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Acute Toxicity of Ammonia to Larval *Metapenaeus ensis*

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Abstract

Metapenaeus ensis larvae at various stages were exposed by static renewal method to different concentrations of ammonia (NH_3 + NH_4^+) in seawater at 33 ppt, pH 8.10 and temperature 30°C. The 24-hour median lethal concentration (LC_{50}) values were 10.33, 4.82, 35.97, 30.33 and 24.39 $mg \cdot l^{-1}$ ammonia-N (un-ionized plus ionized ammonia as nitrogen); and 0.65, 0.30, 2.25, 1.90 and 1.53 $mg \cdot l^{-1}$ NH_3-N (un-ionized ammonia as nitrogen) for nauplius third substage (N3), zoea second substage (Z2), mysis second substage (M2), postlarvae first and tenth substage (PL1, PL10). The 48-hour LC_{50} values for M2 and PL1 were 21.43 and 16.74 $mg \cdot l^{-1}$ ammonia-N, and 1.34 and 1.05 $mg \cdot l^{-1}$ NH_3-N. The *threshold time* was 108 hours and the *incipient* LC_{50} was 3.04 $mg \cdot l^{-1}$ ammonia-N and 0.22 $mg \cdot l^{-1}$ NH_3-N for PL1. The LC_{50} values of ammonia-N and NH_3-N decreased with longer exposure. Among the developmental stages of *M. ensis* larvae tested, zoea larvae were the least tolerant and mysis larvae the most tolerant to ammonia. The concentrations less than 0.30 $mg \cdot l^{-1}$ ammonia-N and 0.02 $mg \cdot l^{-1}$ NH_3-N are considered safe for rearing *M. ensis* in the hatchery.

Introduction

Metapenaeus ensis, Penaeus monodon, P. japonicus and *P. penicillatus* are the common penaeid species currently being cultured in Taiwan (Liao 1986). Though *M. ensis* does not grow as large as the other three penaeids, the demand for this species is high because it is a marketable delicacy preferred by consumers. The culture of this species has expanded very rapidly in Taiwan since 1983 (Liao and Chao 1983).

Ammonia, which originates from the ammonification, deamination and transamination of organic matter in water and ingested food by cultured animals, is the most common toxicant in an intensive culture system. The accumulation of ammonia has detrimental effects on shrimps, fish and molluscs (Colt and Armstrong 1981).

Ammonia-N (un-ionized plus ionized ammonia as nitrogen) and NH_3-N (un-ionized ammonia as nitrogen) could respectively increase to 0.80 mg·l^{-1} and 0.08 mg·l^{-1} in hatcheries and 6.50 mg·l^{-1} and 0.15 mg·l^{-1} in growout ponds of *P. monodon* even with frequent water exchange (Chen et al. 1986, 1989). Therefore, the accumulation of ammonia and its lethal effects on shrimps are the primary concern in an intensive culture system. This paper provides information on the lethal effects of ammonia to *M. ensis* larvae in the laboratory.

Materials and Methods

Two spawners caught in the southern coast of Taiwan released eggs naturally in the laboratory. The hatched larvae were reared to various larval stages for bioassay test. The shrimps used were nauplius third substage (N3), zoea second substage (Z2), mysis second substage (M2), postlarva first and tenth substage (PL1 and PL10).

Seawater (33 ppt) was filtered through sand and gravel and aerated one day before use. The composition of the seawater was the same as that reported by Chen et al. (1989).

Ammonia test solutions were prepared by dissolving 3.82 g of ammonium chloride (Merck reagent grade) with 1 l distilled water to make 1,000 mg·l^{-1} ammonia-N and then diluted to desired concentrations with seawater. The nominal concentrations ranged in geometric progression, from 1 to 32 and 6, 10 and 12 mg·l^{-1} for N3; from 2 to 64 and 12 and 24 mg·l^{-1} for Z2; from 2 to 64 and 24 mg·l^{-1} for M2 (see Table 1); from 2 to 64 and 24 and 96 mg·l^{-1} for PL1; from 16 to 128 and 24 mg·l^{-1} for PL10 (see Table 2). Concentrations of NH_3-N were calculated according to the equations of Bower and Bidwell (1978) based on a salinity of 33 ppt, a pH of 8.10 and a water temperature of 30°C.

The median lethal concentration (LC_{50}) toxicity tests were carried out following the methods described by Hubert (1980) and the American Public Health Association et al. (1985). Shrimps were sampled randomly from the holding tanks and exposed to each test

and control solutions in triplicate. Bioassay experiments to establish tolerance limits were conducted in 1-l test solutions. Each contained 15 test larvae for N3, Z2 and M2; and 10 test larvae for PL1 and PL10. Each beaker was placed in a water bath (30° ± 1°C) and aerated. The shrimps were fed artificial plankton BP (Nippai Co., Ltd., Japan) for Z2, M2, PL1 and PL10. Postlarvae were also fed freshly hatched *Artemia nauplii.* However, the nauplius larvae were not fed.

Each test solution was renewed daily and the static with renewal method was employed for the toxicity tests (Buikema et al. 1982). In all test solutions, dissolved oxygen was 5.6-7.1 $mg \cdot l^{-1}$. The pH values fluctuated from 7.90 to 8.22 in nauplius, 7.85 to 8.17 in zoea, 7.84 to 8.20 in mysis and 7.82 to 8.18 in postlarvae.

Observations were usually made at 12-hour intervals for up to 24 hours for N3 and Z2, 48 hours for M2, 132 hours for PL1 and up to 96 hours for PL10. The median lethal time (LT_{50}), the time required to kill half of the population, was determined. The shrimps were assumed dead when they became immobile and showed no response when beakers were shaken gently. The dose response of test organisms combined from triplicate beakers of each test solution was determined for the LC_{50} values of ammonia-N and NH_3-N and their 95% confidence limits from a microcomputer program (Trevors and Lusty 1985) based upon a method described by Hubert (1980). With this program, the estimated probit line and the result of a chi-square test for goodness of fit were computed.

Results

Tables 1 and 2 list the percentage mortality at different development stages. Only one N3 was killed in the control solution for 24 hours of exposure.

All Z2 exposed to over 32 $mg \cdot l^{-1}$ ammonia-N, and to 24 $mg \cdot l^{-1}$ were killed at 12 and 24 hours, respectively. No Z2 died in the control solution for 24 hours of exposure.

All M2 exposed to 64 $mg \cdot l^{-1}$ ammonia-N were killed at 24 hours. However, those exposed to 16, 8, 4 and 2 $mg \cdot l^{-1}$ resulted in no mortality after 12, 24, 24 and 36 hours, respectively. Only one M2 was killed in the control solution for 48 hours of exposure.

All PL1 exposed to 96, 64, 32 and 24 $mg \cdot l^{-1}$ ammonia-N were killed at 12, 24, 72 and 84 hours, respectively. However, those

Table 1. Percentage mortality of *Metapenaeus ensis* 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 ($mg \cdot l^{-1}$)	Time elapsed (hours) 12	24	36	48
Nauplius third substage (N3)				
1	4.4	11.1		
2	4.4	13.3		
4	13.3	17.8		
6	15.6	20.0		
8	28.9	33.3		
10	37.8	44.4		
12	57.8	66.7		
16	66.7	73.3		
32	88.9	97.8		
Zoea second substage (Z2)				
2	11.1	33.3		
4	26.7	40.0		
8	48.9	64.4		
12	53.3	73.3		
16	53.3	73.3		
24	68.9	100		
32	100	-		
64	100	-		
Mysis second substage (M2)				
2	0	0	0	6.7
4	0	0	4.4	11.1
8	0	0	11.1	24.4
16	0	6.7	24.4	35.6
24	11.1	22.2	35.6	48.9
32	24.4	40.0	53.3	71.1
64	51.1	100	-	-

exposed to 8 $mg \cdot l^{-1}$ after 24 hours; to 4 and 2 $mg \cdot l^{-1}$ after 36 hours survived. No PL1 died in the control solution for 132 hours of exposure.

All PL10 exposed to 128, 64 and 32 $mg \cdot l^{-1}$ ammonia-N were killed at 12, 24 and 48 hours, respectively. Only one PL10 was killed in the control after 96 hours of exposure.

LT_{50} value is an indicator to determine the extent of a toxicant on aquatic animals. The LT_{50} value for Z2 exposed to 8 $mg \cdot l^{-1}$

ammonia-N, and for PL10 exposed to 24 and 32 $mg \cdot l^{-1}$ ammonia-N was 12.8, 36 and 18.8 hours, respectively. The LT_{50} value for M2 and PL1 showed linear relationships with both ammonia-N and NH_3-N (Figs. 1 and 2).

Table 2. Percentage mortality of *Metapenaeus ensis* postlarva first and tenth substage (PL1 and PL10) exposed to different concentrations of ammonia-N after various periods of exposure.

Ammonia-N (mg/l)	Time elapsed (hours) 12	24	36	48	60	72	84	96	108	120	132
Postlarva first substage (PL1)											
2	0	0	0	3.3	26.7	40.0	40.0	40.0	40.0	40.0	40.0
4	0	0	0	16.7	40.0	53.3	53.3	53.3	60.0	60.0	60.0
8	0	0	3.3	23.3	40.0	56.7	56.7	60.0	66.7	66.7	66.7
16	6.7	10	16.7	40.0	60.0	80.0	86.7	86.7	86.7	86.7	86.7
24	6.7	23.3	33.3	46.7	83.3	93.3	100	-	-	-	-
32	23.3	60.0	80.0	86.7	93.3	100	-	-	-	-	-
64	83.3	100	-	-	-	-	-	-	-	-	-
96	100	-	-	-	-	-	-	-	-	-	-
Postlarva tenth substage (PL10)											
16	10.0	20.0	23.3	23.3	26.7	26.7	26.7	26.7			
24	13.3	40.0	50.0	50.0	56.7	60.0	60.0	60.0			
32	16.7	76.7	90.0	100	-	-	-	-			
64	90.0	100	-	-	-	-	-	-			
128	100	-	-	-	-	-	-	-			

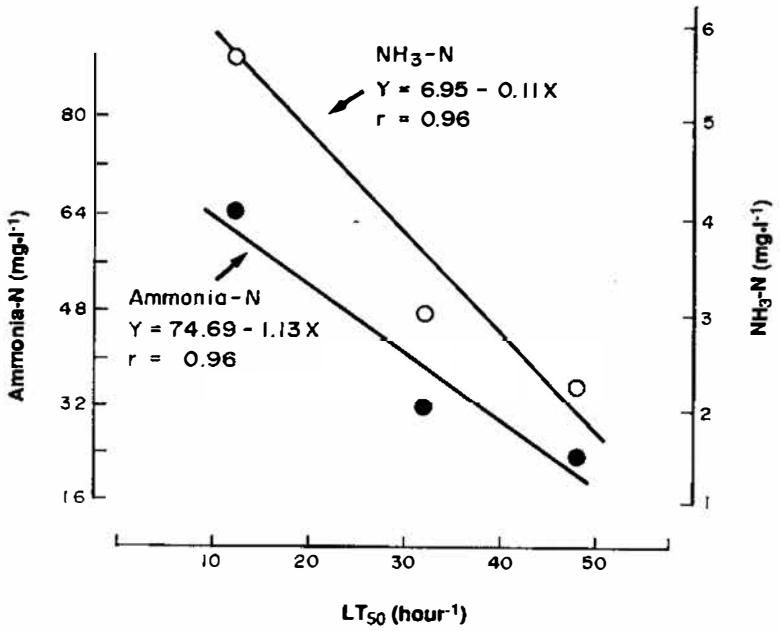

Fig. 1. The LT_{50} (time required to kill half of the population) for ammonia-N and NH_3-N on *Metapenaeus ensis* mysis second substage (M2).

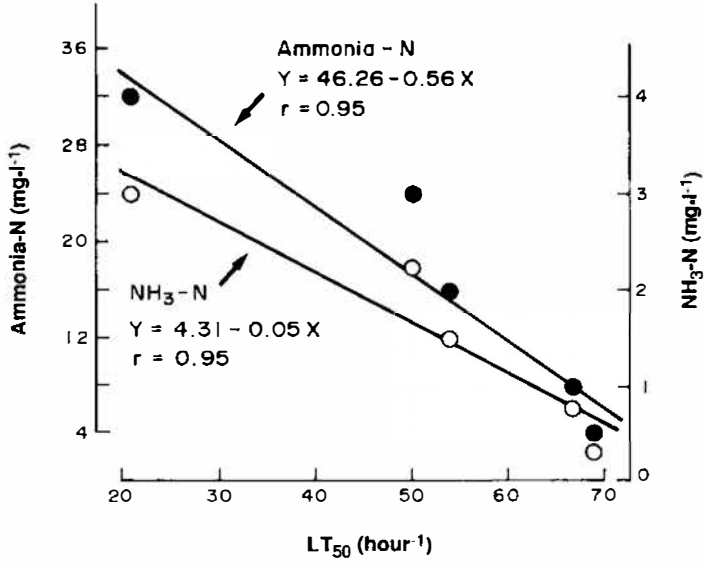

Fig. 2. The LT_{50} (time required to kill half of the population) for ammonia-N and NH_3-N on *Metapenaeus ensis* postlarva first substage (PL1).

The probit of mortality of the larvae exposed to ammonia-N had a positive linear relationship with log ammonia-N concentration at various times of exposure. The results of the chi-square test also indicated that all the estimated lines are satisfactory.

The LC_{50} values and their 95% confidence limits of ammonia-N and NH_3-N for *M. ensis* N3, Z2, M2, PL1 and PL10 are shown in Figs. 3-7. The 24-hour LC_{50} values were 10.33, 4.82, 35.97, 30.33 and 24.48 $mg \cdot l^{-1}$ ammonia-N; and 0.65, 0.30, 2.25, 1.90 and 1.53 $mg \cdot l^{-1}$ NH_3-N for N3, Z2, M2, PL1 and PL10. The 48-hour LC_{50} values for M2 and PL1 were 21.43 and 16.74 $mg \cdot l^{-1}$ ammonia-N, and 1.34 and 1.05 $mg \cdot l^{-1}$ NH_3-N. Susceptibility to ammonia was the greatest at zoea stage and the lowest at mysis stage among the various stages of larvae tested. The LC_{50} decreased with increased exposure time for all stages of *M. ensis* larvae tested. The LC_{50} sharply declined in 24 hours for M2, and in 60 hours for PL1. The *threshold time* (a time which response will be produced and below which it will not) for PL1 was 108 hours. The *incipient* LC_{50} or *threshold concentration* (the LC_{50} for an exposure time in the asymptotic point of the toxicity curve) was determined to be 3.04 $mg \cdot l^{-1}$ ammonia-N and 0.22 $mg \cdot l^{-1}$ NH_3-N for PL1. Unfortunately, we could not obtain LC_{50} values for more than 36 hours of exposure and *incipient* LC_{50} for PL10 from the concentrations tested.

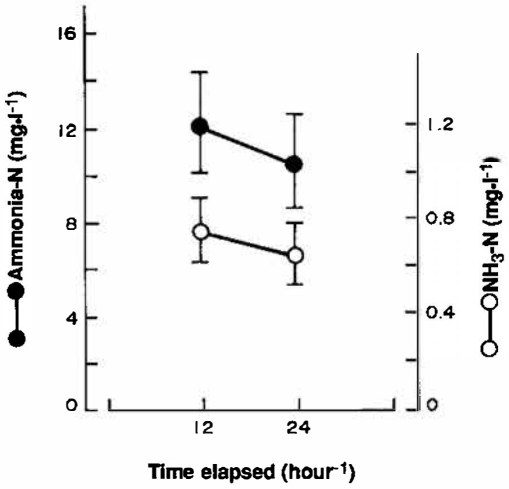

Fig. 3. The toxicities of ammonia-N and NH_3-N and their 95% confidence limits to *Metapenaeus ensis* nauplius third substage (N3) in 33 ppt seawater at a pH of 8.10 and a water temperature of 30°C.

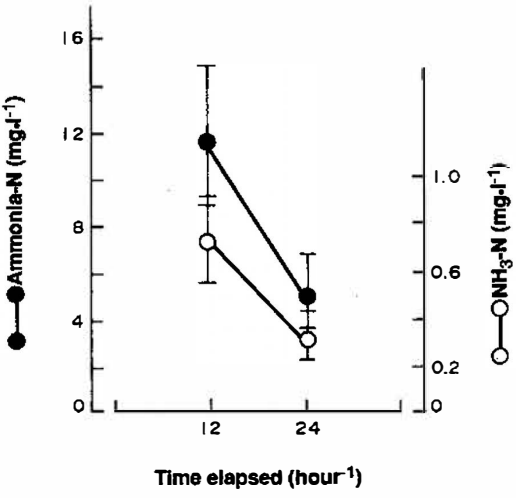

Fig. 4. The toxicities of ammonia-N and NH_3-N and their 95% confidence limits to *Metapenaeus ensis* zoea second substage (Z2) in 33 ppt seawater at a pH of 8.10 and a water temperature of 30°C.

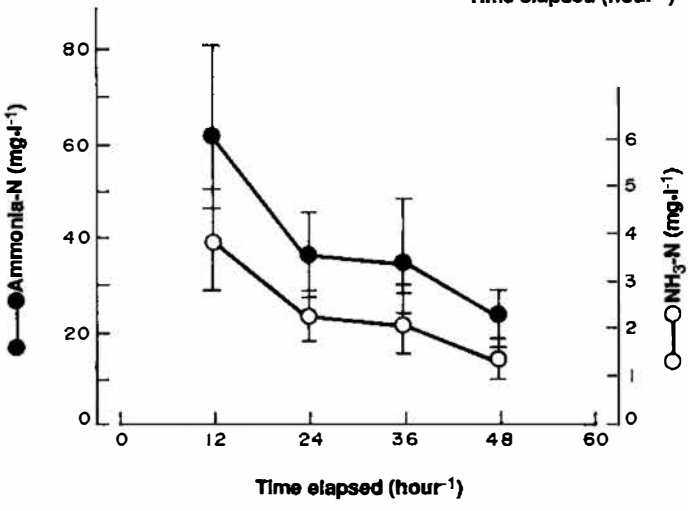

Fig. 5. The toxicities of ammonia-N and NH_3-N and their 95% confidence limits to *Metapenaeus ensis* mysis second substage (M2) in 33 ppt seawater at a pH of 8.10 and a water temperature of 30°C.

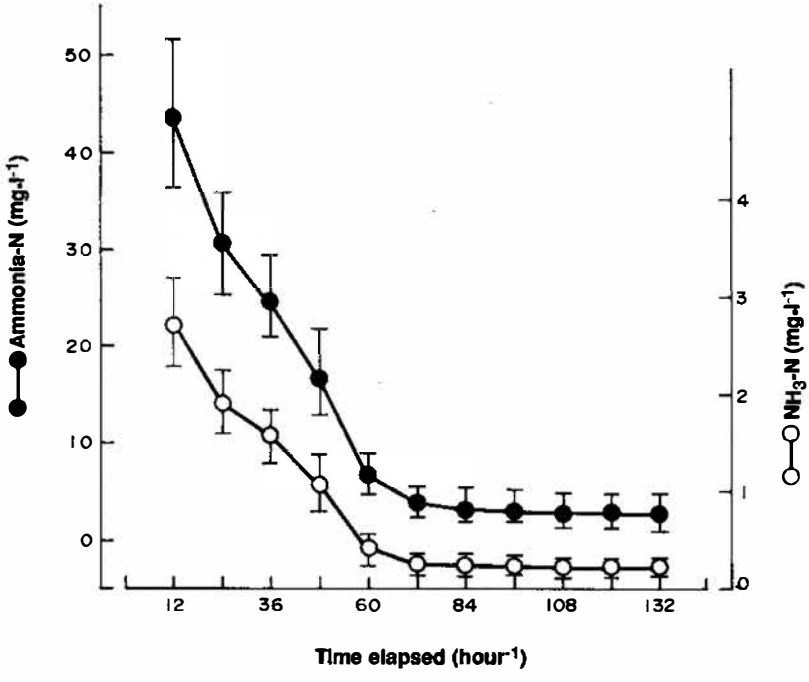

Fig. 6. The toxicities of ammonia-N and NH_3-N and their 95% confidence limits to *Metapenaeus ensis* postlarva first substage (PL1) in 33 ppt seawater at a pH of 8.10 and a water temperature of 30°C.

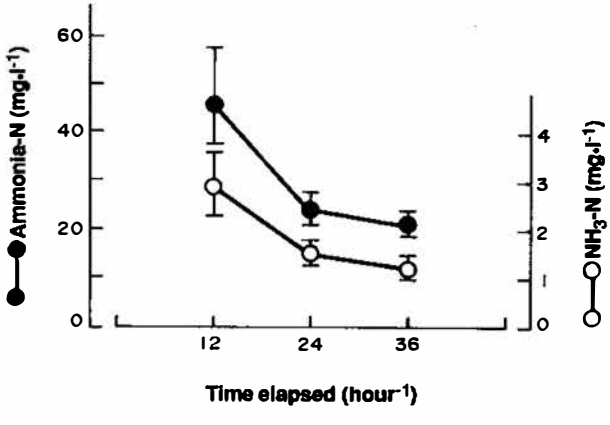

Fig. 7. The toxicities of ammonia-N and NH_3-N and their 95% confidence limits to *Metapenaeus ensis* postlarva tenth substage (PL10) in 33 ppt seawater at a pH of 8.10 and a water temperature of 30°C.

Discussion

Information about the toxicity of ammonia on freshwater fish and invertebrates 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 (Wickins 1976; Delistraty et al. 1977; Jayasankar and Muthu 1983; Chin and Chen 1987).

Wickins (1976) indicated that the 48-hour LC_{50} value of ammonia on seven penaeid larvae (500-1,500 mg) in seawater of 33 ppt and 28°C was 1.29 $mg \cdot l^{-1}$ NH_3-N. Unfortunately, he did not specify the LC_{50} value for each species and at various stages, neither reported LC_{50} values at different times of exposure.

Jayasankar and Muthu (1983) reported that the 24-hour LC_{50} value was 0.29, 0.95 and 3.17 $mg \cdot l^{-1}$ 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. The 48-hour LC_{50} value was 1.18 $mg \cdot l^{-1}$ NH_3-N on *P. indicus* zoea. This revealed that the tolerance of *P. indicus* to ammonia increased progressively as the nauplius metamorphosed to zoea and mysis stage.

Chin and Chen (1987) reported that the 24-hour LC_{50} value was 0.54, 0.76, 2.17 and 4.70 $mg \cdot l^{-1}$ NH_3-N in a salinity of 34 ppt at pH 8.2 and 29.5°C. The 48-, 72- and 96-hour LC_{50} values on *P. monodon* postlarva sixth substage (PL6) were 2.50, 1.54 and 1.04 $mg \cdot l^{-1}$ NH_3-N, respectively. This indicated that the tolerance of *P. monodon* to ammonia also increased progressively as the larvae developed from nauplius to zoea, mysis and postlarva stage. In comparison with the LC_{50} values of NH_3-N on *P. indicus* larvae (Jayasankar and Muthu 1983) and on *P. monodon* larvae (Chin and Chen 1987), the present study indicates that the nauplius larvae of *M. ensis* are more tolerant than those of *P. monodon* and *P. indicus*. However, the zoea and mysis larvae of *M. ensis* were less tolerant than those of *P. monodon* and *P. indicus*. In addition, *M. ensis* PL1 and PL10 were less tolerant than *P. monodon* PL6.

All previous studies showed that the shrimps could resist ammonia as they grew from nauplius to postlarva stage, but this is not true in *M. ensis*. No progressive increase with tolerance to ammonia-N and NH_3-N, was noted as the larvae developed. Mysis was the most resistant and zoea was the least resistant to ammonia. Since the larval development period from nauplius to zoea, mysis,

and finally postlarva lasts only 3-4 days, the LC_{50} values for over 24 hours of exposure for nauplius and zoea were not obtainable.

Sprague (1969) reported that the short-term LC_{50} value could be misleading, and recommended that toxicity be described in terms of *incipient* LC_{50} or *threshold concentration.* If this value cannot be estimated, the 96-hour LC_{50} value is a useful substitute suggested by Armstrong et al. (1978). The present study indicates that the 96-hour LC_{50} and *incipient* LC_{50} for PL1 are 3.50 and 3.04 $mg \cdot l^{-1}$ ammonia-N (0.22 and 0.19 $mg \cdot l^{-1}$ NH_3-N). *Incipient* LC_{50} is an important parameter in calculating a "safe level" from *incipient* LC_{50} by using an empirical "application factor" of 0.1 (Sprague 1971). The "safe level" for rearing *M. ensis* in the hatchery was calculated to be 0.30 $mg \cdot l^{-1}$ ammonia-N and 0.02 $mg \cdot l^{-1}$ NH_3-N in 33 ppt seawater at pH 8.20 and 30°C.

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References

Alabaster, J.S. and R. Lloyd. 1982. Ammonia, p. 85-102. *In* J.S. Alabaster and R. Lloyd (eds.) Water quality for freshwater fish. Food and Agriculture Organization of the United Nations, Butterworths.

American Public Health Association. American Water Works Association and Water Pollution Control Federation. 1985. Standard methods for the examination of water and wastewater. 16th edition. American Public Health Association, Washington, DC.

Armstrong, D.A., M.J. Stephenson and A.W. Knight. 1976. Acute toxicity of nitrite to larvae of the giant Malaysian prawn, *Macrobrachium rosenbergii.* Aquaculture 9: 39-46.

Armstrong, D.A., D. Chippendale, A.W. Knight and J.E. Colt. 1978. Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii.* Biol. Bull. 154: 15-31.

Bower, C.E. and J.P. Bidwell. 1978. Ionization of ammonia in seawater: effects of temperature, pH and salinity. J. Fish. Res. Board Can. 35(7): 1012-1016.

Buikema, A.L. Jr., R.R. Niedertehner and J. Cairns, Jr. 1982. Biological monitoring. Part IV. Toxicity testing. Water Res. 16: 239-262.

Chen, J.C., C.C. Tu and W.S. Yang. 1989. Acute toxicity of ammonia to larval *Penaeus japonicus.* J. Fish. Soc. Taiwan 14: 261-270.

Chen, J.C., T.S. Chin and C.K. Lee. 1986. Effects of amonia and nitrite on larval development of the shrimp *Penaeus monodon,* p. 657-662. *In* J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

Chen, J.C., P.C. Liu, Y.T. Lin and C.K. Lee. 1989. Highly-intensive culture study of tiger prawn *Penaeus monodon* in Taiwan, p. 377-382. *In* N. De Pauw, E. Jaspers, N. Ackefors and N. Wilkins (eds.) Aquaculture: a biotechnology in progress. European Aquaculture Society, Bredene, Belgium.

Chin, T.S. and J.C. Chen. 1987. Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. Aquaculture 66: 247-253.

Colt, J.E. and D.A. Armstrong. 1981. Nitrogen toxicity to crustaceans, fish and molluscs, p. 34-47. *In* L.J. Allen and E.C. Kinney (eds.) Proceedings of the Bio-Engineering Symposium for Fish Culture. Fish Culture Section, American Fisheries Society, Northeast Society of Conservation Engineerings, Bethesda, Maryland.

Delistraty, D.A., J.M. Carlberg, J.C. Van Olst and R.F. Ford. 1977. Ammonia toxicity in cultured larvae of the American lobster *(Homarus americans)*, p. 647-672. *In* J.W. Avault (ed.) Proceedings of the Eighth Annual Meeting, World Mariculture Society, Louisiana State University, Baton Rouge.

Hubert, J.J. 1980. Bioassay. Kendall Hunt Publishing Co., Toronto, Canada.

Jayasankar, P. and M.S. Muthu. 1983. Toxicity of ammonia to the larvae of *Penaeus indicus*. H. Milne Edwards. Indian J. Fish. 30(1): 1-12.

Johnson, E.J. 1985. Ambient water quality for ammonia-1984. EPA 440/5-85-001. U.S. Environmental Protection Agency, Duluth, Minnesota, 217 p.

Liao, I.C. 1986. General introduction to the prawn pond system in Taiwan. Aquacult. Eng. 5: 219-233.

Liao, I.C. and N.H. Chao. 1983. Development of prawn culture and its related studies, p. 127-142. *In* G.L. Rogers, R. Day and A. Lim (eds.) The First International Conference on Warm Water Aquaculture-Crustacea. Brigham Young University Hawaii Campus, Hawaii, USA.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish-I. Bioassay methods for acute toxicity. Water Res. 3: 794-821.

Sprague, J.B. 1971. Measurement of pollutant toxicity to fish-III. Sublethal effects and "safe" concentrations. Water Res. 5: 245-266.

Trevors, J.V. and C.W. Lusty. 1985. A basic microcomputer program for calculating LD_{50} values. Water Air Soil Pollut. 24: 431-442.

Wickins, J.F. 1976. The tolerance of warm-water prawn to recirculated water. Aquaculture 9: 19-37.

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