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Effects of Ammonia on Mortality and Feeding of Postlarvae Shrimp *Litopenaeus vannamei*

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In all aquatic systems, the toxicity of excreted nitrogen compounds is the most limiting parameter once adequate dissolved oxygen levels are maintained (Colt and Armstrong 1981). Ammonia is the main nitrogenous product excreted by crustaceans (Dall et al. 1990), and additionally is produced from the ammonification of organic matter in a culture system (Chen et al. 1990). In an aqueous ammonia solution, total ammonia comprises unionized ammonia (NH_3) and ionized ammonia (NFL_4^+) in equilibrium (Bower and Bidwell 1978). NH; is usually toxic, since it has a high lipid solubility and is able to diffuse quite readily across cell membranes (Chen and Kou 1993) NH_4^+ is also toxic, especially at low pH levels (Allan et al. 1990).

The whiteleg shrimp *Litopenaeus vannamei* is a tropical species that is geographically distributed from Sonora, Mexico, to northern Peru. This decapod is cultured in extensive, intensive, and semi-intensive systems and is, with *Litopenaeus stylirostris,* the most popular shrimp for aquaculture in Mexico and Central and South America countries. The rearing of *L. vannamei* in closed systems results in metabolic wastes (as ammonia) accumulating in the ponds, even with frequent water replacement. The accumulation of ammonia can cause mortality (Chen et al. 1990) and/or cessation of feeding in shrimp. This paper provides information on the mortality and feeding of *L. vannamei* postlarvae exposed at different ammonia levels under laboratory conditions.

MATERIALS AND METHODS

Postlarvae (PL9) were obtained from a larvae production laboratory located at Mazatlán (Mexico), transported to the experimental aquarium, and acclimated for two days before use. During acclimation the postlarvae were fed *Artemia* sp. nauplii. The mean weight of postlarvae was 9.25 ± 4 mg. Seawater used in the bioassays was pumped from the Mazatlan Bay and was filtered through a sand and gravel bed, one cartridge system of 1.1 and 0.8 µm, and finally treated with ultraviolet irradiation. Chemical characteristics of filtered seawater have been previously published (Frías-Espericueta et al. 1999).

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Ammonia test solutions were prepared by dissolving the required amounts of ammonium chloride (Baker GR grade) in seawater. The concentrations of ammonia-N (total inorganic ammonia as nitrogen) ranged from 5 to 100 mg L^{-1}. The concentrations of NH_3-N (unionized ammonia as nitrogen) were calculated according to the equations of Bower and Bidwell (1978) based on a salinity of 34 ppt, a pH of 7.92, and a water temperature of 28°C.

Short-term (12, 24, 48, 72, and 96 hr) median lethal concentration (LC_{50}) toxicity tests were conducted following the methods described by APHA-AWWA-WPCF (1992), Chin and Chen (1987), and Chen and Chin (1989). Postlarvae were sampled randomly from the stocking tank and placed in test and control triplicate Erlenmeyer flasks for one day before bioassays. Each flask containing 500 ml of test solution and 10 test postlarvae, was aerated by an air stone with a blower, and feeding was the same as during the acclimation period. Each test solution and control were renewed daily, in accordance with a static renewal method for toxicity tests (Buikema et al. 1982), and postlarvae were not transferred to new flasks. During the experiment, in all test solutions, dissolved oxygen was 6.7 ± 0.9 mg L^{-1}. Observations and parameter measurements (pH, ammonia, and dissolved oxygen) were carried out at 12-hr intervals. Death was assumed when postlarvae were non-motile and showed no response when touched with a glass rod. Longer tests were not considered appropriate due to stress on shrimp and cannibalism (Allan et al. 1990).

LC_{50} values and their 95% confidence limits for ammonia-N were calculated with a microcomputer program based upon a method described by Finney (1971), which consists of transforming the mortality data in probit mortality. With this method the estimated probit line and results of a chi-square test for goodness of fit were determined. A z-test for the comparison of two LC_{50} values at the 5% level of significance (APHA-AWWA-WPCF 1992) were carried out.

Every 24 hr, 200 *Artemia* sp. nauplii per each test postlarvae were placed in the test solutions. Before replacement of test solution, the quantity of *Artemia* sp. was evaluated taking 5 ml of each test solution and checking the nauplii number by ml and extrapolating the total nauplii in the test solution. With this method, the quantity of food consumed by postlarvae in each test solution and control was calculated.

RESULTS AND DISCUSSION

In *L. vannamei* postlarvae (PL12) exposed to different concentrations of ammonia-N during several periods, no shrimp died in the control and 5 mg L^{-1} ammonia-N solutions after 96 hr exposure. In 10 and 15 mg L^{-1} ammonia-N mortality of 3.3 and 96.6.8% was noted, respectively, during 96 hr exposure. A 100% mortality occurred in the 20 mg L^{-1} at 48 hr of exposure, while in 30, 40 and 50 mg L^{-1} all postlarvae died after 24 hr of exposure.

According to Dall et al. (1990) penaeids molt at intervals of a few days or weeks, so it is virtually a continuous process, with morphological and physiological changes occurring almost daily. Before and immediately after molt, shrimp are more sensitive (Wajsbrot et al. 1990). No mortality was found in 5 mg L^{-1} ammonia-N solutions, but in 10 mg L^{-1} only postlarvae in ecdysis stage died. In 15 and 20 mg L^{-1} the individuals that died during the first 19 and 15 hr, respectively, were also in ecdysis stage. Later, mortalities of the postlarvae (in 15 and 20 mg L^{-1}) were not in ecdysis stage. During late proecdysis and ecdysis shrimp take water to increase their size, resulting in hydration of their tissues and an increase in the blood volume (Dal1 et al. 1990). This, consequently increased the ammonia-N level in postlarvae (haemolymph), and causes the lethal effect (Chen and Kou 1993). According to Wajsbrot et al. (1990) a minimum of 96 hr bioassay for determining toxicity levels in shrimp must be carried out, because in a shrimp population containing different molt stages, a relative high variability is expected in response to ammonia.

LC_{50} values and their 95% confidence limits (error bars) for ammonia-N and NH_3-N are shown in the Fig. 1 and table 1. A comparison between the LC_{50} (APHA-AWWA-WPCF 1992) for ammonia-N at different exposure times, was carried out, This analysis revealed significant difference (P <0.05) between 12-hr and 24-hr, and 24-hr and 48-hr. There was no significant difference (P<0.05) between 48-hr and 72-hr, and 72-hr and 96-hr LC_{50} values.

The statistical analysis showed that the probit of mortality had a positive linear relationship with ammonia-N concentration, and all values of the chi-square test were less than table values (critical value) at P<0.05 level, suggesting that the distribution of every mortality value (probit of mortality) was very close to estimated probit lines (Chen et al. 1991).

Table 1. LC_{50} values (mg L^{-1}) of ammonia-N following 24 to 96 hr exposure for several larvae penaeid species

Species	24-hr	48-hr	72-hr	96-hr	Author
P. monodon (PL6)	52.11	27.73	17.05	11.51	Chin and Chen (1987)
P. indicus (mysis)	46.01				Jayasankar and Muthu (1983)
P. japonicus (PL12)	53.37	33.85		28.89	Chen et al. (1989)
Metapenaeus ensis (PL1)	30.3	16.7			Chen et al. (1991)
P. paulensis (PL1)	24.19	8.59	5.65	5.49	Ostrensky and Wasielesky (1995)
L. vannamei (PL12)	17.9	12.5	12.2	12.2	This study

Colt and Armstrong (1981), in their review, reported that the 96-hr LC_{50} value for un-ionized ammonia ranges from 0.40 to 2.31 mg L^{-1} for crustaceans in general.

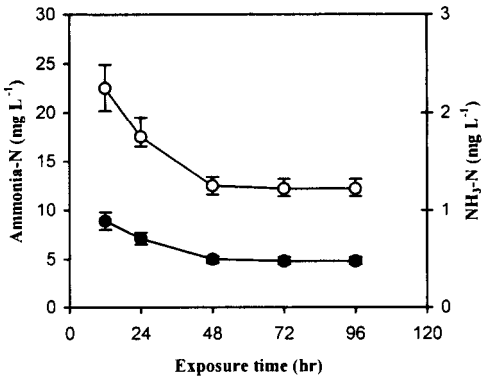

Figure 1. LC_{50} values and 95% confidence limits for ammonia-N (o) and NH_3-N (●) in *L. vannamei* postlarvae (PL12) exposed from 12 to 96 hr

Our value was 0.48 mg L^{-1}, a value within this interval. Regarding ammonia-N, Table 1 shows a comparison of the LC_{50} values for ammonia-N of several larvae penaeids. In this table, clearly at 24-hr of toxic exposure, *L. vannamei* postlarvae is more sensitive to ammonia than *P. monodon, P. indicus, Metapenaeus ensis,* and *P. paulensis.*

An acute toxicity test provides information about the relative lethality of a substance, drug or poison, but can not adequately predict a concentration that has sublethal and chronic effects over organisms; in other words, a concentration where organisms may thrive and not only survive (Buikema et al. 1982). According to Sprague (1971), a "safe" level (a concentration of pollutant which has not an adverse effect on organisms) may be obtained by multiplying a 96-hr LC_{50} value by a factor of 0.1. This factor (0.1) is statistically obtained which is derived as the result of experiment where no perceptible damage (e.g., growth, respiration, reproduction, disease) had been observed. Therefore, the "safe" level for the rearing of *L. vannamei* postlarvae would be 1.22 mg L^{-1} of ammonia-N (0.048 mg L^{-1} of NH_3-N).

Table 2 shows the quantity of *Artemia* sp. nauplii consumed in the test solutions. Clearly, a negative relation between food consumed and ammonia concentration existed in test solutions. According to Dall et al. (1990), ammonia is the major end-product of protein catabolism excreted by a decapod; and by increasing ammonia concentration in the water, ammonia excretion is reduced; consequently, the ammonia level in the blood and tissues increases with serious effects on the physiology of the shrimp. The first reaction of penaeids is the reduction or cessation of feeding to reduce the production of metabolic ammonia (Colt and Armstrong 1981), which could be a serious problem for shrimp aquaculture farming.

In addition, in Table 2, it can be observed that increasing the time of exposure to ammonia, postlarvae in ammonia-N solution (except 30 mg L^{-1}) start to feed. This observation indicates that an acclimation to ammonia occurred. Chen and Nan (1993) found that *Penaeus chinensis* juveniles are able to acclimate if are previously exposed to ammonia-N.

Our LC_{50} values were lower than those LC_{50} values by ammonia-N reported by Frías-Espericueta et al. (1999) who worked with *Litopenaeus vannamei* juveniles. This indicated that *L. vannamei* increased their tolerance to ammonia with age. In this context several authors have found that younger organisms are often more sensitive to toxicant than adults (Buikema et al. 1982; Allan et al. 1990).

Table 2. Amount of *Artemia* sp. Nauplii consumed per each *L. vannamei* postlarvae in the test solutions exposed from 12 to 96 hr

Ammonia-N (mg L^{-1})	12 hr	24 hr	48 hr	72 hr	96 hr
30	0	0	--	--	--
20	0	50	--	--	--
15	50	50	100	--	--
10	150	100	100	150	200
5	150	150	150	200	200
control	200	200	200	200	200

The "safe" level obtained here has important implications for shrimp pond management, especially in grow-out units from the Northwest coast of Mexico where Páez-Osuna et al. (1997) found ammonia levels of 0.161-0.234 mg L^{-1} ammonia-N during the dry and rainy seasons of culture. Wickins (1976) found that 0.45 mg L^{-1} NH_3-N reduced the growth of five penaeid species by 50%. Therefore, the interaction between ammonia and shrimp production is an important consideration for aquaculturists (Frías-Espericueta et al. 1999).

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