

## 廿九、Acute Toxicity of Ammonia to Larval *Penaeus japonicus*

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Larvae of *Penaeus japonicus* at various stages were exposed by static renewal method to different concentrations of ammonia ( $\text{NH}_3 + \text{NH}_4^+$ ) in seawater at 33 ppt, pH at 8.10 and temperature at 30°C. The 24-h  $\text{LC}_{50}$  values of ammonia for nauplius third substage (N3), zoea second substage (Z2), mysis second substage (M2), postlarva second substage (PL2) and postlarva twelfth substage (PL12) were 20.88, 15.54, 17.31, 31.68 and 53.37 mg/L ammonia-N (un-ionized plus ionized ammonia as nitrogen) and 1.31, 0.97, 1.08, 1.98 and 3.34 mg/L  $\text{NH}_3$ -N (un-ionized ammonia as nitrogen), respectively. The 48-h  $\text{LC}_{50}$  values of ammonia for Z2, M2, PL2, PL12 were 7.57, 15.20, 23.80, 33.85 mg/L ammonia-N and 0.47, 0.95, 1.49, 2.12 mg/L  $\text{NH}_3$ -N. The  $\text{LC}_{50}$  values of ammonia-N and  $\text{NH}_3$ -N decreased with longer exposure. The "threshold time" was 108 h for PL2 and PL12. The "incipient  $\text{LC}_{50}$ " was 7.80 mg/L ammonia-N and 0.49 mg/L  $\text{NH}_3$ -N for PL2, and 28.29 mg/L ammonia-N and 1.77 mg/L  $\text{NH}_3$ -N for PL12. Among the developmental stages of *P. japonicus* larvae tested, zoea was the lowest tolerant and PL12 the highest tolerant. Safe values of ammonia was 0.78 mg/L ammonia-N (0.05 mg/L  $\text{NH}_3$ -N), and 2.83 mg/L ammonia-N (0.18 mg/L  $\text{NH}_3$ -N) for rearing *P. japonicus* in the hatchery, and in the nursery, respectively.

### INTRODUCTION

*Penaeus japonicus*, *P. monodon*, *P. penicillatus* and *Metapenaeus ensis* are the common penaeid species currently being cultured in Taiwan (Liao, 1986). Due to mass mortality of *P. monodon* occurred in 1986 (Lightner *et al.*, 1987) and immune of *P. japonicus* to MBV (monodon baculovirus) disease (Fukuda *et al.*, 1988), culture of *P. japonicus* has increased rapidly since the last two years.

Ammonia is the principal end product of protein catabolism in crustaceans (Hartenstein, 1970; Kinne, 1976) and accounts for 40% to 90% of nitrogenous excretions (Parry, 1960). Wickins (1985) found that as the live weight of *P. monodon* decreased from 27 g to 1.6 g, ammonia excretion increased from 0.30 to 0.93 mg  $\text{Ng}^{-1} \text{day}^{-1}$ .

Ammonia is present in both the un-ionized ( $\text{NH}_3$ ) and ionized form ( $\text{NH}_4^+$ ). The un-ionized form is usually toxic (Burkhalter and Kaya, 1977; Armstrong *et al.*, 1978), has high lipid solubility, and thus is able to diffuse quite readily across cell

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membrane (Fromm and Gillette, 1968).

The accumulation of ammonia causes mortality of organisms reared in a semi-static culture system (Spotte, 1979). Ammonia could increase to more than 0.8 mg/L ammonia-N (un-ionized plus ionized ammonia as nitrogen) and 0.08 mg/L  $\text{NH}_3\text{-N}$  (un-ionized ammonia as nitrogen) during the development of larval shrimps in a hatchery, even with frequent water replacement (Chen *et al.*, 1986). Since larvae of *P. japonicus* are raised in a semi-static system in the hatchery and nursery, the accumulation of ammonia is a threat to larval survival. This paper provides information on the lethal effect of ammonia to larval *P. japonicus* in the laboratory.

### MATERIALS AND METHODS

Larvae of *P. japonicus* at nauplius first substage (N1) were shipped from a private hatchery located at I-lan, Taiwan and were reared to different stages of larvae for bioassay test. The shrimps used were nauplius third substage (N3), zoea second substage (Z2), mysis second substage (M2), postlarva second substage (PL2) and postlarva twelfth substage (PL12). Size range was 0.11-0.15 cm for N3, 0.15-0.25 cm for Z2, 0.31-0.43 cm for M2, 0.49-0.61 cm for PL2 and 0.72-0.85 cm for PL12. Sea water pumped from the Keelung coast was filtered through sand and gravel bed and aerated one day before use. The chemical characteristics of sea water are given in Table 1.

Ammonia test solutions were prepared by dissolving requisite amounts of ammonium chloride (Merck reagent grade) in sea water. The nominal concentrations of ammonia-N generally range in geometric progression from 2 to 64 mg/L for N3; from 2 to 128 mg/L for Z2; from 4 to 128 mg/L for M2 (Table 2); from 2 to 128 mg/L for PL2; and from 2 to 128 mg/L for PL12 (Table 3). The concentrations of  $\text{NH}_3\text{-N}$  were calculated according to the equations of Bower and Bidwell (1967), based on a salinity of 33 ppt, a pH of 8.10 and a water temperature of 30°C.

Table 1. The chemical characteristics of sea water used in the bioassay

Salinity	33 ppt
pH	8.1
Total alkalinity	113 mg/L $\text{CaCO}_3$
Total hardness	6250 mg/L as $\text{CaCO}_3$
Ammonia	43 $\mu\text{g/L}$ as ammonia-N
Nitrite	14 $\mu\text{g/L}$ as nitrite-N
Nitrate	32 $\mu\text{g/L}$ as nitrate-N
Phosphate	20 $\mu\text{g/L}$ as orthophosphate-P
Silica	0.8 mg/L as $\text{SiO}_2$
COD	1.22 mg/L
Ca	780 mg/L
Mg	1040 mg/L

Short-term LC<sub>50</sub> (median lethal concentration) toxicity tests were carried out following the methods described by Hubert (1980) and American Public Health Association *et al.* (1985). Larvae were sampled randomly from the holding tanks and exposed to test and control solutions in duplicate beakers. Bioassay experiments to establish tolerance limits were conducted in 1 L polyethylene beakers containing 1 L test solution containing 15 test larvae for each N3, Z2, M2, PL2 and PL12. Each beaker was placed in a water bath (30±1°C) and was aerated by an air stone with blower. During the experiment, zoea, mysis and postlarvae were fed artificial plankton GAP (Genchem Industry Co., Ltd., Tainan, Taiwan). In addition, postlarvae were also fed freshly hatched *Artemia* nauplii. However, nauplius larvae were not fed (American Public Health Association *et al.*, 1985).

Each test solution was renewed daily, and the static renewal method was

Table 2. Percentage mortality of *Penaeus japonicus* nauplius third substage (N3), zoea second substage (Z2) and mysis second substage (M2) exposed to different concentrations of ammonia-N after various periods of exposure

Ammonia-N (mg/L)	Time elapsed (h)			
	12	24	36	48
Nauplius third substage (N3)				
2	0	0	—	—
4	0	0	—	—
8	0	3.3	—	—
12	0	13.3	—	—
16	3.3	16.7	—	—
24	30	63.3	—	—
32	70	86.7	—	—
48	90	100	—	—
64	100	—	—	—
96	100	—	—	—
Zoea second substage (Z2)				
2	0	6.7	10	16.7
4	13.3	13.3	16.7	16.7
8	23.3	33.3	40	46.7
16	36.7	50	70	83.3
32	60	100	—	—
64	100	—	—	—
96	100	—	—	—
128	100	—	—	—
Mysis second substage (M2)				
4	0	0	0	0
8	0	3.3	3.3	3.3
12	0	10	10	10
16	3.3	26.7	40	56.7
24	6.7	90	96.7	96.7
32	26.7	100	—	—
48	86.7	100	—	—
64	100	—	—	—
96	100	—	—	—
128	100	—	—	—



All Z2 exposed to over 64 mg/L ammonia-N, and to 32 mg/L were killed at 12 and 24 h, respectively. However, those exposed to 2 mg/L after 12 h survived (Table 2).

All M2 exposed to over 64 mg/L ammonia-N, and to 48 and 32 mg/L were killed at 12 and 24 h, respectively. However, those exposed to 12, 8 mg/L after 12 h; and to 4 mg/L after 48 h survived (Table 2).

All PL2 exposed to 128 mg/L ammonia-N, to 96 and 64 mg/L, to 48 mg/L, and to 32 mg/L were killed at 12, 24, 48 and 96 h, respectively. However, those exposed to 16 mg/L after 12 h; to 8, 4 mg/L after 24 h; and to 2 mg/L after 36 h survived (Table 3).

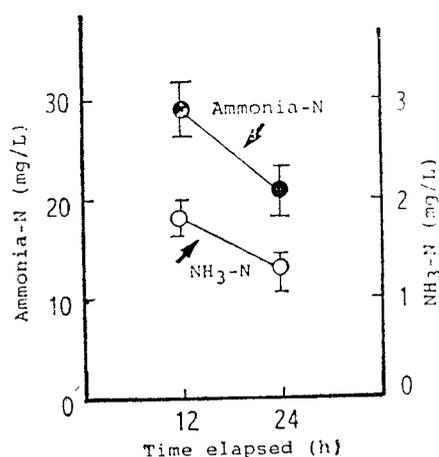


Fig. 1. The LC<sub>50</sub> values of ammonia-N and NH<sub>3</sub>-N and their 95% confidence limits to *Penaeus japonicus* nauplius third substage (N3) in 33 ppt sea water at a pH of 8.10 and a water temperature of 30°C.

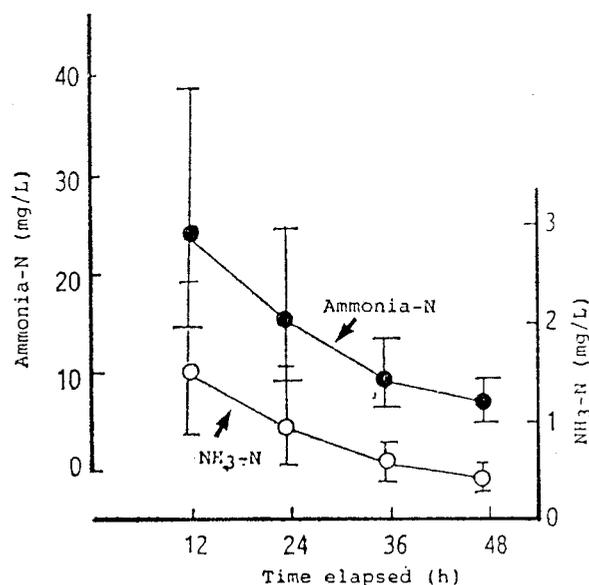


Fig. 2. The LC<sub>50</sub> values of ammonia-N and NH<sub>3</sub>-N and their 95% confidence limits to *Penaeus japonicus* zoes second substage (Z2) in 33 ppt sea water at a pH of 8.10 and a water temperature of 30°C.

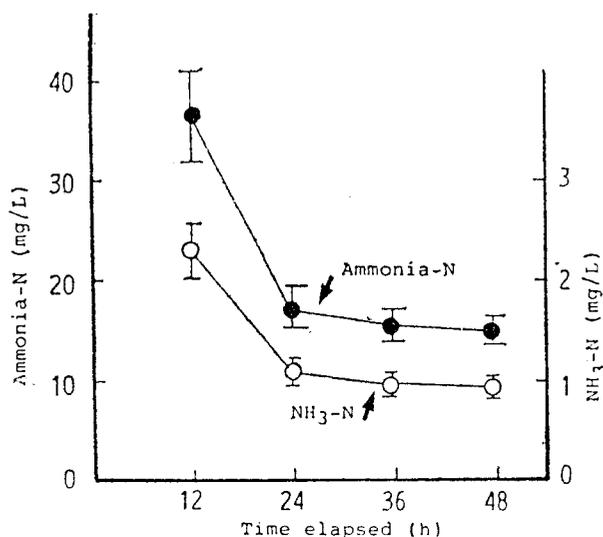


Fig. 3. The  $LC_{50}$  values of ammonia-N and  $NH_3$ -N and their 95% confidence limits to *Penaeus japonicus* mysis second substage (M2) in 33 ppt sea water at a pH of 8.10 and a water temperature of 30°C.

All PL12 exposed to 128 mg/L ammonia-N, to 96 mg/L, to 64 mg/L, and to 48 mg/L were killed at 24, 36, 48 and 96 h, respectively. However, those exposed to 48 mg/L after 12; to 32 and 24 mg/L after 24 h; to 16 mg/L after 60 h; and to 8, 4, 2 mg/L after 120 h survived (Table 3).

No larva died in the control solution for N3, Z2, M2, PL2 and PL12 after 24, 48, and 120 h, respectively.

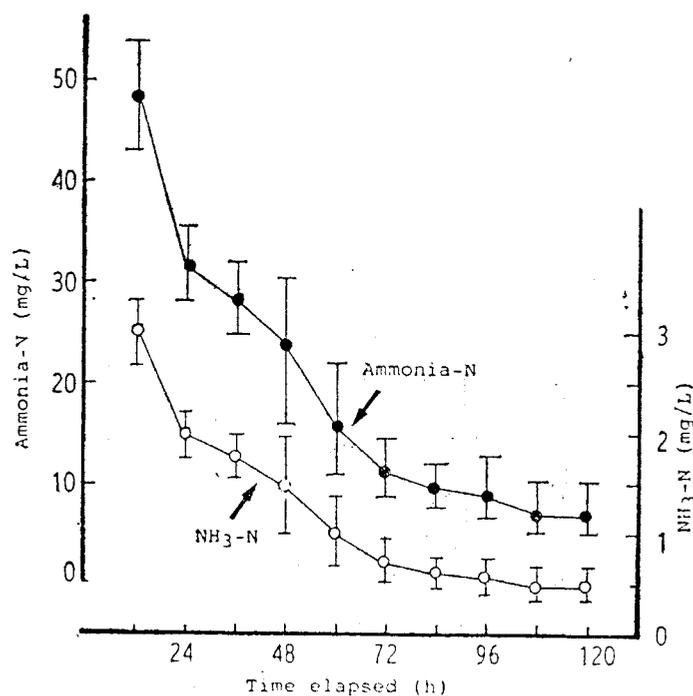


Fig. 4. The  $LC_{50}$  values of ammonia-N and  $NH_3$ -N and their 95% confidence limits to *Penaeus japonicus* postlarva second substage (PL2) in 33 ppt sea water at a pH of 8.10 and a water temperature of 30°C.

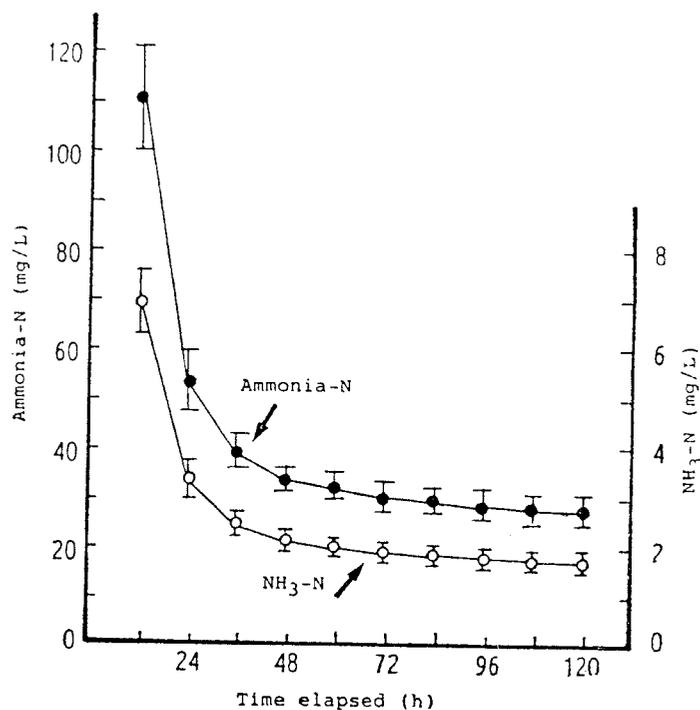


Fig. 5. The  $LC_{50}$  values of ammonia-N and  $NH_3$ -N and their 95% confidence limits to *Penaeus japonicus* postlarva twelfth substage (PL12) in 33 ppt sea water at a pH of 8.10 and a water temperature of 30°C.

$LT_{50}$  value is an indicator to determine the extent of a toxicant on aquatic animals. The  $LT_{50}$  value for N3 exposed to 24 mg/L ammonia-N, and for Z2 exposed to 16 mg/L ammonia-N was 38 and 24 h, respectively. The  $LT_{50}$  value for M2 exposed to 16 and 24 mg/L ammonia-N was 43.5 and 18.5 h, respectively. The  $LT_{50}$  value for PL2 exposed to 16 and 32 mg/L ammonia-N was 79.5 and 36 h, respectively. The  $LT_{50}$  value for PL12 exposed to 32 and 48 mg/L ammonia-N was 68 and 27 h, respectively.

The probit of mortality of the larvae exposed to ammonia-N had a positive linear relationship with log ammonia-N at various time of exposure. The results of the chi-square test also indicated that values of chi are less than the table value, suggesting that all the estimated lines are satisfactory (Trevors and Lusty, 1985).

The  $LC_{50}$  values and their 95% confidence limits for ammonia-N and  $NH_3$ -N on *P. japonicus* N3, Z2, M2, PL2 and PL12 are shown in Figures 1-5. The  $LC_{50}$  declined sharply in 36 h for Z2, in 24 h for M2, in 72 h for PL2 and in 48 h for PL12. The 24-h  $LC_{50}$  value of ammonia was 20.88, 15.54, 17.31, 31.68, 53.37 mg/L ammonia-N and 1.31, 0.97, 1.08, 1.98, 3.34 mg/L  $NH_3$ -N for N3, Z2, M2, PL2 and PL12, respectively. Susceptibility to ammonia was the greatest at zoea stage and the lowest at PL12 stage among the various stages of larvae tested. The  $LC_{50}$  decreased with increase of exposure time for all stages of *P. japonicus* tested. The "threshold time" (a time above which response will be produced and below which

it will not) was found at 108-h for both PL2 and PL12. The "incipient LC<sub>50</sub>" or "threshold concentration" (the LC<sub>50</sub> for an exposure time in the asymptotic point of the toxicity curve) was determined to be 7.80 mg/L ammonia-N and 0.49 mg/L NH<sub>3</sub>-N for PL2, and 28.29 mg/L ammonia-N and 1.77 mg/L NH<sub>3</sub>-N for PL12.

## DISCUSSION

Information about the toxicity of ammonia on freshwater fish and invertebrate has been reported by many investigators and reviewed by Alabaster and Lloyd (1982) and Johnson (1985). However, data on the toxic level of ammonia to marine crustaceans are limited (Chin and Chen, 1987; Delistraty *et al.*, 1977; Jayasankar and Muthu, 1983; Wickins, 1978).

Wickins (1976) indicated that 48-h LC<sub>50</sub> value of ammonia on seven penaeid larvae (500-1500 mg) in sea water of 33 ppt and 28°C was 1.29 mg/L NH<sub>3</sub>-N, which corresponded to 24 mg/L ammonia-N at pH 8.0 or 227 mg/L ammonia-N at pH 7.0. Unfortunately, he did not specify LC<sub>50</sub> value of each species and at various stages. Neither reported LC<sub>50</sub> value at different time of exposure.

Jayasankar and Muthu (1983) reported that 24-h LC<sub>50</sub> value was 3.58, 17.86 and 46.01 mg/L ammonia-N, and 0.29, 0.95 and 3.17 mg/L NH<sub>3</sub>-N for nauplius, zoea and mysis larvae of *P. indicus* in 30-34 ppt salinity, pH 8.12-8.17 and 27-29°C. The 48-h LC<sub>50</sub> value was 16.80 mg/L ammonia-N and 1.18 mg/L NH<sub>3</sub>-N on *P. indicus* zoea. This fact revealed that tolerance of *P. indicus* to ammonia increased progressively as nauplius larvae metamorphosed to zoea and mysis stage.

Chin and Chen (1987) reported that 24-h LC<sub>50</sub> value was 6.00, 8.48, 24.04 and 52.11 mg/L ammonia-N and 0.54, 0.76, 2.17 and 4.70 mg/L NH<sub>3</sub>-N in a salinity of 34 ppt at pH 8.2 and 29.5°C. The 48-, 72-, and 95-h LC<sub>50</sub> on *P. monodon* postlarva sixth substage (PL6) were 27.73, 17.05 and 11.51 mg/L ammonia-N and 2.50, 1.54 and 1.04 mg/L NH<sub>3</sub>-N, respectively. This fact also indicated that tolerance of *P. monodon* to ammonia increased progressively as the larvae developed from nauplius to zoea, mysis and postlarva stage. In comparison with the LC<sub>50</sub> values of NH<sub>3</sub>-N on *P. indicus* larvae (Jayasankar and Muthu, 1983), and on *P. monodon* larvae (Chin and Chen, 1987), present study indicated that nauplius and zoea larvae of *P. japonicus* were more tolerant than those of *P. monodon* and *P. indicus*. However, mysis larvae of *P. japonicus* were less tolerant than those of *P. monodon* and *P. indicus*. In addition, *P. japonicus* PL2 were less tolerant than *P. monodon* PL6.

All previous studies showed that the shrimps could resistant to ammonia as they grow from nauplius to postlarva stage. However, in present study, as the larval shrimps developed, they did not show a progressive tolerance to ammonia-N and NH<sub>3</sub>-N. PL12 was the highest resistant and zoea was the lowest resistant to ammonia. Since the larvae developed from nauplius to zoea, to mysis, and to postlarva take only 3-4 days, we did not obtain LC<sub>50</sub> value more than 36 h of exposure for nauplius.

Sprague (1969) reported that the short-term LC<sub>50</sub> can be very misleading and recommended that toxicity should be described in terms of "incipient LC<sub>50</sub>" or "threshold concentration". If this value cannot be estimated, the 96-h LC<sub>50</sub> value is a useful substitute suggested by Armstrong *et al.* (1978). Present study indicated that 96-h LC<sub>50</sub> and incipient LC<sub>50</sub> for PL2 were 9.32 mg/L and 7.80 mg/L ammonia-N, (0.58 mg/L and 0.49 mg/L NH<sub>3</sub>-N) respectively. The 96-h LC<sub>50</sub> and incipient LC<sub>50</sub> for PL12 were 28.89 and 28.29 mg/L ammonia-N, (1.81 mg/L and 1.77 mg/L NH<sub>3</sub>-N), respectively. Incipient LC<sub>50</sub> is a very important parameter in connection with "safe level" which is calculated from incipient LC<sub>50</sub> by using an empirical "application factor" of 0.1 (Sprague, 1971). The "safe level" for rearing *P. japonicus* in the hatchery was calculated to be 0.78 mg/L ammonia-N and 0.05 mg/L NH<sub>3</sub>-N. Since the postlarvae developed to PL10-12, were moved out from inside hatchery, the "safe level" for rearing *P. japonicus* in the nursery was calculated to be 2.83 mg/L ammonia-N and 0.18 mg/L NH<sub>3</sub>-N in 33 ppt at a pH 8.10 and a water temperature 30°C.

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## 氨對斑節蝦蝦苗之急性毒性

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將不同時期之斑節蝦蝦苗於 33 ppt 鹽度，pH 8.10 及水溫 30°C 下以靜止換水方式 (static renewal method) 暴露於不同氨濃度中。對於 N3 (第 3 期無期幼蟲)，Z2 (第 2 期眼幼蟲)，M2 (第 2 期糠蝦苗)，PL2 (第 2 期後期幼蟲)，PL12 (第 12 期後期幼蟲) 之 24 小時 LD<sub>50</sub> (半致死濃度) 分別為 20.88, 15.44, 17.31, 31.68, 53.37 mg/L 氨-氮及 1.31, 0.97, 1.08, 1.98, 3.34 mg/L 未解離氨-氮。對於 Z2, M2, PL2, PL12, 48 小時 LC<sub>50</sub> 分別為 7.57, 15.20, 23.80, 33.85 mg/L 氨-氮及 0.47, 0.95, 1.49, 2.12 mg/L 未解離氨-氮。LC<sub>50</sub> 隨著暴露時間增長而減小。對於 PL2 及 PL12 之「閾值時間」均在 108 小時。對於 PL2 之 "incipient LC<sub>50</sub>" 為 7.80 mg/L 氨-氮，0.49 mg/L 未解離氨-氮。對於 PL12 之 "incipient LC<sub>50</sub>" 為 28.29 mg/L 氨-氮，1.77 mg/L 未解離氨-氮。在所有檢試之斑節蝦蝦苗中，眼幼蟲之抵抗力最弱，PL12 之抵抗力最強。0.78 mg/L 氨-氮，0.05 mg/L 未解離氨-氮以及 2.83 mg/L 氨-氮，0.18 mg/L 未解離氨-氮分別為繁殖場與育苗場培育斑節蝦蝦苗之安全基準。