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LETHAL AND SUBLETHAL EFFECTS OF AMMONIA TO *PENAEUS PENICILLATUS* JUVENILES

JIANN-CHU CHEN and JIN-NIEN LIN

Department of Aquaculture,
National Taiwan Ocean University,
Keelung, Taiwan 20224, Republic of China

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Jiann-Chu Chen and Jin-Nien Lin (1991) Lethal and sublethal effects of ammonia to *Penaeus penicillatus* juveniles. *Bull. Inst. Zool., Academia Sinica* **30**(2): 73-80. *Penaeus penicillatus* juveniles were exposed by a static renewal method to different concentrations of ammonia ($NH_3+NH_4^+$) in seawater at 25 ppt, pH 8.2 and 26°C. The 24, 48, 96, 120, 144 and 180 h LC_{50} of ammonia to *P. penicillatus* juveniles (0.41 $\pm$0.09 g wet weight) were 108, 71.1, 46.6, 43.0, 39.4 and 33.9 mg/ℓ for ammonia-N (un-ionized plus ionized plus ionized ammonia as nitrogen) and 8.16, 5.37, 3.52, 3.25, 2.98 and 2.56 ml/ℓ for NH_3-N (un-ionized ammonia as nitrogen), respectively. The "incipient LC_{50}" was 32.0 mg/ℓ for ammonia-N and 2.42 mg/ℓ for NH_3-N from a toxicity curve approaching an asymptote. Relative weight gains of *P. penicillatus* juveniles (0.20$\pm$0.03 g wet weight) exposed to 5 and 10 mg/ℓ ammonia-N (0.38 and 0.76 mg/ℓ NH_3-N) were 123.5% and 58.5%. Relative length increases of *P. penicillatus* juveniles exposed to 5 and 10 mg/ℓ ammonia-N were 65.1% and 78.5%, compared to those exposed to the control solution. Based on the "incipient LC_{50}" and an application factor of 0.1, the "safe level" for rearing *P. penicillatus* juveniles was 3.20 mg/ℓ ammonia-N and 0.24 mg/ℓ NH_3-N.

Key words: *Penaeus penicillatus*, Juveniles, Ammonia, Lethal, Sublethal.

In an intensive culture system, ammonia enters the water primarily from ammonification of organic matter such as unconsumed food and dead organisms, and from deamination and transamination through the digestion and assimilation of ingested food by cultured animals.

Ammonia is a common toxicant in a culture system and is detrimental to fishes and crustaceans. The 96-h LC_{50} of NH_3-N (un-ionized ammonia as nitrogen) ranges from 0.40 to 2.31 mg/ℓ for crustaceans (Colt and Armstrong, 1981).

Toxicities of ammonia to different stages of *Penaeus monodon* larvae have been reported (Chin and Chen, 1987; Chen and Chin, 1988). However, there is no information on the lethal and sublethal effects of ammonia on *P. penicillatus*, which is considered an ideal species for intensive cultivation due to its resistance to low temperatures (Liao, 1988). This study provides information on lethal and sublethal effects of ammonia on *P. penicillatus* juveniles.

MATERIALS AND METHODS

Acute Toxicity Test

P. penicillatus juveniles (3.36-4.53 cm, 0.17-0.50 g wet weight) from a private nursery were acclimated in the laboratory for one week. The shrimps used had an average length of 4.16±0.46 cm and weight of 0.41±0.09 g (wet weight+Std). Seawater pumped from the Keelung coast was filtered through sand gravel filters and aerated for two days before use. Salinity was adjusted to 25 ppt with tap water which had been dechlorinated with sodium thiosulfate. The chemical characteristics of the water used were the same as those reported before (Chen *et al.*, 1990).

Ammonia test solutions were prepared by dissolving 3.82 g of ammonium chloride (Merck reagent grade) in 1 ℓ distilled water to make 1,000 mg/ℓ ammonia-N (unionized plus ionized ammonia as nitrogen), and then diluted to desired concentrations. The nominal concentrations of ammonia-N used were 10 to 40 mg/ℓ at 5 mg/ℓ increments, 60, 80, 120 mg/ℓ (Table 1).

The shrimps were exposed to each test and the control solution in duplicate tanks. Bioassay experiments to establish tolerance limits were conducted in 15 ℓ circular tanks containing 10 ℓ of test solution (Hubert, 1980; American Public Health Association, 1985). Each tank contained 15 test animals and was aerated by an air stone with a blower. Each test solution was renewed daily, in accordance with the static renewal method (Buikema *et al.*, 1982).

Water temperature was maintained at 25-27°C, dissolved oxygen was 5.46-6.12 mg/ℓ and pH varied from 8.1 to 8.3 during the experiment. The shrimps were fed with commercial shrimp food (Tairoun Products Co. Ltd., Taipei, Taiwan) three times a day (8:00, 16:00 and 22:00) at a ration of 7% of their body weight each time. The dose response of test organisms from each test solution was determined and the LC_{50} values of ammonia-N and their 95% confidence limits were computed using a computer program (Trevors and Lusty, 1985). Concentration of NH_3-N was calculated according to equations of

Table 1
Percentage mortality of *Penaeus penicillatus* juveniles after various periods of exposure to different concentrations of ammonia-N

Time Elapsed (h)	Concentration of Ammonia-N (mg/ℓ)								
	15	20	25	30	35	40	60	80	120
12	0	0	0	0	0	3.3	6.7	16.7	60
24	0	0	0	0	3.3	10	13.3	36.7	100
36	0	0	0	3.3	3.3	16.7	23.3	70	—
48	0	0	0	6.7	6.7	20	36.7	100	—
60	0	0	3.3	6.7	6.7	23.3	43.3	—	—
72	0	0	3.3	6.7	6.7	26.7	56.7	—	—
84	0	0	3.3	6.7	6.7	33.3	70	—	—
96	0	0	6.7	6.7	10	33.3	83.3	—	—
108	0	0	6.7	6.7	13.3	33.3	83.3	—	—
120	0	0	6.7	6.7	13.3	36.7	93.3	—	—
132	0	3.3	10	10	16.7	40	93.3	—	—
144	0	6.7	16.7	13.3	23.3	50	93.3	—	—
156	0	6.7	16.7	13.3	33.3	53.3	96.7	—	—
168	6.7	10	20	20	53.3	56.7	100	—	—
180	6.7	10	20	56.7	56.7	56.7	—	—	—
192	6.7	10	20	56.7	66.7	63.3	—	—	—
204	6.7	10	20	56.7	66.7	63.3	—	—	—
216	6.7	10	56.7	56.7	66.7	63.3	—	—	—

Bower and Bidwell (1978) based on a salinity of 25 ppt, a pH of 8.2 and 26°C.

Chronic Toxicity Test

Shrimps (3.52 ± 0.17 cm, 0.20 ± 0.03 g wet weight) were exposed to the control, 5 and 10 mg/ℓ ammonia-N in duplicate tanks by the static renewal method for 240 h, the same as the acute toxicity test. The weight and length of the shrimps were recorded at the beginning and the end of the experiment. Duncan's Multiple Range Test was used to evaluate the sublethal effects of ammonia. This comparison was accomplished by using the statistical analysis system on a PC computer.

RESULTS

Acute Toxicity Test

All of the shrimps exposed to 120, 80 and 60 mg/ℓ ammonia-N were killed after 24, 48 and 168 h, respectively. However, those exposed to 35, 30, 25, 20 and 15 mg/ℓ survived after 12, 24, 48, 120 and 156 h (Table 1). No shrimp died in the control solution after 216 h of exposure.

The LT_{50}'s (median lethal time, time required to kill half of the population) for juveniles exposed to 80, 60, 40 and 35 mg/ℓ ammonia-N (6.08, 4.56, 3.04 and 2.66 mg/ℓ NH_3-N), were 28.6, 66, 144 and 166 h (Fig. 1).

The probit of mortality of the larvae exposed to ammonia-N had a positive linear relationship with log ammonia-N at various times of exposure (Table 2). The results of the chi-square test also indicated that all values of chi-square are less than the table values suggesting that all of the estimated lines are satisfactory (Trevors and Lusty, 1985).

The LC_{50} of ammonia-N and NH_3-N and their 95% confidence limits are shown in Fig. 2. This revealed that the LC_{50} decreased with increasing exposure times. The LC_{50} declined from 12 to 36 h and then stabilized to reach an asymptote. The

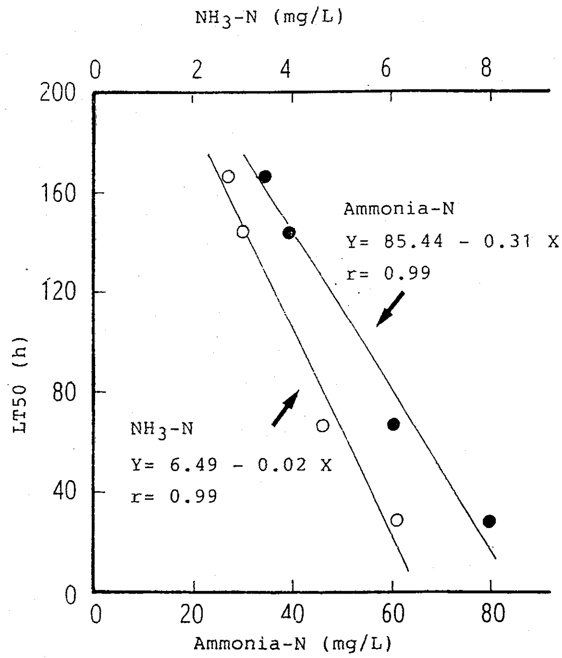

Fig. 1. The LT_{50} (median lethal time, time required to kill half of the population) of ammonia-N and NH_3-N for *Penaeus penicillatus* juveniles.

"incipient LC_{50}" (the LC_{50} for an exposure time at the asymptotic point of the toxicity curve) was determined to be 32.0 mg/ℓ for ammonia-N and 2.43 mg/ℓ for NH_3-N (Fig. 2).

Chronic Toxicity Test

All of the shrimps exposed to control, 5 and 10 mg/ℓ ammonia-N survived after 240 h. Weight gain percentages of the shrimps exposed to control, 5 and 10 mg/ℓ ammonia-N (0.38 and 0.76 mg/ℓ NH_3-N) were 81.0%, 100% and 47.4% (Table 3). However, no significant difference ($p > 0.05$) in weight gain was indicated between the shrimps exposed to control and 5 mg/ℓ ammonia-N. Weight gain of the shrimps exposed to 10 mg/ℓ ammonia-N was significantly lower ($p < 0.05$) than those exposed to 5 mg/ℓ ammonia-N and control (Table 3).

Length increase percentages of the shrimps exposed to control, 5 and 10 mg/ℓ ammonia-N were 23.3%, 15.2% and 18.3%

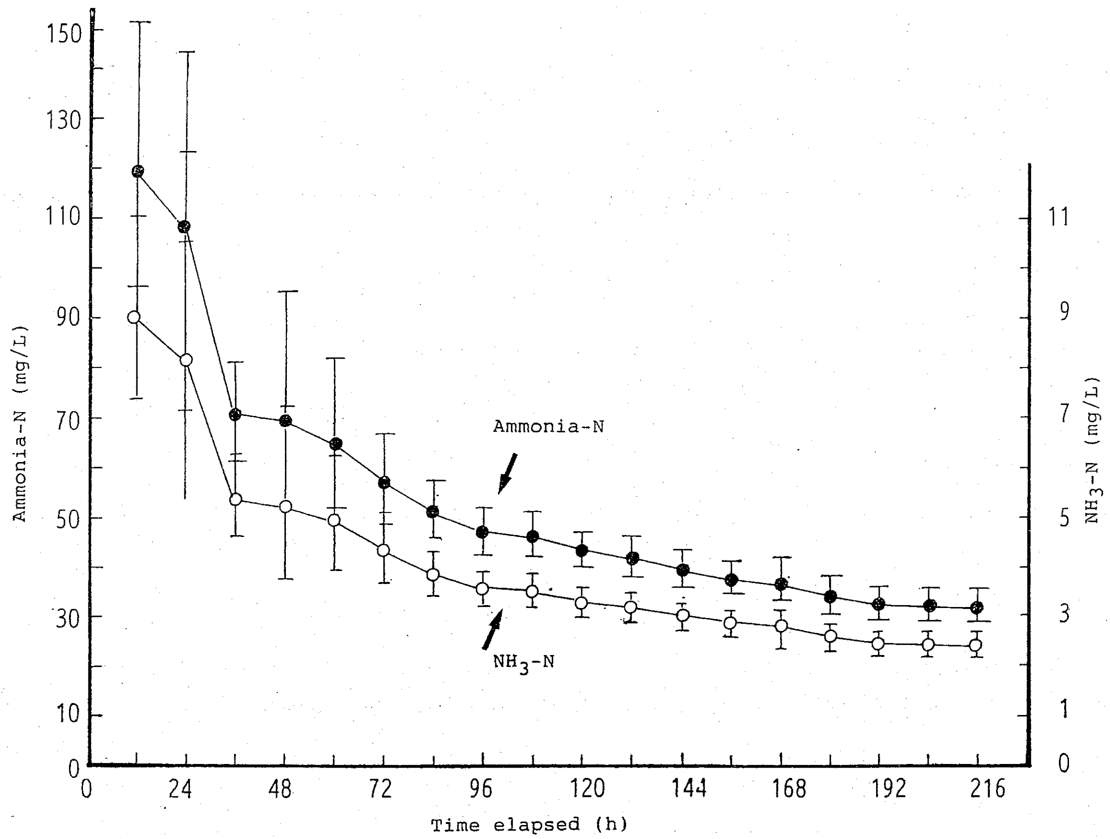

Fig. 2. The toxicities of ammonia-N and NH_3-N and their 95% confidence limits to *Penaeus penicillatus* juveniles in 25 ppt seawater at pH 8.20 and 26°C. Data expressed as mean±SEM ($n=2$).

Table 2
Relationship between probit of mortality (Y) and log ammonia-N concentration as mg/ℓ (X) at various exposure times for *Penaeus penicillatus* juveniles

Time (h)	$Y=a+bX$	N	r	χ^2
12-h	$Y=-4.279+4.474X$	4	0.959	2.603
24-h	$Y=-2.266+3.576X$	4	0.938	1.571
36-h	$Y=-4.944+5.388X$	5	0.953	5.061
48-h	$Y=-2.933+4.284X$	4	0.944	1.217
60-h	$Y=-3.501+4.693X$	5	0.960	1.792
72-h	$Y=-4.837+5.603X$	5	0.963	2.053
84-h	$Y=-6.420+6.692X$	5	0.959	3.349
96-h	$Y=-6.907+7.136X$	5	0.960	4.345
108-h	$Y=-6.829+7.106X$	5	0.970	3.465
120-h	$Y=-8.922+8.524X$	5	0.964	5.052
132-h	$Y=-6.056+6.826X$	6	0.954	6.889
144-h	$Y=-4.950+6.234X$	6	0.950	7.281
156-h	$Y=-5.783+6.858X$	6	0.960	6.562
168-h	$Y=-1.607+4.160X$	6	0.946	4.295
180-h	$Y=-2.216+4.715X$	6	0.950	5.529
192-h	$Y=-2.896+5.247X$	6	0.957	5.267
204-h	$Y=-2.896+5.247X$	6	0.957	5.267
216-h	$Y=-2.896+5.247X$	6	0.957	5.267

N denotes number of nominal concentrations for calculation.
r dentoes correlation coefficient.

Table 3
Effect of ammonia after 240 h of exposure on weight of *Penaeus penicillatus* juvenile (0.20±0.03 g wet weight) at salinity 25 ppt, pH 8.2 and temperature 26°C

Concentration (mg/ℓ)		No. of	Initial Weight	Final Weight	Weight Gain	Relative Weight
Ammonia-N	NH_3-N	Animals	(g)	(g)	(%)	Gain
Control	—	30	0.21±0.007[a]	0.38±0.013[a]	81.0[a]	100
5	0.38	30	0.17±0.006[a]	0.34±0.011[a]	100.0[a]	123.5
10	0.76	30	0.19±0.006[a]	0.28±0.009[b]	47.4[b]	58.5

Data in the same column having different superscripts are significantly different ($p<0.05$). Data expressed as mean±SEM ($n=30$).

Table 4
Effect of ammonia after 240 h of exposure on length of *Penaeus penicillatus* juvenile (0.20±0.03 g wet weight) at salinity 25 ppt, pH 8.2, and temperature 26°C

Concentration (mg/ℓ)		No. of	Initial Length	Final Length	Length Increase	Relative Length
Ammonia-N	NH_3-N	Animals	(cm)	(cm)	(%)	Increase
Control	—	30	3.69±0.64[a]	4.55±0.66[a]	23.3[a]	100
5	0.38	30	3.36±0.38[a]	3.87±0.53[b]	15.2[b]	65.1
10	0.76	30	3.39±0.62[a]	4.01±0.64[b]	18.3[b]	78.5

Data in the same column having different superscripts are significantly different ($p<0.05$). Data expressed as mean±SEM ($n=30$).

(Table 4). Length increases of the juveniles exposed to 5 and 10 mg/ℓ ammonia-N were significantly lower ($p<0.05$) than those exposed to control. However, no significant differences ($p>0.05$) were found in the length increase percentages between the shrimps exposed to 5 and 10 mg/ℓ ammonia-N (Table 4).

DISCUSSION

Concerning the toxicities of ammonia to penaeid shrimps, Wickins (1976) reported that the 48 h LC_{50} of NH_3-N to seven penaeid species postlarvae (0.5-1.5 g wet weight), *P. aztecus*, *P. japonicus*, *P. occidentalis*, *P. orientalis*, *P. schmitti*, *P. semisulcatus* and *P. setiferus* was 1.29 mg/ℓ which corresponded to 24 mg/ℓ ammonia-N at pH 8.0, and 227 mg/ℓ ammonia-N at pH 7.0 in 33 ppt seawater at 28°C. However, he did not specify the acute toxicity of ammonia for each species and did not report the LC_{50} at different times of exposure.

Information about the toxicities of ammonia to different stages of penaeid larvae has been provided for *P. indicus* (Jayasankar and Muthu, 1983) and for *P. monodon* (Chin and Chen, 1987; Chen and Chin, 1988). Both data indicated that the LC_{50} of ammonia-N and NH_3-N decreased with increases in exposure time. Both data also indicated that as the larval shrimps developed, they showed a progressive increase in tolerance to both ammonia-N and NH_3-N.

Huang (1979) determined the 96 h LC_{50} of ammonia for *P. monodon* juveniles (0.17 g wet weight) to be 100 mg/ℓ NH_4Cl (26.67 mg/ℓ ammonia-N). Lai and Ting (1984) reported that the 24, 48 and 72 h LC_{50}s of ammonia for *P. monodon* juveniles (0.07–0.19 g wet weight) were 61.05, 48.08 and 37.69 mg/ℓ NH_4Cl (15.99, 11.81 and 9.88 mg/ℓ ammonia-N), respectively. However, they did not report the LC_{50} of NH_3-N, neither did they provide levels of salinity and pH in their studies. Our present study indicated that the 24, 48 and 72 h LC_{50}s of ammonia for *P. penicillatus* juveniles were 108, 71.1 and 57.0 mg/ℓ ammonia-N (8.16, 5.37 and 4.31 mg/ℓ NH_3-N). This fact indicates that *P. penicillatus* juveniles tolerate ammonia much more than *P. monodon* juveniles.

Wickins (1976) stated that the EC_{50} (concentration that reduced the growth of the control by 50%) of NH_3-N for *P. japonicus*, *P. occidentalis*, *P. schmitti*, *P. semisulcatus* and *P. setiferus* postlarvae (0.5–1.5 g) was 0.37, 0.40, 0.69, 0.22 and 0.59 mg/ℓ, respectively, with an average of 0.45 mg/ℓ at 30–34 ppt and 28°C for 3 weeks of exposure. He also reported that a level above 0.1 mg/ℓ NH_3-N reduced growth of *Macrobrachium rosenbergii* to 60–70% of that of the control at a salinity of 0.5 to 4 ppt for 6 weeks of exposure time.

P. penicillatus juveniles exposed to 5 mg/ℓ ammonia-N (0.38 mg/ℓ NH_3-N) in this study appeared to show a weight gain. However, no significant difference ($p>0.05$) in weight gain was found between those exposed to 5 mg/ℓ ammonia-N and the control. The length increase of the *P. penicillatus* juveniles exposed to 5 and 10 mg/ℓ ammonia-N was significantly lower ($p<0.05$) than those exposed to the control. Unfortunately, we could not obtain the EC_{50} of ammonia for both weight and length of *P. penicillatus* juveniles.

Ammonia exponentially increased in an intensive culture system of *P. penicillatus* and jumped to 7.788 mg/ℓ ammonia-N and 0.159 mg/ℓ NH_3-N after 101 days, and to 46.11 mg/ℓ ammonia-N and 0.869 mg/ℓ NH_3-N after 115 days of cultivation (Chen *et al.*, 1988). In the present study, the fact that *P. penicillatus* juveniles exposed to 10 mg/ℓ ammonia-N significantly reduced both their weight and length suggests the importance of preventing an increase in the ammonia level in an intensive system. Wickins (1976) reported that the maximum acceptable level which reduced growth of the controls by 1–2% for penaeid postlarvae was 0.1 mg/ℓ NH_3-N. It is necessary to determine the effect of ammonia on growth and determine the EC_{50} of ammonia for different species of penaeid, since each penaeid may have different susceptibilities to ammonia toxicity.

Sprague (1969) reported that the short-term LC_{50} can be very misleading and recommended that toxicity should be described in terms of "incipient LC_{50}". If this value can not be estimated, the 96 h LC_{50} is a useful substitute. Armstrong *et al.* (1976, 1978) studied the toxicity of ammonia to the freshwater prawn, *Macrobrachium rosenbergii* and reported that 144 or 192 h LC_{50} can be considered equivalent to "incipient LC_{50}". The present study indicates that 96 h LC_{50} and "incipient LC_{50}" are 46.6 and 32.0 mg/ℓ ammonia-N (3.52 and 2.42 mg/ℓ NH_3-N). "Incipient LC_{50}" is an important parameter in connection with a "safe level" which is calculated from "incipient LC_{50}" by using an empirical "application factor" of 0.1 (Sprague, 1971). The "safe level" for rearing *P. penicillatus* juveniles is 3.2 mg/ℓ ammonia-N and 0.24 mg/ℓ NH_3-N at 25 ppt, pH 8.2 and 26°C.

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氨對紅尾蝦幼蝦之致死及半致死影響

陳 建 初　　林 俊 年

將紅尾蝦幼蝦於鹽度 25 ppt、pH 8.20 及水溫 26°C 下以靜止換水方式 (Static renewal method) 暴露於不同氨濃度中。氨對於幼蝦 (0.41±0.09 g) 之 24、48、96、120、144 及 180 小時之 LC_{50} (半致死濃度) 分別爲 108、71.1、46.6、43.0、39.4 及 33.9 mg/ℓ 氨-氮及 8.16、5.37、3.52、3.25 及 2.56 mg/ℓ 未解離氨-氮。"Incipient LC_{50}" 爲 32.0 mg/ℓ 氨-氮及 2.42 mg/ℓ 未解離氨-氮。暴露於對照組、5 及 10 mg/ℓ 氨-氮 240 小時後之相對增重率與相對增長率分別爲 100、123.5 及 58.5 與 100、65.1 及 78.5。由"incipient LC_{50}" 估得培育紅尾蝦幼苗之安全值爲 3.20 mg/ℓ 氨-氮，0.24 mg/ℓ 未解離氨-氮。