabolism originated from excretion and organic matter decomposition. Crustaceans excrete 60-70 % of nitrogen as ammonia through their gills through passive diffusion and the rest is made up of small amounts of ammonic acid, urea and uric acid (Chen and Kou 1996). High ammonia concentrations in tanks stocked in high densities of larvae is a potential danger to aquatic organisms due to high toxicity (Chin and Chen 1987, Ostrensky and Wasielesky 1995), which may cause death or slow down prawn growth rate at sublethal levels (Wickins 1976, Armstrong *et al*. 1978, Daniels *et al*. 1992, Miranda-Filho *et al.* 2009). In aqueous solution, ammonia can be present in ionized (NH_4^+) and/or unionized (NH_3) form, condition that is pH, temperature and salinity dependent.

In crustaceans, elevated ammonium concentration might produce hemolymph alcalinization as a consequence of increased internal concentration of ammonium (Campbell 1973, Chen and Kou 1993, Chen and Lin 1995, Mugnier and Justou 2004). Other reported effects are respiratory inhibition (Alcaraz *et al*. 1999, Malassen and Valenti 2005), reduction of osmoregulatory capacity (Young-Lai *et al*. 1991, Lin *et al*. 1993, Mugnier and Justou 2004) and reduction of survival (Mallasen and Valenti 2005, Naqvi *et al*. 2007, Schuler *et al*. 2010, Barbieri 2010, Liao *et al*. 2011).

In animals with gills, sensitivity to ammonia is greater during the early developmental stages, because gill surface ratio to body weight is bigger and also because the physiological detoxifying mechanisms are still immature (Rand and Petrocelli 1985).

By understanding tolerances of *Macrobrachium tenellum* (Smith) to ammonia its culture system can be improved to optimized survival. In this study LC_{50} values were obtained for larvae at various exposure times, to increase our knowledge about the water quality requirements of this species for aquaculture systems.

MATERIALS AND METHODS

A static bioassay was performed to assess the acute toxicity of ammonia (LC_{50} values) on *M. tenellum* larvae over a period of 72 h, with toxic renewal. The experiment was designed to assess the effect of different concentrations of ammonia on survival of larvae. The different concentrations of ammonia were obtained by first making a stock solution of reagent grade ammonium chloride (NH_4Cl, Baker™). Test concentrations of ammonia were then made up as total ammonia nitrogen (TAN) by measuring a specified quantity of NH_4Cl, dissolving it in culture water in a volumetric flask and then making up the solution with more culture water to 5 L in a plastic container. Stock solutions with measured ammonia concentrations were then further diluted with brackish water (20 g/L, Reefsalt™ of Seachem™) according to the individual concentrations required for each treatment. Concentrations tested ranging from 2.89 to 185.48 mg NH_4-N/L (0.103, 0.206, 0.412, 0.823, 1.540, 3.292, 6.585 mg NH_3-N/L). Ammonia was measured using a Hach Model DR-2000 spectrophotometer (Hach Company, Ames, Iowa, USA). The concentrations of unionized ammonia (NH_3-N) were calculated according to the equations of Thurston, Khoo and Whitfield modified by Boueres (2001) based on salinity 20 g/L, water temperature 28 ºC and pH 7.8. Each treatment was stocked with five days old larvae, at zoea III stage, obtained from a single ovigerous *M. tenellum* female reared in laboratory ponds. Three replicates of ten larvae each were used for treatment. For the assay, larvae were placed in 250 mL beakers containing 150 mL of test solution without aeration. Salinity was 20 g/L, pH 7.79 and temperature 28 ºC. Larvae were fed on *Artemia* nauplii before and during the experiment. Food debris was removed from the beakers daily to prevent decomposition. Test solutions in the beakers was completely replaced every 24 h with new solution prepared fresh each day

Mortality of larvae was recorded after 1, 2, 3, 6, 12, 24, 48 and 72 h of exposure, following the parameters established by Armstrong *et al*. (1976), considering ceasing of the heartbeat as death sign for the first 24 hours, and opacity and lack of movement after 24 h.

Statistical analysis

One-way ANOVA was used to investigate the effect of ammonia concentration on survival and comparisons amongst means were made using T post hoc test (Sokal and Rholf 1981).

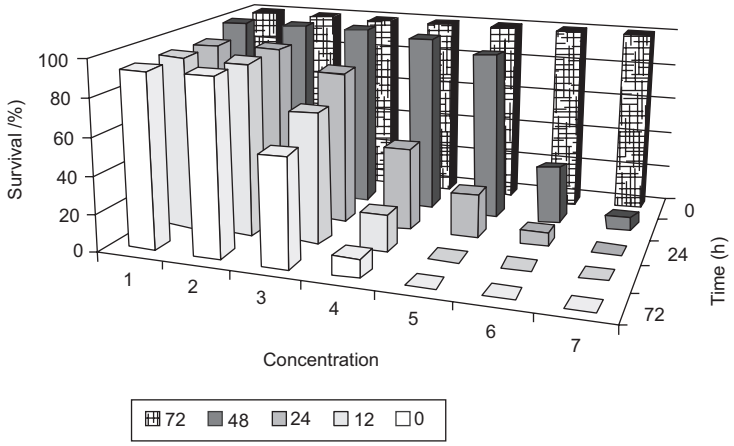

Fig. 1. Average survival for larvae in stage III, during the 72 h exposition period to different concentrations of total amonia, at pH 7.79. 1: 2.898 (0.103), 2: 5.796 (0.206), 3: 11.593 (0.412), 4: 23.185 (0.823), 5: 43.37 (1.540), 6: 92.74 (3.292), 7: 185.48 (6.585) mg/L NH_4-N (NH_3-N)

The reported LC_{50} values and 95% confidence limits were obtained on the statistical software EPA Probit Analysis Program ver. 1.5.

RESULTS

There was no mortality in control treatments during the experiment. Neither was observed any deaths, during the first six hours of exposure for all ammonia concentrations. All larvae exposed to 5.796 mg NH_4-N/L (0.206 mg NH_3-N/L) and 11.593 mg NH_4-N/L (0.412 mg NH_3-N/L) survived for 24 and 12 h, respectively.

However, exposure to total ammonia had a significant effect (ANOVA, $P< 0.01$) on larvae survival, causing mortality in concentrations as low as 2.898 mg NH_4-N/L (0.103 mg NH_3-N/L) at 48 h (**Fig. 1**).

A mortality of 100 % was observed at concentrations of 43.37 mg NH_4-N/L (1.540 mg NH_3-N/L) and 92.74 mg NH_4-N/L (3.292 mg NH_3-N/L) after 48 h. and in 185.48 mg NH_4-N/L (6.585 mg NH_3-N/L) at 24 h (**Table I**).

The LC_{50} values obtained decreased with increasing exposure time, from 75.95 mg NH_4-N/L (2939 mg NH_3-N/L) for 12 h, 23.98 mg NH_4-N/L (0.749 mg NH_3-N/L) for 24 h, 14.25 mg NH4-N/L (0.477 mg NH_3-N/L) for 48 h, and 12.66 mg NH_4-N/L (0.409 mg NH_3-N/L) for 72 h exposure (**Table II**).

TABLE I. PERCENT MORTALITY OF *M. tenellum* LARVAE EXPOSED TO DIFFERENT AMMONIA-N AND NH_3-N CONCENTRATIONS, AT DIFFERENT EXPOSURE TIME AND 20 g/L OF SALINITY

NH_4^+-N (mg/L)	NH_3-N (mg/L)	Exposure time (h)			
		12	24	48	72
2.898	0.103	0.0	0.0	6.6	6.6
5.796	0.206	0.0	0.0	6.6	6.6
11.593	0.412	0.0	16.6	30	43
23.185	0.823	3.3	56.6	80	90
43.37	1.540	10	86.6	100	100
92.74	3.292	70	93.3		
185.48	6.585	83.3	100		

TABLE II. ESTIMATED LC_{50} (mg/L) VALUES AND THEIR 95 % CONFIDENCE LIMITS FOR NH_3-N

h		Conc. NH_3-N (mg/L)	95% Confidence limits	
			Lower	Upper
12	LC_{50}	2.939	2.434	3.603
24	LC_{50}	0.749	0.448	1.252
48	LC_{50}	0.477	0.314	0.715
72	LC_{50}	0.409	0.341	0.490

DISCUSSION

Previous studies have shown that ionized and unionized ammonia toxicity varies with water pH (Armstrong *et*. 1978), with development stage (Neil *et al*. 2005), and among decapods species (Allan *et al*. 1990), but also with temperature, salinity, atmospheric pressure and dissolved oxygen (Allan *et al*. 1990).

In ammonia toxicity assays with fishes, toxic concentrations are expressed as unionized ammonia only. Nevertheless, it has been shown that both forms of ammonia are toxic. At a higher pH, ammonia is predominantly in the unionized form and

it is responsible for the toxicity; the opposite occurs at a low pH, when NH_4 is the main form present (Armstrong *et al*. 1978). An increase in ammonia toxicity with increased pH has been reported in *Macrobrachium rosenbergii* and other crustaceans, during larval and juvenile stages (Noor-Hamid *et al*. 1994, Mallasen and Valenti 2005, Neil *et al*. 2005).

In brackish water species, salinity exerts an important effect on ammonia internal concentration. Research experiments have shown that sodium has a lower affinity than NH_4 for the enzyme responsible for the active transport into the intracellular milieu. Apparently the Km values for Na^+ transport are tenfold higher in marine species compared to freshwater species (Shaw 1960). Barbieri (2010) observed that *Litopenaeus schmitti* juveniles experienced an increase in susceptibility to TAN up 69 % as the salinity decreased from 35 g/L to 5 g/L for 96 h exposure.

The present study was performed at 20 g/L salinity (5 166 mg Na^+/L), ammonia concentrations considered toxic ranged from 9.39 to 85.16 mg/L. The ratio of NH_4^+ to Na^+ was 0.0018-0.016: 1. For *Macrobrachium rosenbergii* larvae (Armstrong *et al*. 1978), it was determined a 0.01-0.02: 1 ratio, at 12 g/L salinity. Those results agree with our results, since an increase from 12 to 20 g/L, approximately, produces a hundredfold increase in ammonia toxicity. In freshwater decapods larvae it has been determined an inverse relationship, Shaw (1960) obtained a ratio of 10:1.

Several toxic effects of ammonia on crustacean decapod larvae and adults have been reported. For *M. rosenbergii* larvae, development slowed down and mortality rate increased in alkaline water (pH 9) with increasing ammonia concentration and larval tolerance to high ammonia and pH levels decreased for the last zoeal stages (Mallasen and Valenti 2005). Ammonia stress has been associated with decreased haemolymph osmotic concentrations in *Penaeus japonicus* (Chen and Chen 1996), changes in nitrogenous excretion in *M. rosenbergii* adult prawns (Chen and Kou 1996), decreased survival and slowed down larval development in *P. monodon* (Noor-Hamid *et al*. 1994), changes in oxygen consumption (Alcaraz *et al*. 1999, Barbieri 2010) and decreased growth (Armstrong *et al*. 1978, Chen and Kou 1992).

Recent studies with other decapods species report that tolerance to ammonia decreases for the last zoeal stages or even in later development stages (Mallasen and Valenti 2005), but the opposite has also been found (Chin and Chen 1987, Ostrensky and Wasielesky 1995). In this experiment the tolerance to ammonia concentrations was tested on zoea III larvae in order to control for other environmental factors, i. e., larval nutritional status or damage associated to larvae handling during the rearing period.

These results suggest that *M. tenellum* exhibits a slightly higher tolerance to ammonia in the zoea stage when compared to most of the prawn and shrimp species complied in Ostrensky and Wasielesky (1995).

Sprague (1969, 1971) pointed out the effects of a given toxicant could be described in terms of "safe level", that can be obtained using an application factor of 0.1. According to our results, safe level would be below 0.6 mg/L for TAN and 0.075 NH_3-N/L on the basis of the 24 h LC_{50} value at pH 7.79 and 20 g/L salinity for *Macrobrachium tenellum* larval rearing under controlled conditions. The results suggest TAN and unionized ammonia must be daily measured since a little increase, combined with an increase pH, could result in a high mortality.

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