Bull. Inst. Zool., Academia Sinica **30**(4): 289-297 (1991)

LETHAL DOSES OF AMMONIA ON *PENAEUS CHINENSIS* LARVAE

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(Accepted April 19, 1991)

Jiann-Chu Chen and Chi-Yuan Lin (1991) Lethal doses of ammonia on *Penaeus chinensis* Larvae. *Bull. Inst. Zool., Academia Sinica* **30**(4): 289-297. *Penaeus chinensis* larvae at different developmental stages were exposed to a series of ammonia-N (un-ionized plus ionized ammonia as nitrogen) concentrations in static renewal toxicity tests. Larvae at the nauplius stage were the most susceptible, while post-larvae were the most tolerant to ammonia among the larvae tested. The 24-h LC_{50}s were 5.54, 7.42, 20.52 and 30.49 mg/ℓ for ammonia-N and 0.25, 0.34, 1.08 and 1.85 mg/ℓ for NH_3-N (un-ionized ammonia as nitrogen) for the nauplius second substage (N2), the zoea second substage (Z2), the mysis second substage (M2) and the postlarva fourth substage (PL4), respectively in seawater (salinity, 34 ppt; pH, 8.16; and water temperature, 21-25°C). The 72-h LC_{50}s for M2 and PL4 were 4.05 and 11.98 mg/ℓ for ammonia-N and 0.21 and 0.73 mg/ℓ for NH_3-N, respectively. The "threshold" was found at 96 h for PL4 on a toxicity curve approaching an asymptote. Based on the "incipient LC_{50}" and an application factor of 0.1, the "safe level," for rearing *P. chinensis* larvae was estimated to be 0.62 mg/ℓ for ammonia-N and 0.04 mg/ℓ for NH_3-N in seawater, 34 ppt in salinity, pH 8.16 and 25°C.

Key words: *Penaeus chinensis*, Larvae, Ammonia, Toxicity.

Penaeus monodon, *P. japonicus*, *P. penicillatus*, *P. semisulcatus* and *Metapenaeus ensis* are the indigenous penaeids currently being cultured commercially in Taiwan (Liao, 1986).

The fleshy prawn *P. chinensis* (also known as *P. orientalis*) which was originally distributed in Po Hai and the Yellow Sea between the area west of Korea and east of China (Liu, 1983; Yu and Chan, 1986), was first introduced from Mainland China in February 1989. Their offspring have been successfully reared and they are propagated in Taiwan today (Tzeng *et al.*, 1990).

Penaeus chinensis possesses the culture attributes of cold tolerance and a fast growth rate in this subtropical warm temperate region. An attempt has been made to culture this species as a winter alternative to *P. monodon.* Fundamental knowledge regarding the oxygen consumption, optimal oxygen, pH and salinity levels were provided for *P. chinensis* (Liu, 1983). Toxicities of ammonia and nitrite to *P. chinensis* juveniles were also reported by Chen *et al.* (1990). But to date there has been no documentation on the safe and toxic levels of ammonia on *P. chinensis* larvae.

Ammonia origniates from cultured

animal excretions and from ammonification of unconsumed food or organic detritius. Ammonia, which is the most common toxicant, and the main inorganic forms of nitrogen in a culture system may deteriorate water quality, resulting in high mortality and low growth rate of penaeid (Colt and Armstrong, 1981). Therefore, accumulation of this toxicant and its toxic effect are the primary concerns in an intensive culture system. The purpose of this study was to determine the toxicity and safe levels of ammonia to *P. chinensis* larvae in the laboratory.

MATERIALS AND METHODS

Fertilized eggs obtained from a single brood of second generation *Penaeus chinensis* in Taiwan were incubated, hatched and reared to different stages in the laboratory in the month of January, 1990. The larvae included nauplius second substage (N2), zoea second substage (Z2), mysis second substage (M2) and postlarva fourth substage (PL4).

Filtered seawater, 34 ppt, was used in the toxicity tests. The composition of the seawater used has been reported elsewhere (Chin and Chen, 1987).

Ammonia stock solution was made of ammonium chloride at a concentration of 1,000 mg/l ammonia-N (un-ionized plus ionized ammonia as nitrogen), which was then diluted to the desired test concentrations with seawater. The nominal concentration of ammonia-N, varying with larval development stages, ranged in geometric progression as summarized in Table 1. The concentration of NH_3-N was calculated from equations of Bower and Bidwell (1978) with reference to salinity 34 ppt, pH 8.16 and temperature 21–25°C.

Short-term LC_{50} (median lethal concentration) toxicity tests were carried out according to the methods described by Hubert (1980) and the American Public Health Association (1985). The larvae were randomly sampled from the stock and experimented on in triplicate. Toxicity experiments to establish tolerance limits, were conducted in 1 l beakers containing 1 l of the test solution. Each beaker contained 20 test larvae for N 2, Z 2, M 2 and PL4, respectively. Each test solution was renewed daily, in accordance with the static renewal method suggested for toxicity tests (Buikema *et al.*, 1982), and the temperature used were maintained by water batch, 21°C for N 2 and Z 2; 23°C for M2; and 25°C for PL 4 (Tzeng *et al.*, 1990). All beakers were aerated by air-stone with 3.69 ± 0.14 (mean ± SD) ml/sec. The larvea were fed with formulated feed powder designed and prepared for penaeid larvae by Hsin-Da Co. Ltd, (Pingtung, Taiwan). However, nauplius larvae were not fed. Dissolved oxygen was maintained at 5.8 ± 0.3 mg/l and pH at 8.16 ± 0.02 for all test conditions.

Observations were usually made at 12 h intervals up to 48 h for N 2, Z 2 and M 2; and up to 108 h for PL 4. Death was assumed when the larvae became immobile and showed no response. The concentration response of test organisms was represented by LC_{50} of ammonia-N and NH_3-N and their 95% confidence limits were determined. The estimated probit line and the result of a chi-square test for goodness-of-fit were also computed (Trevors and Lusty, 1985).

RESULTS

Accumulated mortality of *Penaeus chinensis* larvae at different stages exposed to different concentrations of ammonia-N during varying periods are shown in Table 1. All M 2 exposed to 24, 32 and 48 mg/l died in 60, 24, 24 h; and

Table 1
Accumulated percentage mortality of *Penaeus chinensis* nauplius second substage (N2), zoea second substage (Z2), mysis second substage (M2) and postlarva fourth substage (PL4) exposed to different concentrations of ammonia-N after various periods of exposure. Sixty individuals were used in each ammonia concentration

Ammonia-N	Time elapsed (h)								
(mg/ℓ)	12	24	36	48	60	72	84	96	108
Nauplius second substage (N2)									
1	25	28.3	40	45					
2	40	45	48.3	53.3					
4	46.7	50	51.7	55					
8	50	51.7	55	63.3					
16	51.7	58.3	60	71.7					
24	58.3	65	76.7	78.3					
32	66.7	70	88.3	95					
Zoea second substage (Z2)									
1	8.3	26.7	40	46.7					
2	16.7	38.3	46.7	50					
4	36.7	46.7	51.7	58.3					
8	41.7	51.7	56.7	63.3					
16	46.7	56.7	61.7	70					
24	51.7	61.7	66.7	73.3					
Mysis second substage (M2)									
4	10	20	35	43	51.7	55			
8	13.3	25	41.7	53.3	58.3	66.7			
12	16.7	30	45	68.3	73.3	80			
16	26.7	43.3	55	81.7	86.7	93.3			
24	35	61.7	66.7	95	100	—			
32	56.7	100	—	—	—	—			
48	63.3	100	—	—	—	—			
Postlarva fourth substage (PL4)									
4	0	0	5	11.7	16.7	20	30	40	40
8	0	0	10	15	25	31.7	41.7	51.7	51.7
12	0	3.3	16.7	25	33.3	43.3	58.3	68.3	68.3
16	5	16.7	30	36.7	43.3	58.3	66.7	83.3	83.3
24	10	33.3	46.7	51.7	63.3	80	85	100	—
32	23.3	53.3	63.3	75	83.3	100	—	—	—
48	40	80	91.7	100	—	—	—	—	—
64	53.3	91.7	100	—	—	—	—	—	—
128	100	—	—	—	—	—	—	—	—

all PL 4 exposed to 24, 32, 48, 64 and 128 mg/l died in 96, 72, 48, 36 and 12 h, respectively. Only three N2, two Z 2 and M 2 died in the control solution after 48 h exposure (Table 1).

LT_{50} value is an indicator representing the toxicity of the concluded factor on aquatic animals. Statistical analysis indicated that the LT_{50} value for N 2, Z 2, M 2 and PL 4 showed either linear or

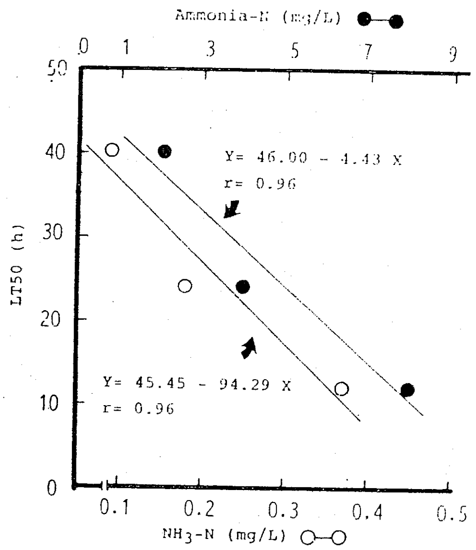

Fig. 1. The relationship between concentrations of ammonia-N and NH_3 and LT_{50} (h) for *Penaeus chinensis* nauplius second substage (N2).

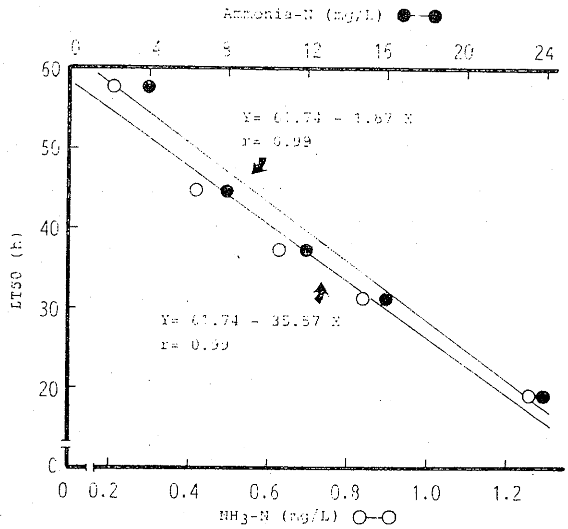

Fig. 3. The relationship between concentrations of ammonia-N and NH_3-N and LT_{50} (h) for *Penaeus chinensis* mysis second substage (M2).

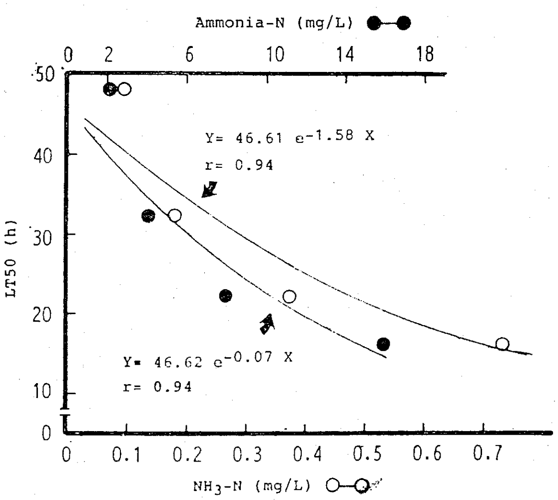

Fig. 2. The realtionship between concentrations of ammonia-N and NH_3-N and LT_{50} (h) for *Penaeus chinensis* zoea second substage (Z2).

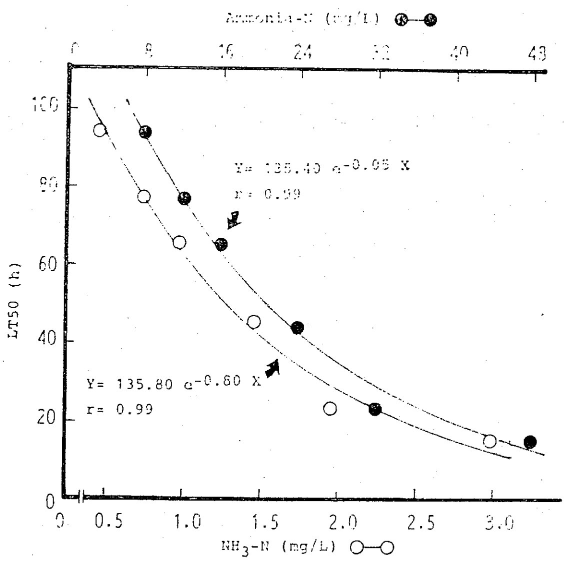

Fig. 4. The relationship between concentrations of ammonia-N and NH_3-N and LT_{50} (h) for *Penaeus chinensis* post-larva fourth substage (PL4).

exponential relationships with both ammonia-N and NH_3-N (Figs. 1-4).

The probit of mortality for *Penaeus chinensis* larvae exposed to ammonia-N had a positive linear relationship with log ammonia-N concentrations at varying exposure times (Table 2). The results of the chi-square test also suggested that all the assumed lines are satisfactory (Trevors and Lusty, 1985).

Table 2
Relationship between probit of mortality (Y) and log ammonia-N concentration as mg/ℓ (X) at various exposure times for *Penaeus chinensis* nauplius second substage (N2), zoea second substage (Z2), mysis second substage (M2) and postlarva fourth substage (PL4)

Time (h)	$Y = a + bX$	N[a]	r^b	Chi-square
Nauplius second substage (N2)				
12-h	$Y = 3.872+0.582X$	7	0.953	2.610*
24-h	$Y = 3.924+0.617X$	7	0.961	1.998*
36-h	$Y = 3.854+0.776X$	7	0.889	9.199*
48-h	$Y = 3.818+0.899X$	7	0.861	10.989*
Zoea second substage (Z2)				
12-h	$Y = 2.792+0.973X$	7	0.962	5.431*
24-h	$Y = 3.788+0.648X$	7	0.984	0.971*
36-h	$Y = 4.174+0.541X$	7	0.970	1.048*
48-h	$Y = 4.141+0.672X$	7	0.943	3.915*
Mysis second substage (M2)				
12-h	$Y = 0.868+1.626X$	7	0.956	5.713*
24-h	$Y = 1.780+1.393X$	5	0.930	3.970*
36-h	$Y = 2.928+0.999X$	5	0.949	1.579*
48-h	$Y = 0.949+2.276X$	5	0.944	6.410*
60-h	$Y = 2.232+1.668X$	4	0.927	3.303*
72-h	$Y = 1.628+2.098X$	4	0.933	3.627*
Postlarva fourth substage (PL4)				
12-h	$Y = -0.253+2.959X$	5	0.995	0.519*
24-h	$Y = -1.313+4.253X$	6	0.995	1.822*
36-h	$Y = -1.295+2.682X$	7	0.962	9.777*
48-h	$Y = 0.247+2.045X$	6	0.951	7.658*
60-h	$Y = 0.488+2.057X$	6	0.952	7.507*
72-h	$Y = 0.628+2.104X$	5	0.964	3.834*
84-h	$Y = 1.205+1.942X$	5	0.958	3.645*
96-h	$Y = 1.556+1.922X$	4	0.950	2.386*
108-h	$Y = 1.556+1.922X$	4	0.950	2.386*

[a] number of nominal concentration for calculation.
[b] coefficient of correlation.
* significance at 95% level.

The LC_{50} values and their 95% confidence limits of ammonia-N and NH_3-N for *P. chinensis* N 2, Z 2, M 2 and PL 4 are shown in Figs. 5-8. The 24-h LC_{50} was 5.54, 7.42, 20.52 and 30.49 mg/l ammonia-N; and 0.25, 0.34, 1.08 and 1.85 mg/l NH_3-N for N 2, Z 2, M 2 and PL 4, respectively. The 72-h LC_{50} was 4.05 and 11.98 mg/l ammonia-N, and 0.21 and 0.73 mg/l NH_3-N for M 2 and PL 4. Susceptibility to ammonia was the greatest at the nauplius stage and the lowest at the postlarva stage among the various stages of larvage tested. The LC_{50} decreased with increasing exposure times for all stages of *P. chinensis* larvae tested. The LC_{50} sharply declined in 36 h for N 2 (Fig. 5), Z 2 (Fig. 6) and PL 4 (Fig. 8); and in 48 h for M 2 (Fig. 7). The "threshold" (a time when a response will be produced and below which it will

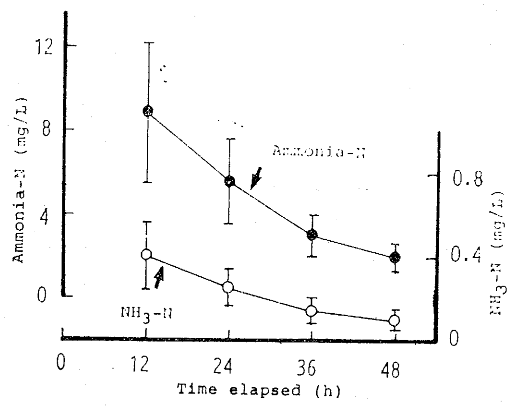

Fig. 5. The LC_{50} of ammonia-N and NH_3-N and their 95% confidence limits for *Penaeus chinensis* nauplius second substage (N2) at different periods of exposure.

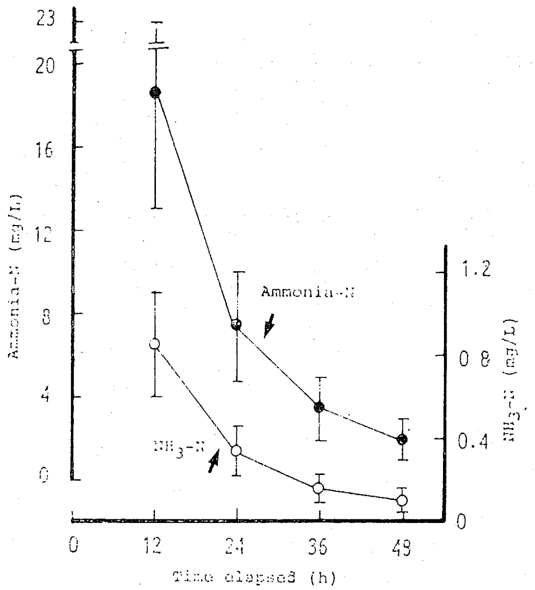

Fig. 6. The LC_{50} of ammonia-N and NH_3-N and their 95% confidence limits for *Penaeus chinensis* zoea second substage (Z2) at different periods of exposure.

not) for PL 4 was 96 h. The "incipient LC_{50}" (the LC_{50} for an exposure time in the asymptotic point of the toxicity curve) was determined to be 6.19 mg/l ammonia-N and 0.38 mg/l NH_3-N for PL 4 in 34 ppt at pH 8.16 and 25°C.

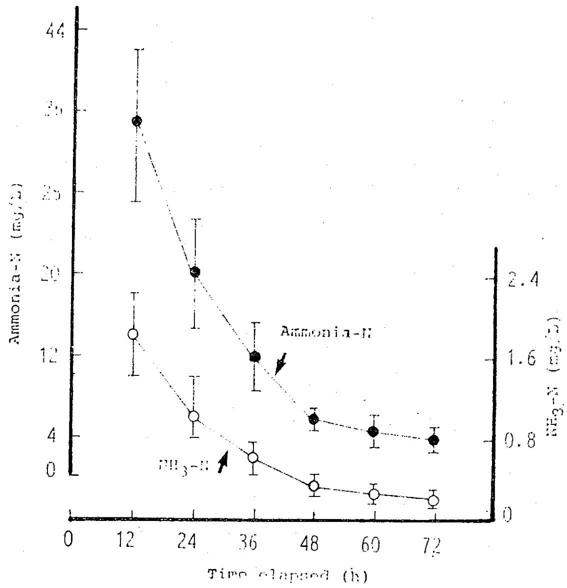

Fig. 7. The LC_{50} of ammonia-N and NH_3-N and their 95% confidence limits for *Penaeus chinensis* mysis second substage (M2) at different periods of exposure.

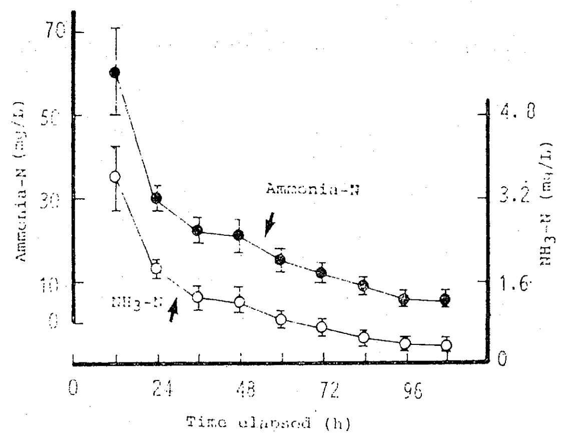

Fig. 8. The LC_{50} of ammonia-N and NH_3-N and their 95% confidence limits for *Penaeus chinensis* postlarva fourth substage (PL4) at different periods of exposure.

DISCUSSION

The toxicity of ammonia to various freshwater teleosts was reviewed (Alabaster and Lloyd, 1982; Johnson, 1985). Data on the toxic level of ammonia to freshwater prawns and penaeid postlarvae was reported ranging from 0.40 to 2.31 mg/l NH_3-N (Colt and Armstrong, 1981).

Wickins (1976) indicated that 48-h LC_{50} of ammonia on seven panaeid larvae (500–1,500 mg) in seawater, 33 ppt in salinity, pH 8.0, and 28°C, was 1.29 mg/l for NH_3-N and 24 mg/l for ammonia-N. The LC_{50} for each species at various developmental stages was not specified, neither were LC_{50} values for varying exposure durations reported.

The toxicities of ammonia on different stages of various penaeid larvae and the "safe levels" of ammonia for larval rearing have been reported elsewhere (Jayasankar and Muthu, 1983 for *Peneaus indicus*, Chin and Chen, 1987 for *P. monodon* and Chen *et al.*, 1989 for *P. japonicus*).

The tolerance of penaeid larvae to ammonia increased progressively from the nauplius stage towards the zoea stage. The 24-h LC_{50} was reported to be 0.29, 0.95 and 3.17 mg/l NH_3-N for nauplius, zoea and mysis larvae of *P. indicus* in 33–34 ppt salinity, pH 8.12–8.17 and 27–29°C (Jayasankar and Muthu, 1983). Chin and Chen (1987) reported that 24-h LC_{50} was 0.54, 0.76, 2.17 and 4.70 mg/l NH_3-N for *P. monodon* N 3, Z2, M2 and PL 6 in a salinity of 34 ppt at pH 8.2 and 29.5°C. The 48, 72 and 96-h LC_{50}s were 2.50, 1.54 and 1.04 mg/l NH_3-N for *P. monodon* PL6, respectively.

The 24-h LC_{50} on *P. japonicus* N 3, Z 2, M 2, PL 2 and PL 12 was 1.31, 0.97, 1.08, 1.98 and 3.34 mg/l for NH_3-N in 33 ppt at pH 8.10 and 30°C (Chen *et al.*, 1989). In comparison with the LC_{50} value of NH_3-N on *P. indicus* larvae (Jayasankar and Muthu, 1983), *P. monodon* larvae (Chin and Chen, 1987) and on *P. japonicus* larvae (Chen *et al.*, 1989), the results obtained from the present study indicate that nauplius larvae of *P. chinensis* are the most susceptible to ammonia. Furthermore, the tolerance of *P. chinensis* M 2 and PL 4 to ammonia was almost the same as that of *P. japonicus* M 2 and PL2.

All previous studies and the present study show that prawns can resist ammonia in greater amounts as they grew from the nauplius to the postlarva stage. In Contrast, *P. japonicus* zoea was the least tolerant, and no progressive tolerance increases to ammonia-N and NH_3-N was noted as the larvae developed.

Sprague (1969) reported that the short-term LC_{50} value could be misleading, and recommended that toxicity should be described in terms of "incipient LC_{50}". If this value cannot be estimated, the 96-h LC_{50} suggested by Armstrong *et al.*, (1978) is a useful substitute. The present study indicates that the 96-h LC_{50} and "incipient LC_{50}" for PL 4 are identical to 6.19 mg/l for ammonia-N and 0.38 mg/l for NH_3-N. "Incipient LC_{50}" is an important parameter in calculating a "safe level" from "incipient LC_{50}" by using an empirical "application facter" of 0.1 (Sprague, 1971). The "safe level" for rearing *P. chinensis* larvae in the hatchery was calculated to be 0.62 mg/l for ammonia-N and 0.04 mg/l for NH_3-N in 34 ppt at pH 8.16 and 25°C.

Acknowledgements: This research was supported partially by the National Science Council (Grant No. NSC 80-0209-B019-01). We would like to thank Dr. Y. Y. Ting and Dr. M. N. Lin, Tainan Branch, Taiwan Fisheries Research Institute and Mr. C. D. Chen, Hsin-Da Feed Company for providing animals and formulated powder feed.

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氨對中華對蝦蝦苗之致死劑量之研究

陳 建 初　　林 基 源

將不同時期之中華對蝦蝦苗以靜止 (static renewal) 換水方式暴露於一連串氨-氮 (未解離氨及解離氨以氮表示) 溶液中。在所有測試之蝦苗中，以無節幼蟲對氨最敏感，而以後期幼蟲之抵抗力最强。對於 N2 (無節幼蟲第二期)、Z2 (眼幼蟲第二期)、M2(糠蝦蟲第二期)、PL4 (後期幼蟲第四期) 之 24 小時 LC_{50} 分別爲 5.54、7.42、20.52、30.49 mg/ℓ 氨-氮 (鹽度 34 ppt、pH 8.16、水溫 21-25°C)，以及 0.25、0.34、1.08、1.85 mg/ℓ 未解離氨-氮 (未解離氨以氮表示)。對於 M2 及 PL4 之 72 小時 LC_{50} 分別爲 4.05 及 11.98 mg/ℓ 氨-氮以及 0.21 及 0.73 mg/ℓ 未解離氨-氮。氨對於 PL4 之“閾值”在 96 小時。對於 PL4 之 “incipient LC_{50}” 分別爲 6.19 mg/ℓ 氨-氮及 0.38 未解離氨-氮。0.62 mg/ℓ 氨-氮及 0.04 mg/ℓ 未解離氨-氮爲繁殖場培育中華對蝦蝦苗之安全基準。