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Ammonia tolerance of *Litopenaeus vannamei* (Boone) larvae

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Abstract

The tolerance of *Litopenaeus vannamei* larvae to increasing concentrations of total ammonia nitrogen (TAN) using a short-term static renewal method at 26°C, 34 g L^{-1} salinity and pH 8.5 was assessed. The median lethal concentration (24 h LC_{50}) for TAN in zoea (1-2-3), mysis (1-2-3) and postlarvae 1 were, respectively, 4.2-9.9-16.0; 19.0-17.3-17.5 and 13.2 mg L^{-1}TAN (0.6-1.5-2.4; 2.8-2.5-2.6 and 1.9 mg L^{-1} NH_3-N). The LC_{50} values obtained in this study suggest that zoeal and postlarval stages are more sensitive to 24 h ammonia exposure than the mysis stage of *L. vannamei* larvae. On the basis of the ammonia toxicity level (24 h LC_{50}) at zoea 1, we recommend that this level does not exceed 0.42 mg L^{-1} TAN – equivalent to 0.06 mg L^{-1} NH_3-N – to reduce ammonia toxicity during the rearing of *L. vannamei* larvae.

Keywords: *Litopenaeus vannamei* (Boone), ammonia, toxicity, larval stages

Introduction

Currently, the Pacific whiteleg shrimp *Litopenaeus vannamei* (Boone) is the most important cultivated penaeid shrimp species in the world. Semi-intensive and intensive shrimp culture systems are a common activity in many countries. However, these culture practices usually result in degradation of the culture water by uneaten food and waste products of the shrimps. Thus, water quality management and knowledge of water quality requirements are essential to any culture system.

One of the most important limiting factors in intensive culture systems is the build-up of toxic nitrogenous waste. Ammonia is the main end-product of protein catabolism in crustaceans and can account for 60–70% of nitrogen excretion with only small amounts of amino acids, urea and uric acid (Chen & Kou 1996a,b). Of the nitrogenous compounds, ammonia is the most toxic with nitrite and nitrate being less toxic to shrimp (Chen, Liu & Lei 1990). In water, ammonia is present in both ionized (NH_4^+) and un-ionized (NH_3) state, with NH_3 as the toxic form due to its ability to diffuse across cell membranes (Fromm & Gillete 1968; Emerson, Russo, Lund & Thurston 1975). The relative proportions of NH_3 and NH_4^+ depend on pH, temperature and to a lesser extent on salinity (Bower & Bidwell 1978).

In penaeid shrimp, high concentrations of ammonia may affect growth rates and survival, and can in extreme cases cause mortality (Wickins 1976; Zin & Chu 1991; Chen & Lin 1992). Ammonia damages the gills and reduces the ability of haemolymph to transport oxygen while increasing oxygen consumption by tissues (Chien 1992; Racotta & Hernández-Herrera 2000). Osmoregulatory capacity decreases with increasing ammonia concentration and exposure time (Lin, Thuet, Trilles, Mounet-Guillaume & Charmantier 1993). Ammonia may also increase the moulting frequency of shrimps (Chen & Kou 1992).

Several studies have been carried out to determine toxicity levels of ammonia in different life stages of penaeid shrimp, such as *Penaeus monodon* (Fabricius), *Marsupenaeus japonicus* (Bate), *Farfantepenaeus paulensis* (Peréz-Farfante), *Fenneropenaeus*

chinensis (Osbeck) and *Litopenaeus setiferus* (Burkenroad) (Chin & Chen 1987; Chen & Lin 1991a, b; Lin *et al.* 1993; Ostrensky & Wasielesky 1995; Alcaraz, Chiappa-Carrara, Espinoza & Vanegas 1999). In *L. vannamei,* studies on the acute toxicity of ammonia have been conducted on juveniles (Frías-Espericueta, Harfush-Melendez, Osuna-López & Páez-Osuna 1999; Lin & Chen 2001) and on post-larval stages (Magallón-Barajas, Servín, Portilllo, García & López 2006), but so far not on larval stages. However, larviculture is a process of primary importance from which qualified larvae must be produced to obtain maximum yields during post-larval, nursery and grow-out culture. In traditional larviculture systems, water quality can rapidly degrade leading to increased mortality and a higher incidence of diseases, particularly in high density cultures. Therefore, the objective of this study was to determine the acute toxicity of ammonia in the larval stages of *L. vannamei*, contributing to the optimization of water quality management.

Materials and methods

Experimental animals and larval rearing conditions

Nauplii 5 (N5) of *L. vannamei* were obtained from EGIDIOSA hatchery (San Pablo, Santa Elena Province, Ecuador) and transported to the research facilities of ESPOL-CENAIM Foundation. The nauplii were disinfected with 100 ppm of ARGENTYNE® (Argent, Redmond, WA, USA) for 1 min and acclimatized to the experimental conditions. N5 were stocked at 200 N5 L^{-1} in 4–500 L round-shaped fibreglass tanks filled with sand-filtered and UV-treated seawater of 34 g L^{-1} and a pH of 8.1. Temperature was maintained at 28 ± 1°C. Dissolved oxygen concentration was kept above 4 ppm. Larvae were fed with the microalgae *Chaetoceros gracilis* from N5 until PL1 (1-day postlarvae). From zoea 2 (Z2) until PL1, also rotifers (*Brachionus rotundiformis*), frozen and live *Artemia* nauplii and artificial food EPIFEED-LHF® (Epicore, Eastampton, NJ, USA), FRIPPAK® (Inve Aquaculture, Dendermonde, SA, Belgium) and LARVA Z PLUS® (Zeigler, Gardners, PA, USA) were supplied.

Experimental design and toxicity tests

Short-term toxicity tests (24 h) were carried out to determine the acute total ammonia toxicity levels according to the method described by APHA, AWWA, WPCF (1992). Test solutions of ammonia were prepared by dissolving 9.5518 g of Ammonium Chloride (NH_4Cl) in 250 mL distilled water to make 10 000 mg L^{-1} stock solution and then diluting it to the desired concentration. Ammonia stock solution was added with an automatic pipette (1–10 mL) directly to a series of 2-L glass beakers filled with 1 L of UV-treated seawater of 34 g L^{-1} salinity. Flasks were placed into a container filled halfway with water. The water bath was heated to 26 ± 1°C. Before the start of the actual toxicity tests, range-finding tests were carried out to define concentrations for the final toxicity test. The total ammonia nitrogen (TAN) nominal concentrations used for every larval substage are presented in Table 1. Water from two test flasks was sampled to determine actual concentration of the testing solutions. Ammonia analysis was performed according to the method described by Solórzano (1969). For every bioassay, pH of the seawater was adjusted to 8.5 with a 2 N sodium hydroxide (NaOH) solution. Groups of 20 larvae at each larval stage were taken randomly from the culture tank and transferred to the experimental units, once acclimatized for temperature. All treatment concentrations had five replicates. Larvae were not fed and the water was not renewed during the 24-h test, following the procedure of Buikema, Niederlehner and Cairns (1982). Mortality was checked after 24 h of exposure. Death was assumed when the larvae became immobile and showed no response. Based on the mortality, the 24-h Median Lethal Concentration (LC_{50}) and its 95% confidence intervals were cal-

Table 1 Nominal concentrations (mg L^{-1}) of total ammonia nitrogen and ammonia NH_3-N used for toxicity tests of *Litopenaeus vannamei* (Boone) larval stages

Larval stages and sub-stages	Nominal concentration of TAN (mg L^{-1})	Nominal concentration of NH_3-N (mg L^{-1})
Zoea 1, 2, 3	5.0, 7.5, 10.0, 12.5, 15.0, 20.0	0.7, 1.1, 1.5, 1.8, 2.2, 2.9
Mysis 1, 2, 3 and PL 1	5.0, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0	0.7, 1.5, 1.8, 2.2, 2.9, 3.7,4.4

culated using a Logistic regression using a JMP Statistical Package Program. The toxicity of un-ionized ammonia (NH_3-N) was calculated according to the equations proposed by Whitfield (1974), based on a salinity of 34 g L^{-1}, a temperature of 26 ± 1°C and a pH of 8.5. The recommended levels to rear *L. vannamei* larvae were calculated using one-tenth of the 24-h LC_{50} values.

Results

Survival of *L. vannamei* larvae exposed to increasing concentrations of TAN at the different larval stages zoea 1 (Z1) to PL1 for 24 h are presented in Fig.1. High survival (80–100%) was observed in the control group. Z1 showed lower survival than the control from the lowest concentration tested (5 mg L^{-1} TAN), whereas an increased tolerance to ammonia was observed for Z2 and zoea 3 (Z3) sub-stages. Survivals above 50% were obtained for TAN concentrations of 10 and 15 mg L^{-1} for Z2 and Z3 respectively. Ammonia tolerance increased with larval developmental stages, particularly at mysis, which displayed the highest tolerance with survivals up to 70% for 15 mg L^{-1} TAN. No variation was observed within mysis sub-stages. The increased ammonia resistance observed with larval development did not extend to the PL1 stage, which showed a survival of 60% at 12.5 mg L^{-1} TAN.

LC_{50} values for TAN and their 95% confidence intervals for *L. vannamei* larval stages are shown in Table 2. The 24-h LC_{50} for Z1 was 4.2 mg L^{-1} TAN (0.6 mg L^{-1} NH_3-N); this increased with larval development to 9.9 and 16.0 mg L^{-1} TAN (1.5 and 2.4 mg L^{-1} NH_3-N) for Z2 and Z3 respectively. Higher TAN LC_{50} were obtained for mysis sub-stages, being 19.0, 17.3 and 17.5 mg L^{-1} TAN equivalent to 2.8, 2.5 and 2.6 mg L^{-1} of un-ionized NH_3-N for mysis 1, mysis 2 and mysis 3, respectively. The 24-h LC_{50} for postlarvae 1 was 13.2 mg L^{-1} TAN and 1.9 mg L^{-1} NH_3-N. The recommended levels for rearing zoea stages were estimated to be between 0.4 and 1.6 mg L^{-1} TAN (0.06–0.24 mg L^{-1} NH_3-N). Mysis recommended levels were estimated to be from 1.73 to 1.90 mg L^{-1} TAN (0.25–0.28 mg L^{-1} NH_3-N) and for PL1, values of 1.32 and 0.19 mg L^{-1} for TAN and NH_3-N were estimated respectively.

Discussion

Several researchers have examined ammonia toxicity in various species of penaeid shrimp and at different developmental stages, especially for juveniles. Chen and Lei (1990) determined that for *P. monodon* juvenile, toxicity of ammonia decreased with exposure time. Chen and Lin (1992) observed an increased susceptibility to ammonia as salinity decreased from 30 to 10 g L^{-1} in *F. chinensis* juveniles. For *Penaeus semisulcatus* (De Haan) juveniles exposed to different concentrations of ammonia-N in a series of acute toxicity tests at four different water temperatures, Kir, Kumlu and Erodolgan (2004) found that lower temperatures clearly increased tolerance of the shrimp to ammonia. Growth rates of *M. japonicus* juveniles

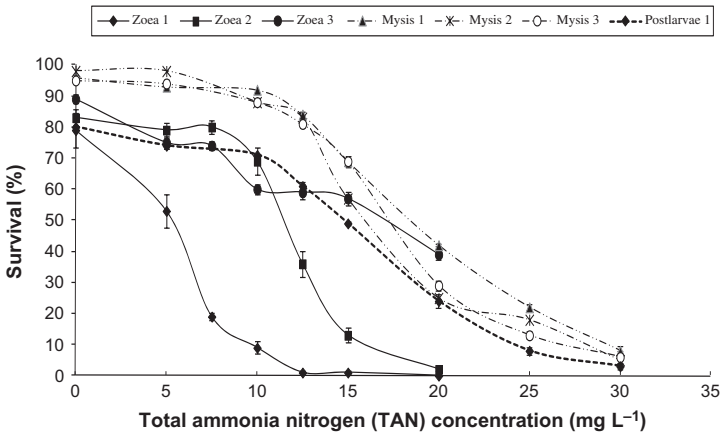

Figure 1 Survival of *Litopenaeus vannamei* (Boone) larval stages from zoea 1 to postlarvae 1 exposed for 24 h to increasing concentrations (mg L^{-1}) of total ammonia nitrogen

Table 2 The 24 h-LC_{50} values (mg L^{-1}) of total ammonia nitrogen (NH_3-N + NH_4^+-N) and NH_3-N for *Litopenaeus vannamei* (Boone) larval stages and sub-stages. 95% confidence intervals are shown between parentheses

Larval stages and sub-stages	24-h LC_{50} TAN	24-h LC_{50} NH_3-N
Zoea 1	4.2 (3.5–4.8)	0.6 (0.5–0.7)
Zoea 2	9.9 (9.2–10.7)	1.5 (1.4–1.6)
Zoea 3	16.0 (14.3–18.4)	2.4 (2.1–2.7)
Mysis 1	19.0 (18.1–20.0)	2.8 (2.7–2.9)
Mysis 2	17.3 (16.5–18.1)	2.5 (2.4–2.7)
Mysis 3	17.5 (16.7–18.4)	2.6 (2.5–2.7)
Postlarvae 1	13.2 (12.1–14.2)	1.9 (1.8–2.1)

exposed to different ammonia concentrations were investigated by Chen and Kou (1992). The authors concluded that ammonia had a stronger effect on weight rather than length. Studies in *L. vannamei* juveniles have been conducted to evaluate acute toxicity levels of ammonia (Frías-Espericueta *et al.* 1999) and at different salinity levels (15–35 g L^{-1}) (Lin & Chen 2001). Racotta and Hernández-Herrera (2000) evaluated the metabolic responses of ammonia exposure in *L. vannamei* juveniles, while Magallón-Barajas *et al.* (2006) determined the daily variations in short-term ammonia toxicity in *L. vannamei* postlarvae (1–30 days old).

Compared to juveniles, scarce information is available about acute toxicity levels of ammonia in larval stages of penaeid shrimp. In Table 3, the 24-h LC_{50} of un-ionized ammonia on several penaeid species at larval stages are given.

A progressive tolerance to ammonia with larval development was observed for *P. monodon* (Chin & Chen 1987), *M. japonicus* (Lin *et al.* 1993) and *F. chinensis* (Chen & Lin 1991b), concluding that zoea stages were the least tolerant and postlarvae the most tolerant to ammonia. In contrast, our results from short-term toxicity tests in *L. vannamei* revealed that mysis stage was considerably more resistant to ammonia than the other stages, coinciding with the findings of Ostrensky and Wasielesky (1995) for *F. paulensis* larvae.

The LC_{50} values for un-ionized ammonia in *L.vannamei* zoea and mysis stages were higher than those reported for *P. monodon, M. japonicus and F. chinensis* (Chin & Chen 1987; Chen & Lin 1991b; Lin *et al.* 1993; respectively), suggesting that *L. vannamei* is more resistant to ammonia at these stages. Similar 24-h LC_{50} values for ammonia through the larval stages were obtained in this study compared to those reported for *F. paulensis* larvae (Ostrensky & Wasielesky 1995), and moreover the same pronounced decrease in ammonia tolerance in the post-larval stage was also observed. During the post-larval stage, the final and most important metamorphosis occurs where the larvae become juvenile shrimp. This could explain the lower ammonia tolerance.

Increase in pH levels favour the formation of the more toxic un-ionized form of ammonia or enhance the toxic effects (Colt & Armstrong 1981). Chen and Sheu (1990 a,b) reported that if the pH level gets higher than 8.2, increasing pH in a given ammonia solution could increase ammonia toxicity to *P. monodon* M2 sub-stage and *M. japonicus* postlarvae. Similar findings were observed by Chen and Chin (1989), when studying the effect of ammonia at different pH levels in *P. monodon* postlarvae. The median lethal time (LT_{50}) values showed that ammonia toxicity to *P. monodon* postlarvae increased as pH increased. Bower and Bidwell (1978) stated that the fraction of NH_3 does not only depend on pH but also on temperature and to a lesser extent salinity. As

Table 3 The 24-h LC_{50} (mg L^{-1}) of un-ionized ammonia (NH_3-N) in several penaeid species for early larval stages

	Larval stages			Test conditions			
Penaeid species	Zoea	Mysis	Postlarvae (PL days old)	pH	Temperature (°C)	Salinity (g L^{-1})	Reference
Penaeus monodon	0.76	2.17	4.70 (PL6)	8.2	29.5	34	Chin and Chen (1987)
Marsupenaeus japonicus	0.97	1.08	1.98 (PL1)	7.9	25.0	36	Lin *et al.* (1993)
Fenneropenaeus chinensis	0.65	0.9	1.30 (PL4)	8.2	*	34	Chen and Lin (1991a,b)
Farfantepenaeus paulensis	1.79	2.91	1.40 (PL4)	†	25.0	28	Ostrensky and Wasielesky (1995)
Litopenaeus vannamei	1.47	2.63	1,94 (PL1)	8.5	26.0	34	This study

*Temperature for each larval stage: zoea (21°C); mysis (23°C); postlarvae (25°C).
†pH for each larval stage: zoea (8.24); mysis (7.90); postlarvae (8.1).

pH and temperature rises, proportion of NH_3 to NH_4^+ increases and the toxicity of ammonia to animals increases. Magallón-Barajas *et al.* (2006) investigated the daily variations in short-term (4 h) toxicity of ammonia to *L. vannamei* postlarvae (1–30 days old) exposed to several ammonia concentrations in two different scenarios, scenario 1 (pH 8, 26°C) and scenario 2 (pH 9, 30°C). In scenario 1, ammonia concentrations up to 18 mg L^{-1} did not provoke mortality, whereas for scenario 2, mortality was recorded at all post-larval ages. In this study, the pH was higher than scenario 1 resulting in a higher survival; this suggests that *L. vannamei* larvae are resistant to ammonia under this water conditions.

The mean recommended levels calculated in this study for rearing *L. vannamei* larvae were 0.15, 0.26 and 0.19 mg L^{-1} NH_3-N for zoea, mysis and post-larval stages, respectively, based on pH 8.5, temperature 26°C and salinity 34 g L^{-1}. A similar value was observed by Chin and Chen (1987) for the 'safe level' (0.10 mg L^{-1} NH_3-N) for *P. monodon* larvae; however, they calculated this value from the 96-h LC_{50} value for postlarvae with the application factor of 0.1 (Sprague 1971), and based on pH 8.2, temperature 29.5°C and salinity of 34 g L^{-1}. Ostrensky and Wasielesky (1995) stated that the 'safe level' for rearing *F. paulensis* in the hatchery was estimated to be 0.03 mg L^{-1} NH_3-N, a value 5 to almost 10 times lower than the one obtained in our study. They calculated the 'safe level' using the 24-h LC_{50} of embryos (0.30 mg L^{-1} NH_3-N) for rearing larvae, based on pH 8.1, temperature of 25°C and salinity of 28 g L^{-1}. A comparison of 'safe level' between penaeid species becomes difficult as different methodologies, test conditions, time exposure and larval stages were used among related studies.

In conclusion, our results suggest that *L. vannamei* is a resistant species to ammonia at larval stages. The results obtained in this study could be used as a baseline for future studies and would be helpful in water quality management protocols of *L. vannamei* hatcheries.

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