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Acute Toxicity of Ammonia to Juvenile Shrimp *Penaeus vannamei* Boone

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The whiteleg shrimp *Penaeus vannamei* Boone, is a tropical species that is geographically distributed from Sonora, Mexico, to northern Peru. This decapod and *P. stylirostris* are cultured in extensive, intensive, and semi-intensive systems and are the most popular shrimps for aquaculture in North, Central and South America. These penaeids constitute most of the shrimp that Mexico exports to the United States of America and Europe.

The rearing of marine and brackish-water organisms in closed systems results in metabolic wastes accumulating in the environment. Ammonia is the major end-product of protein catabolism excreted by fish, crustaceans, and molluscs and can increase exponentially in the hatchery and in the grow-out farm, even with frequent water replacement. The accumulation of ammonia can cause mortality of organisms reared in closed culture systems (Chen et al. 1990a). In an aqueous ammonia solution, total ammonia comprises un-ionized ammonia (NHs) and ionized ammonia (NH_4^+) in equilibrium (Bower and Bidwell 1978). The former is usually most toxic, as it has high lipid solubility and is able to diffuse readly across cell membranes; NH_4^+ is also toxic, especially at low pH levels (Allan et al. 1990). This paper provides information on the acute toxicity of ammonia to *P. vannamei* juveniles at different body weights in the laboratory.

MATERIALS AND METHODS

P. vannamei juveniles were obtained from shrimp-farming ponds (Southern Mazatlan, Sinaloa, Mexico), transported to the experimental aquarium (Allan et al. 1990; Chen et al. 1990b) and acclimated for at least a week before experiments commenced. During acclimation the shrimp were fed with commercial shrimp feed twice a day at a rate of 10% of body weight per day. Juveniles were selected at two different sizes and had average weights of 0.99 ± 0.01 and 3.8 ± 0.38 g. Seawater used in the bioassays was pumped from the Mazatlan Bay and was filtered through a sand and gravel bed (Table 1).

Ammonia test solutions were prepared by dissolving the required amounts of ammonium chloride (Baker GR grade) in seawater. The concentrations of

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ammonia-N (total inorganic ammonia as nitrogen) ranged from 20 to 100 mg L^{-1} in both experiments. The concentrations of ammonia were determined by method proposed by Solorzano (1969), while NH_3-N (un-ionized ammonia as nitrogen) concentrations were calculated according to the equations of Bower and Bidwell (1978) based on a salinity, pH, and temperature of 34, 8.08 and 26°C for juveniles of 0.99 g; and 34, 7.70, and 23°C for juveniles of 3.8 g, respectively.

Short-term (24, 48, 72, 96, 108 and 120 hr) median lethal concentration (LC_{50}) toxicity tests were conducted following the methods described by APHA-AWWA-WPCF (1992), Chen et al. (1990a), and Chen et al. (1990b). Juveniles were shipped to the laboratory from a private farm located at 20 km, and acclimated in a stocking tank (700 L) for 1 week before use. After that, juveniles were sampled randomly and placed in triplicate polyethylene tanks (30 L). Each tank contained eight shrimp (similar to shrimp in pond culture: 20 organisms/m^2), was aerated continuously, and shrimps were acclimated for three days. Each test solution and control was renewed daily in accordance with a static renewal method for toxicity testing (Buikema et al. 1982). Juveniles were not transferred to new tanks. During the experiment, in all test solutions, dissolved oxygen was maintained at 5.29 ± 0.63 and 4.72 ± 1.4 mg L^{-1} for juveniles of 0.99 g and 3.8 g, respectively; and they were fed with commercial shrimp feed. Observations and parameter measurements (PH, ammonia, and dissolved oxygen) were made at 12-hr intervals. Death was assumed when juveniles were non-motile and showed no response when touched with a glass rod. This method was the same for both size shrimp. Longer tests were not considered appropriated due to stress of 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 tit 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.

Table 1. Chemical characteristic of seawater used in bioassays

Parameter	Dissolved metals (μg L^{-1})
Total alkalinity: 2.1 meq L^{-1}	Cd: 0.32
pH: 8.5	Co: 0.09
Ammonia-N: 10 μg L^{-1}	Cr: 0.1
Nitrite-N: 4.5 μg L^{-1}	Cu: 5.5
Nitrate-N: 250 μg L^{-1}	Fe: 0.7
Phosphate-N: 85.3 μg L^{-1}	Mn: 1.05
BOD_{48}: 0.7 mg L^{-1}	Ni: 0.77
	Zn: 68.7

RESULTS AND DISCUSSION

In *P. vannamei* juveniles of 0.99 g (wet weight) exposed to different concentrations of ammonia-N during several periods, no shrimp died in the control and 20, 30, 40, and 50 mg L^{-1} ammonia-N solutions after 120 hr exposure. In 60 and 70 mg L^{-1} ammonia-N mortality of 50.0 and 95.8% was noted, respectively, during a 120 hr exposure. A 100% mortality occurred in the 80 and 100 mg L^{-1} of ammonia-N groups at 96 and 60 hr of exposure, respectively.

No 3.8 g shrimp died in the control and 20, 30, 40, and 50 mg L^{-1} ammonia-N groups after 120 hr of exposure. Shrimp (3.8 g) in 60 and 80 mg L^{-1} ammonia-N groups experienced 29.1 and 91.6 % mortality, respectively, after 120 hr of exposure. Only in the 100 mg L^{-1} ammonia-N group were all shrimp dead after 84 hr of exposure.

LC_{50} values and their 95% confidence limits (error bars) for ammonia-N and NH_3-N are shown in the Figs. 1 and 2; for *P. vannamei* juveniles of 0.99 and 3.8 g, respectively.

A comparison between the LC_{50} (APHA-AWWA-WPCF 1992) for ammonia-N at different exposure times, was carried out. 0.99 g juvenile revealed significant difference ($p<0.05$) between 48-hr and 72-hr, 72-hr and 96-hr, and 96-hr and 108-hr. There was not significant difference ($p<0.05$) between 24-hr and 48-hr, and 108-hr and 120-hr LC_{50}. The 3.8 g juvenile LC_{50} differed between 72-hr and 96-hr ($p<0.05$). Other LC_{50} comparisons were not significant ($p<0.05$).

For juveniles 0.99 and 3.8 g, 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, modeling adequately the data (Chen et al. 1991, Hubert 1992).

Wickins (1976), found an approximate 48-hr LC_{50} for NH_3-N for seven penaeid species (*P. aztecus, P. japonicus, P. occidentalis, P. orientalis, P. schmitti, P. semisulcatus and P. setiferus*), which corresponds approximately to 24 mg L^{-1} NH_4^+-N at pH 8.0 in sea water at 33 and 28 °C. 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.

A comparison of the LC_{50} values for ammonia-N of several juvenile penaeids revealed that only LC_{50} of *Penaeus monodon* (4.87 ± 1.4 g) (Chen et al. 1990a) was similar to values found by this study (Table 2). The LC_{50} 96-hr value of *P. vannamei* juveniles (0.99 g) was higher than those reported by Allan et al. (1990) for juveniles of *Metapenaeus macleayi* (2.0 g) and *Penaeus monodon* (2.2 g); by Wajsbrot et al. (1990) for *Penaeus semisulcatus* (0.3-2.4 g), and by Ostrensky and Wasielesky (1995) for *Penaeus paulensis* (5.45 ± 0.4 g). This indicates that

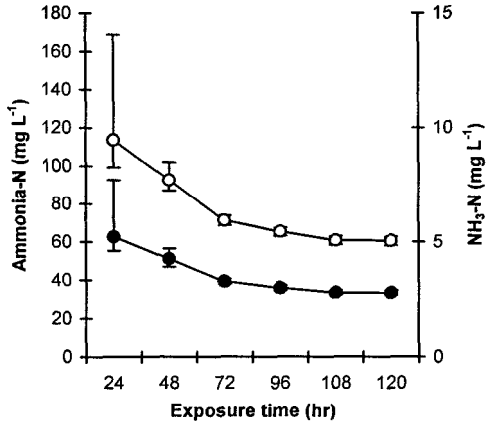

Figure 1. LC_{50} values and 95% confidence limits of ammonia-N (O) and NH_3-N (●) for *P. vannamei* juveniles (0.99 g) exposed from 24 to 120 hr

Penaeus vannamei juveniles are more resistant to ammonia than other species.

Sprague (1969) noted that LC_{50} values could be misleading and recommended that toxicity tests must be described in terms of incipient LC_{50} or threshold concentration (a concentration above which a response will be produced and below which it will not). For 0.99 and 3.8 g juveniles, the incipient LC_{50} were 108-hr and 96-hr LC_{50} (60.7 and 70.9 mg L^{-1} ammonia-N), respectively.

An acute toxicity test provides information about the relative lethality of a substance, drug or poison (Buikema et al. 1982), 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. 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 an application of 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 *P. vannamei* juveniles would be 6.52 and 7.09 mg L^{-1} of ammonia-N (0.29 and 0.13 mg L^{-1} of NH_3-N) for juveniles at 0.99 and 3.8 g, respectively. Chen et al. (1990b) with *Penaeus chinensis* juveniles and Chen et al. (1990a) with *Penaeus monodon* adolescents, reported an "safe" level for rearing of these species of 3.51 (0.14 mg L^{-1} of NH_3-N) and 4.26 (0.08 mg L^{-1} of NH_3-N) mg L^{-1} of ammonia-N, respectively. Chen and Lin (1991) reported a value of 0.24 mg L^{-1} NH_3-N, for *Penaeus penicillatus* juveniles.

Regarding ammonia-N, Fig. 1 and Fig. 2 indicated that *P. vannamei*, increased

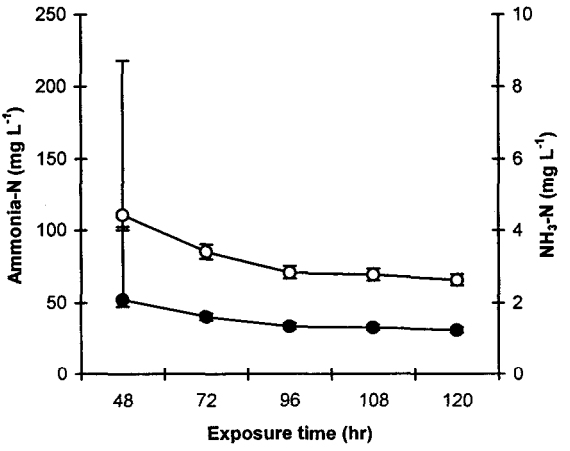

Figure 2. LC_{50} values and 95% confidence limits of ammonia-N (○) and NH_3-N (●) for *P. vannamei* juveniles (3.8g) exposed from 48 to 120 hr

their tolerance to ammonia with age. Several authors have expressed that younger organisms are often more sensitive to toxicant than adults (Buikema et al. 1982; Allan et al. 1990; Chen et al. 1990b). Regarding to NH_3-N the LC_{50} value for 0.99 g *P. vannamei* juveniles was higher than 3.8 g juveniles, probably because pH values differed between experiments; and this difference likely influenced on mortality (and hence the proportion of ionized and un-ionized form) and unrelated to shrimp size. Chen and Chin (1989) found that increasing pH value of test solution increases the proportion of NH_3-N and the toxicity of ammonia. in *P. monodon* postlarvae. Undoubtedly, differences in our experiments were due to differences in pH and temperature values and/or differences in shrimp size.

Table 2. LC_{50} values (mg L^{-1}) of ammonia-N for several juvenile penaeid species

Species	24-hr	48-hr	72-hr	96-hr	Author
P. monodon (4.87 g)	97.9	88	53.4	42.6	Chen et al. (1990a)
P. chinensis (0.36 g)	79.9	51.1	37	35.1	Chen et al (1990b)
M. macleayi (2 g)				26.3	Allan et al. (1990)
P. monodon (2.2 g)				37.4	Allan et al. (1990)
P. semisulcatus (0.3-2.4 g)				23.7	Wajsbrot et al. (1990)
P. paulensis (5.45 g)	51.8	43.1	40.1	38.7	Ostrensky and Wasielesky (1995)
P. vannamei (0.99 g)	113.4	92.5	71.2	65.2	This study
P. vannamei (3.8 g)		110.6	85.3	70.9	This study

The "safe" level obtained here has important implication 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.

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