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# Acute toxicity of ammonia to various life stages of the São Paulo shrimp, *Penaeus paulensis* Pérez-Farfante, 1967

Antonio Ostrensky[a,*], Wilson Wasielesky Jr.[b]

[a]*Laboratório de Carcinocultura, Centro de Estudos do Mar, Universidade Federal do Paraná, Av. Beira-Mar S/N°, Pontal do Sul, Paraná, 83255-000 Brazil*

[b]*Laboratório de Zoofisiologia, Departamento de Ciências Fisiológicas, Fundação Universidade do Rio Grande, Rio Grande do Sul, Brazil*

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**Abstract**

Each life stage of the São Paulo shrimp, *Penaeus paulensis*, was exposed to ammonia in a series of acute toxicity tests, by the semistatic method, at 25°C and salinity at 28 ppt. A pronounced sensitivity was observed in the egg stage. In this phase, 0.30 mg/l $NH_3$-N caused death of 50% of the embryos. The 24-h $LC_{50}$ values for nauplii, zoeae, myses and postlarvae were, respectively, 102.30, 22.93, 74.87 and 24. 19 mg/l total ammonia-N (4.25, 1.79, 2.91 and 1.40 mg/l $NH_3$-N). The 96-h $LC_{50}$ values for zoeae, myses and postlarvae were 9.39, 21.98, 5.49 mg/l total ammonia-N (0.73, 0.85 and 0.32 mg/l $NH_3$-N). The $LC_{50}$ of ammonia for juveniles (5.45 $\pm$ 0.4 g mean live-weight) and for adults (31.43 $\pm$ 1.3 g mean live-weight) were also measured. The 24-h $LC_{50}$ values for juveniles and adults were, respectively, 51.87 and 61.63 mg/l total ammonia-N (1.47 and 1.59 mg/l $NH_3$-N) and the 96-h $LC_{50}$ values were 38.72 and 42.49 mg/l total ammonia-N (1.10 and 1.06 mg/l $NH_3$-N). An analysis of variance showed that there was no direct relationship between shrimp development and its tolerance to ammonia ($P > 0.05$) and that eggs, zoeae and postlarvae are very sensitive to ammonia:. The "safe level" for rearing *P. paulensis* was estimated to be 0.03 mg/l $NH_3$-N. In general, *P. paulensis* is less resistant to ammonia than other species of shrimp.

*Keywords*: *Penaeus paulensis*; Ammonia; Toxicity

## 1. Introduction

The maintenance of water quality and knowledge of tolerance limits of a species regarding water quality are indispensable requirements in any rearing system (Spotte, 1970; Kinne,

* Corresponding author.

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1976), decisively influencing the success or failure of this productive activity. One of the most important limiting factors in intensive culture systems is the build-up of toxic nitrogenous waste, such as ammonia, nitrite and urea. In an intensive shrimp culture system, ammonia and nitrite increase exponentially over time in grow-out ponds. High concentrations of ammonia may result in retardation of shrimp growth, and in extreme cases can cause mortality (Wajsbrot et al., 1990; Zin and Chu, 1991).

In an aqueous solution ammonia is present in the ionized ($NH_4^+$) and un-ionized ($NH_3$) forms; the sum of both constitutes the total ammonia ($NH_4^+ + NH_3$). The proportion of each in aqueous ammonia is dependent upon the pH and, to a lesser degree, upon temperature and salinity (Thurston, 1980). Most researchers agree that the chemical form which is the most toxic is un-ionized ammonia, due to its ability to diffuse readily across cell membranes (Fromm and Gillette, 1968), and that the effects of ionized ammonia are considerably less pronounced (EIFAC, 1983; Yu and Hirayama, 1986).

The present work utilized the procedures adopted by the Laboratorio de Carcinocultura, of Rio Grande University (FURG) for rearing São Paulo shrimp, *Penaeus paulensis*, with the objective of analysing their resistance to ammonia.

This species occurs from Cabo Frio, Southeast Brazil, to Buenos Aires Province, Argentina, in subtropical waters. Adult shrimp of *P. paulensis* are rarely found below 130 m (Pérez-Farfante, 1988) and typically inhabit off-shore waters, while juveniles generally occur in protected coastal habits. *P. paulensis* have a great potential to be exploited by shrimp farms in Brazil, besides contributing in a large part to marine shrimp catches in this region.

This analysis will furnish important allowances for the optimization of the management and increase in productivity of *P. paulensis* hatcheries.

## 2. Materials and methods

The experimental part of this work was conducted in the Laboratorio de Carcinocultura, located in Cassino Beach, Rio Grande, RS, Brazil, between March 1990 and May 1992. Reproducing shrimp were collected along the Santa Catarina coast and maintained in the laboratory under controlled conditions. The females were induced to spawn with unilateral eyestalk ablation. The larvae were maintained in polyethylene tanks with a 180-litre capacity and reared in these tanks until they reached the desired stage for experimentation, or until they reached the postlarval (PL) stage, upon which they were transferred to circular tanks with a 12 000-litre capacity. The shrimp remained in these tanks until they reached the juvenile stage and then they were used in the bioassays. The adults used were also collected along the Santa Catarina coast and acclimated in the laboratory before being used in the bioassays.

Water used in the tests was pumped from the Cassino coast next to the laboratory and filtered through a sand bed and a 5-$\mu$m mesh filter.

The methodology used in the short-term $LC_{50}$ (median lethal concentration) toxicity tests was based on the manuals of the Environmental Protection Agency of The United States (Peltier and Weber, 1985; EPA, 1985) and American Public Health Association et al. (1985).

The experiments were performed in two phases, one using 5 different concentrations of ammonia in duplicate, and the second with 10 concentrations without duplication, all of which were compared with a control group, tested in duplicate, which had an absence of inorganic ammonia. When the mortality rate in the controls exceeded 10%, the tests were discarded.

The test solutions of ammonia were prepared by dissolving ammonium chloride ($NH_4Cl$) (Merck reagent grade) in seawater with a salinity of 28 ppt. The concentrations tested in each larval stage were selected based on preliminary tests using a geometric scale with a factor of 10 (0.01, 0.1, 1, 10, 100 mg/l TAN (total ammonia nitrogen). In the final tests, the concentrations of ammonia-N were determined by a geometric scale with a factor of two, from the quantitative limits established in the preliminary tests.

Eggs and larvae were sampled randomly from the holding tanks and transferred to test and control solutions in 500-ml beakers. Bioassays with juveniles were conducted in 30-litre and adults in 180-litre polyethylene tanks. The number of individuals in each beaker was 30, in the case of eggs, nauplii, zoeae and myses, and 20 for the postlarval stage. All the larvae submitted to the tests were initially in the first sub-stage of each stage (nauplius, $N_I$; zoea, $Z_I$; mysis, $M_I$; postlarva, $PL_I$). The juveniles ($5.45 \pm 0.4$ g mean live-weight) were tested in groups of 9 and the adults ($31.43 \pm 1.3$ g mean live-weight) in groups of 8 per aquarium.

No food was supplied during the tests with nauplii. The zoeae were fed with *Tetraselmis chuii*, the myses and postlarvae with *Artemia* sp. nauplii, and the juveniles and adults with clams (*Mesodesma mactroides*).

Bioassay tests were conducted using the "static renewal method", with test solutions renewed daily (Buikema et al., 1982). All test-solutions were aerated continuously by an air-stone with blower, the temperature was maintained at $25 \pm 1$ °C, salinity at 28 ppt, luminous intensity of 1800 lux and a photoperiod of 12 h light/12 h darkness. The individuals were considered dead when they showed no movement or no response when mechanically stimulated with a micro-pipette.

The results, obtained at intervals of 24 h, were analyzed by a Probit Analysis program, developed by Dr. Jesus Santiago Moure, of the Zoology Department of Paraná Federal University, based on Finney (1952) and Sokal (1958), and to the trimmed Spearman Karber method (Hamilton et al., 1977) for the calculation of the $LC_{50}$ and its 95% confidence limits. The toxicity of un-ionized ammonia ($NH_3$-N) was calculated according to the equations proposed by Whitfield (1974) as modified by Ostrensky et al. (1992), based on a salinity of 28 ppt, temperature of 25°C and on the mean pH values obtained in each experiment (Table 1).

The terms used in this study are: total ammonia = total inorganic ammonia (un-ionized ammonia + ionized ammonia); ammonia-N = total inorganic ammonia as nitrogen; $NH_3$-N = un-ionized ammonia as nitrogen; $NH_4^+$ = ionized ammonia as nitrogen.

## 3. Results

An analysis of the $LC_{50}$ of $NH_3$-N (Fig. 1) or total ammonia-N (Table 1) shows that during embryonic development, the shrimp are very sensitive to the ammonia. A concen-

Table 1
$LC_{50}$ (mg/l) of total ammonia-N for all the life stages of *Penaeus paulensis* and their 95% confidence limits

| Stage | Time (h) | | | | Mean pH |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| Egg (E) | 6.25 | – | – | – | 8.02 |
| | (5.10–7.60) | – | – | – | |
| Nauplius (N) | 102.30 | 41.80 | 25.59 | – | 7.95 |
| | (91.10–112.30) | (93.10–112.30) | (17.30–44.62) | – | |
| Zoea (Z) | 22.93 | 14.53 | 10.94 | 9.39 | 8.24 |
| | (16.05–29.05) | (10.24–20.64) | (7.43–19.32) | (6.38–16.50) | |
| Mysis (M) | 74.87 | 41.80 | 32.85 | 21.98 | 7.92 |
| | (66.69–84.37) | (34.24–51.18) | (25.29–44.70) | (14.44–38.85) | |
| Postlarva (PL) | 24.19 | 8.59 | 5.65 | 5.49 | 8.10 |
| | (19.62–26.25) | (7.63–9.64) | (4.06–7.86) | (4.52–6.63) | |
| Juvenile (J) | 51.87 | 43.11 | 40.05 | 38.72 | 7.78 |
| | (48.17–55.86) | (41.31–44.99) | (37.48–42.79) | (35.61–42.09) | |
| Adult (A) | 61.63 | 50.51 | 46.57 | 42.49 | 7.71 |
| | (54.60–69.56) | (46.21–55.20) | (43.31–50.08) | (38.78–46.56) | |

Homogeneous groups: E < PL < (Z = J = A = M) < (M = N)*

Conditions: 28 ppt seawater, 25°C and mean pH for each test.
*Ammonia tolerance relationship obtained by Scheffé's posteriori multiple range test using the $LC_{50}$ values calculated for each life stage of São Paulo shrimp *Penaeus paulensis*.

tration of 0.30 mg/l $NH_3$-N resulted in the death of 50% of the embryos. Above 2.8 mg/l $NH_3$-N, none of the embryos completed the necessary divisions needed to develop into nauplii.

Besides the lethal effects, the development of the hatched nauplii in sub-lethal concentrations was monitored to see if larval development was alterated by ammonia. This was manifested in alterations in the natatory behavior and a lowering in the percent of molting shrimp.

The nauplius stage required, in general, a total period of 72–75 h to develop into successive stages. At 24, 48 and 72 h, $LC_{50}$ values of $NH_3$-N were 4.25, 1.73 and 1.06 mg/l, respectively. These values were among the highest obtained in the tests.

No direct relationship was observed between shrimp development and their tolerance to ammonia. For example, for zoeae the $LC_{50}$ values ranged from 1.79 to 0.73 mg/l $NH_3$-N in 24 and 96 h, respectively; for myses, the $LC_{50}$ values ranged from 2.91 to 0.85 mg/l $NH_3$-N, in the same period. Mysis, unlike the other stages, did not exhibit a tendency to stabilization in the values of toxicity in the period of 96 h.

After the embryonic phases, the postlarval stage was, of all of those tested, the most sensitive stage to ammonia. The 72-h and 96-h $LC_{50}$ values were 0.33 and 0.32 mg/ $NH_3$-N, which were lower than that quantified for nauplii, zoeae and myses. The 95% confidence limits (Fig. 1) were much wider for the first three larval stages than for the postlarval to adult stages.

For both the juveniles and adults, the 24-h $LC_{50}$ values were slightly higher than those calculated for 48 h. The lethal effects of ammonia appeared to stabilize during these periods.

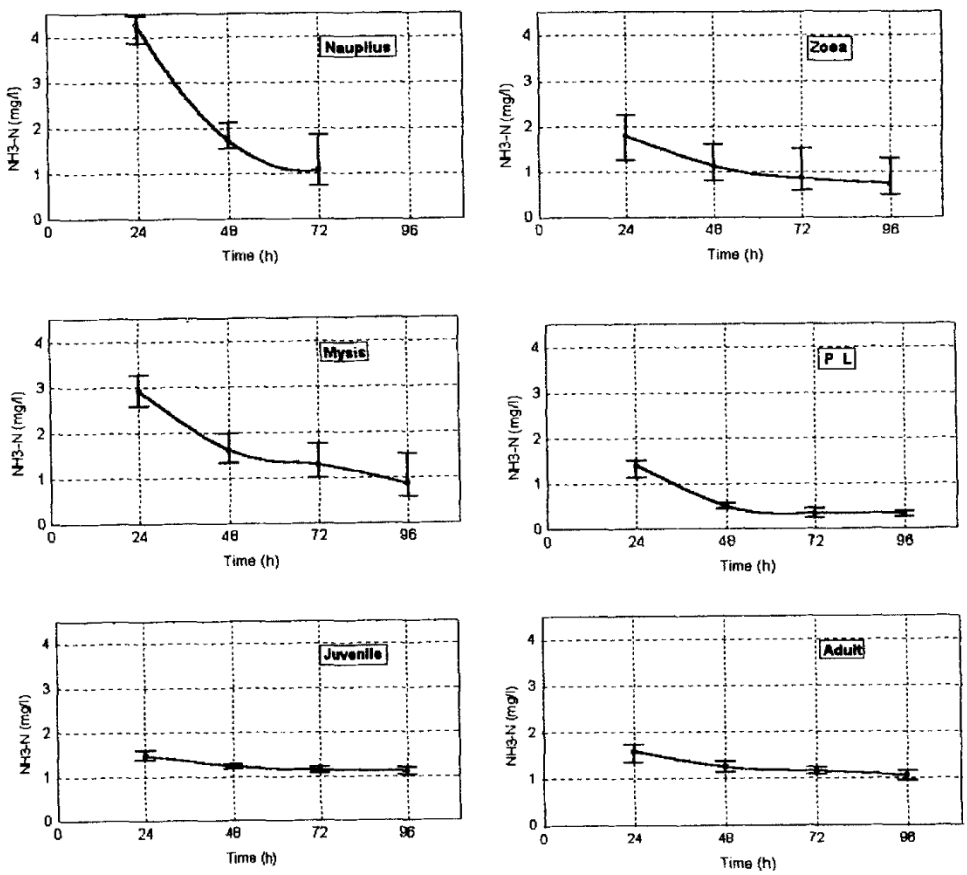


Fig. 1. $LC_{50}$ (median lethal concentrations) of $NH_3$-N (mg/l) for all the life stages of *Penaeus paulensis* and their 95% confidence limits.

This is reflected in the low variability of the $LC_{50}$, which ranged from 1.47 to 1.10 mg/l $NH_3$-N in 24-h and 96-h, respectively, for juveniles and from 1.59 to 1.11 mg/l $NH_3$-N for adults during the same periods.

The results of two-way analysis of variance performed with the $LC_{50}$ and their 95% confidence limits for $NH_3$-N on each life stage are summarized in Table 1. In the embryonic phases, *P. paulensis* was significantly less tolerant to ammonia than in its other life stages. In second position was the postlarval stage, whicht was more tolerant than the embryonic phases, but less tolerant than the others. Among zoea and adult stages, there was no significant difference ($P > 0.05$) in ammonia tolerance. The nauplius stage, meanwhile, was more tolerant than almost all of the others life stages of *P. paulensis*.

## 4. Discussion

Our data indicate that special care should be taken with the water used in hatching of *P. paulensis*. This water should be practically ammonia-free, or larval production may be

Table 2
The 24-h $LC_{50}$ values of $NH_3$-N for several penaeids species

| Species | Stage | | | | | Author |
|---|---|---|---|---|---|---|
| | Nauplius | Zoea | Mysis | Postlarva | Juvenile | |
| *P. indicus* | 0.29 | 0.95 | 3.17 | | – | Jayasankar and Muthu (1983) |
| *P. monodon* | 0.54 | 0.76 | 2.17 | 4.70 | – | Chin and Chen (1987) |
| *P. japonicus* | 1.31 | 0.97 | 1.08 | 1.98 | – | Chen et al. (1989) |
| *P. chinensis* | 0.25 | 0.34 | 1.08 | 1.85 | – | Chen and Lin (1991b) |
| *M. ensis* | 0.65 | 0.30 | 2.25 | 1.90 | – | Chen et al. (1991) |
| *P. paulensis* | 4.25 | 1.79 | 2.91 | 1.40 | 1.47 | Present study |
| *P. monodon* | – | – | – | – | 2.68 | Chen and Lei (1990) |
| *M. ensis* | – | – | – | – | 2.19 | Nan and Chen (1991) |
| *P. chinensis* | – | – | – | – | 3.88 | Chen and Lin (1992) |
| *P. penicillatus* | – | – | – | – | 3.25 | Chen and Lin (1991a) |

seriously compromised. Our results also suggest that there should be a daily monitoring of the rearing tanks during the postlarval stage, so that decomposition of unconsumed food does not change the water quality, since liberation of ammonia may be very intense under such conditions (Ostrensky et al., 1991).

In the present study, nauplius and mysis stages were considerably more resistant than the other stages, based on the 24-h and 48-h $LC_{50}$ values. In contrast, Chen and Chin (1987) concluded that the nauplius stage of *P. monodon* was the least resistant to ammonia and the postlarvae the most tolerant, coinciding with observations made by Jayasankar and Muthu (1983), who worked with P. indicus. Results of short-term experiments conducted by other authors also revealed that there is a progressive increase in the tolerance of the larval stages of several species of penaeids to ammonia (Table 2).

Meanwhile, Mawatari and Hirayama (1975) observed a higher tolerance to ammonia in the nauplius stage of two species, *P. japonicus* and *P. orientalis*. These authors suggested that by not depending on exogenous food, the nauplii are less dependent on the environment, being able to endure more environmental adversities.

The results indicated that tolerance of juveniles to ammonia is more similar to adults than to larval stages, since postlarvae would be a transitional stage in terms of sensitivity to ammonia.

Wickins (1976) experimented with 7 species of penaeids in the juvenile stage and calculated the 48-h $LC_{50}$ to be 1.29 mg/l $NH_3$-N for all species combined. He estimated that the maximal acceptable level of ammonia during the rearing of individuals in the juvenile stage was 0.10 mg/l $NH_3$-N.

Chen and Lin (1991a) stated that 24-, 48-, 72- and 96-h $LC_{50}$ values were 8.16, 5.37, 3.52 and 3.25 mg/l $NH_3$-N, respectively, for *P. penicillatus* juveniles. According to Wajsbrot et al. (1990), the 96-h $LC_{50}$ for *P. semisulcatus* juveniles was 1.43 mg/l $NH_3$-N. Chen et al. (1990) stated that 24 and 72-h $LC_{50}$ values were 1.76 and 1.20 mg/l $NH_3$-N, respectively, for *P. monodon* adolescents, suggesting that the shrimp increases in tolerance to ammonia with age. Allan et al. (1990) found that the 96-h $LC_{50}$ values of ammonia for juvenile *Metapenaeus macleayi* and for *P. monodon* were 1.39 and 1.69 mg/l $NH_3$-N,

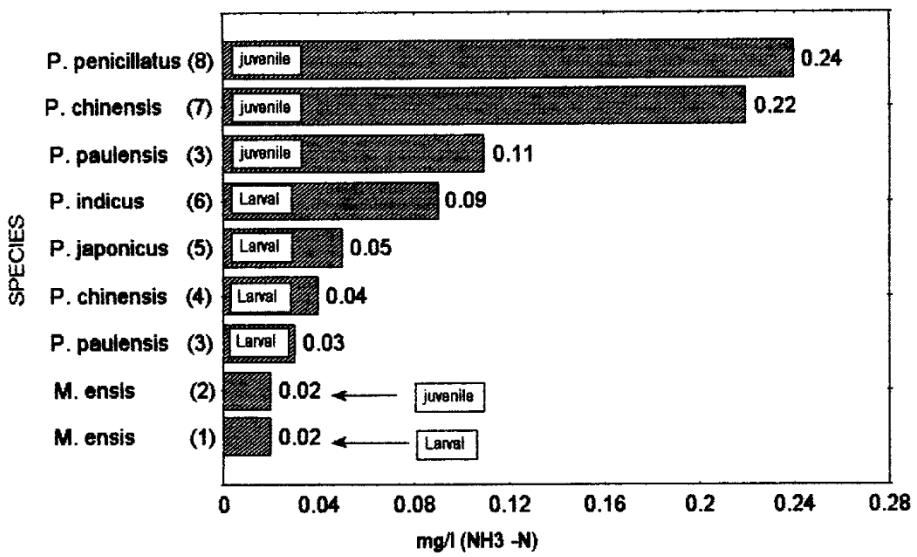


Fig. 2. "Safe levels" calculated for larvae and juveniles of penaeids: (1) Chen et al., 1991, (2) Nan and Chen, 1991; (3) Present study; (4) Chen and Lin, 1991b; (5) Chen et al., 1989; (6) Jayasankar and Muthu, 1983; (7) Chen and Lin, 1992; (8) Chen and Lin, 1991a).

respectively. Chen and Lin (1992) reported that 24-, 48- and 96-h $LC_{50}$ for *P. chinensis* juveniles were 3.88, 3.13 and 2.47 mg/l $NH_3$-N, respectively, at 30 ppt.

Chen and Kou (1992) studied the effects of prolonged exposure of juvenile *P. japonicus* to ammonia and concluded that the growth rate, after 40 days of exposure at 0.70 mg/l $NH_3$-N, was reduced significantly as compared to the control group. These authors also documented that ammonia decreased the intermoult period. The maximum acceptable toxicant concentration (MATC) for juveniles over 30 days was 0.35 mg/l $NH_3$-N.

Sprague (1969) pointed out that the effects of a given toxicant could be described in terms of "safe level", that can be obtained by multiplying an empirical "application factor" of 0.1 (Sprague, 1971). The "safe level" for rearing *P. paulensis* in the hatchery was calculated to be 0.55 mg/l TAN and 0.03 mg/l $NH_3$-N, in 28 ppt, at pH 8.10 and water temperature 25°C. Since the juveniles were moved out of the hatchery, the "safe level" for rearing *P. paulensis* in earthen ponds was calculated to be 3.9 mg/l TAN and 0.11 mg/l $NH_3$-N, in 28 ppt, at pH 7.78 and water temperature 25°C.

Fig. 2 shows that the "safe level" calculated for larval rearing of *P. paulensis* is lower than the value calculated for the larval rearing of *P. indicus* (Jayasankar and Muthu, 1983), *P. japonicus* (Chen et al., 1989) and *P. chinensis* (Chen and Lin 1991b). Also the "safe level" calculated for *P. paulensis* juvenile is considerably lower than for *P. penicillatus* juveniles (Chen and Lin, 1991a) and *P. chinensis* juveniles (Chen and Lin, 1992). *M. ensis* (Chen et al., 1991; Nan and Chen, 1991) is among the few species that exhibit lower values of "safe levels" than that calculated for *P. paulensis*.

These comparisons demonstrate that *P. paulensis* is more sensitive to ammonia than most species of penaeids and these results underline the necessity for research and development

of local technology for the rearing of native species. The application of technology conceived for exotic species, with different biological requirements and physiological characteristics, could affect the productivity and, probably, the success of the activity in Brazil.

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