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NOTE

Effects of ambient ammonia on ammonia-N and protein concentrations in hemolymph and ammonia-N excretion of *Penaeus chinensis*

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ABSTRACT: Individual *Penaeus chinensis* adults (26.91 ± 4.66 g) were subjected to 0.03, 5.10, 10.11 and 20.04 mg l^{-1} ammonia-N (un-ionized plus ionized ammonia as nitrogen) in 30 ppt seawater and 25 °C. Changes in ammonia-N excretion, hemolymph ammonia-N and hemolymph protein were monitored for 1 to 24 h. Net ammonia-N uptake occurred when shrimp were exposed to 10.11 and 20.04 mg l^{-1} ammonia-N after 4 h. Relationships among ammonia-N excretion rate, hemolymph ammonia-N, hemolymph protein, concentration of ambient ammonia-N and exposure time were determined. An increase of ammonia-N and a decrease of protein in the hemolymph of *P. chinensis* following a 4 h exposure to ambient ammonia-N as high as 10 mg l^{-1} may cause catabolism of protein and amino acids to balance the osmoregulation, as well as dysfunction of Na^+/NH_4^+ exchanges.

The fleshy prawn *Penaeus chinensis* Osbeck is a typical penaeid which matures and spawns in seawater, spends postlarval and juvenile phases in brackish water, then returns to the sea as a preadult. It is also an important penaeid currently being cultured in China, Taiwan and Korea (Chen 1990). This species can be reared at salinities ranging from 20 to 32 ppt which was considered the optimal level by Liu (1983).

Ammonia, the end-product of protein catabolism, accounts for more than half of the nitrogenous waste of decapod crustaceans and is continually released. Ammonia is excreted mainly through the gill epithelium (Regnault 1987).

Protein and amino acids can serve as a significant source of metabolic energy for crustaceans (Claybrook 1983). A decrease in the ambient osmotic concentration increased catabolism of amino acids, causing an increase in ammonia-N excretion (Lange 1972). Ammonia has been reported to affect osmoregulation of crustaceans (Young-Lai et al. 1991). However, there is no information concerning its effect on protein and amino acid level in the hemolymph. This study was designed to monitor hemolymph ammonia-N, hemolymph protein and ammonia-N excretion of *Penaeus chinensis* adults exposed to different concentrations of ammonia-N.

Materials and methods. *Penaeus chinensis* adults obtained from Tainan Branch, Taiwan Fisheries Research Institute, Keelung, were acclimated in a holding tank for 1 wk and fed commercial shrimp feed (39 % crude protein) designed for *P. monodon* (Tairoun Products Co., Taipei, Taiwan) once a day. Shrimp were not fed for 2 d prior to the experiment.

Starved shrimp were randomly removed from the holding tank and individually transferred to a 20 l circular plastic tank containing 10 l of each test solution. Individual tanks were aerated through an air blower attached to an aeration stone. Only shrimp in the intermolt stage (Wassenberg & Hill 1984) were selected for the study. There were 20 treatments, 4 test solutions combined with 5 exposure times: 1, 4, 8, 16 and 24 h. For each treatment there were 5 replicates. The total number of shrimp used was 105, 5 for each treatment plus 5 for an initial control solution. Shrimp used ranged from 21.25 to 30.52 g with an average weight of 26.91 ± 4.66 g ($\bar{x}$ ± SD) and no significant difference in weight was found among treatments. In addition, 10 tanks containing aerated test solution only (duplicates for each test solution) were used as blanks.

Ammonia test solutions consisting of concentrations of 0.03 (control), 5.10, 10.11 and 20.04 mg l^{-1} ammonia-N were prepared according to the procedure reported previously (Chen & Nan 1992). The experiment started at 09:00 h and lasted for 1, 4, 8, 16 and 24 h under a photoperiod of 12 h light : 12 h dark with a light intensity of 160 to 250 lux during the day. The concentration of ammonia-N was determined by the phenolhypochlorite method (Solorzano 1969) at the beginning and end of the experiment. Ammonia-N excretion was cal-

Table 1. *Penaeus chinensis*. Mean (± SE) concentration of ammonia-N over time for blank solutions[a] (B) and test solutions containing shrimp (T)

Initial ammonia-N conc. (mg l^{-1})	Ammonia-N concentration (mg l^{-1}) after:				
	1 h	4 h	8 h	16 h	24 h
0.03 (B)	0.042	0.036	0.043	0.037	0.043
0.03 (T)	0.125 (0.002)	0.145 (0.001)	0.376 (0.003)	0.513 (0.004)	0.649 (0.007)
5.10 (B)	5.042	4.896	4.771	4.365	4.189
5.10 (T)	5.688 (0.004)	5.779 (0.004)	5.811 (0.023)	5.335 (0.013)	5.156 (0.006)
10.11 (B)	10.011	9.941	9.681	8.954	8.412
10.11 (T)	10.429 (0.004)	9.893 (0.001)	9.585 (0.004)	8.852 (0.004)	8.306 (0.006)
20.04 (B)	19.854	19.254	18.901	16.899	16.451
20.04 (T)	20.064 (0.004)	19.022 (0.003)	18.576 (0.006)	16.568 (0.008)	16.116 (0.009)

[a] Mean value of 2 blanks

culated as the difference between the test ammonia-N concentration (at 1, 4, 8, 16 and 24 h) and the mean value of the 2 blanks of each test solution. This value was then converted to the ammonia-N excretion rate (mg g^{-1} h^{-1}) by multiplying water volume (10 l), and then dividing by wet weight (g) and time lapsed (h). During the experimental period, mean water temperature ($\bar{x}$ ± SD), mean pH and mean dissolved oxygen were 25.0 ± 0.5°C, 7.93 ± 0.24 and 6.34 ± 0.43 mg O_2 l^{-1}, respectively.

Hemolymph samples were individually removed by syringe from the pericardial cavity through the intersegmental membrane between the cephalothorax and the abdominal segment. Determination of hemolymph ammonia-N followed a procedure described previously (Chen & Kou 1991). Hemolymph protein was determined using a Bio-Rad Protein Assay Kit (Bio-Rad Number 500-0002, Richmond, CA, USA) and bovine serum albumin as a standard, according to a method derived from Bradford (1976).

Effects of ambient ammonia-N and exposure time on ammonia-N excretion rate, hemolymph ammonia-N and hemolymph protein were subjected to 1-way and 2-way analysis of variance (Steel & Torrie 1980). If significant differences were indicated at the 0.05 level, then Duncan's multiple range test was used to identify significant difference among treatments (Duncan 1955). The linear relationships among ambient ammonia-N, exposure time and either ammonia-N excretion rate, hemolymph ammonia-N, or hemolymph protein were tested using the General Linear Model Procedure and Regression Procedure, version 6.03 of the SAS sta-

Table 2. *Penaeus chinensis*. Mean (± SE) ammonia-N excretion in shrimp exposed to different concentrations of ammonia-N over 24 h. Excretion was calculated as the difference between the mean ammonia-N concentration and the blank (see Table 1). Data in the same column followed by different letters are significantly different ($p < 0.05$)

Initial ammonia-N conc. (mg l^{-1})	Ammonia-N excretion (mg l^{-1}) after:				
	1 h	4 h	8 h	16 h	24 h
0.03	0.083 d (0.001)	0.110 b (0.002)	0.333 b (0.002)	0.477 b (0.004)	0.607 b (0.007)
5.10	0.647 a (0.004)	0.883 a (0.004)	1.041 a (0.023)	0.969 a (0.013)	0.967 a (0.006)
10.11	0.418 b (0.004)	−0.048 c (0.001)	−0.096 c (0.004)	−0.102 c (0.005)	−0.106 c (0.006)
20.04	0.210 c (0.004)	−0.232 d (0.003)	−0.326 d (0.006)	−0.331 d (0.008)	−0.335 d (0.009)

Table 3. *Penaeus chinensis*. Mean (± SE) ammonia-N excretion rate in shrimp exposed to different concentrations of ammonia-N over 24 h (5 replicates for each test solution and time period). Data in the same column followed by different letters are significantly different

Initial ammonia-N conc. (mg l^{-1})	Ammonia-N excretion rate (mg g^{-1} h^{-1}) after:				
	1 h	4 h	8 h	16 h	24 h
0.03	0.064 d (0.004)	0.023 b (0.003)	0.032 b (0.002)	0.022 b (0.002)	0.019 b (0.002)
5.10	0.463 a (0.014)	0.165 a (0.015)	0.108 a (0.009)	0.046 a (0.003)	0.033 a (0.005)
10.11	0.295 b (0.008)	−0.010 c (0.001)	−0.009 c (0)	−0.005 c (0)	−0.004 c (0.001)
20.04	0.157 c (0.013)	−0.043 d (0.003)	−0.032 d (0.003)	−0.016 d (0.001)	−0.011 d (0.002)

tistical system (SAS 1988). All statistical significance tests were at the $p < 0.05$ level.

Results. Concentrations of ammonia-N over 24 h for blanks (B) and test solutions containing shrimps (T) are given in Table 1. When *Penaeus chinensis* were exposed to 0.03 and 5.10 mg l^{-1} ammonia-N for 1, 4, 8, 16 and 24 h, ammonia-N was higher than that in the blanks. However, when shrimp were exposed to 10.11 and 20.04 mg l^{-1}, ammonia-N was lower than that in the blanks after 4, 8, 16 and 24 h. Nitrogen produced from bacteria and feces was not significant.

Excretion of ammonia-N by shrimp exposed to 0.03 and 5.10 mg l^{-1} increased over time and was 0.607 and 0.967 mg l^{-1} after 24 h, respectively (Table 2). Ammonia-N excretion was 0.083, 0.647, 0.418 and 0.210 mg l^{-1} for shrimp exposed to 0.03, 5.10, 10.11 and 20.04 mg l^{-1} after 1 h. However, ammonia-N excretion was lower than ammonia-N uptake in shrimp exposed to 10.11 and 20.04 mg l^{-1} after 4 h.

Shrimp exposed to 5.10 mg l^{-1} ammonia-N had a significantly higher ammonia-N excretion rate than those exposed to 0.30, 10.11 and 20.11 mg l^{-1} (Table 3). Ammonia-N excretion rates were 0.295 and 0.157 mg g^{-1} h^{-1} for the shrimp exposed to 10.11 and 20.04 mg l^{-1} ammonia-N after 1 h, respectively and became negative after 4 h of exposure.

Major changes in ammonia-N excretion rates occurred between 1 and 4 h. The ammonia-N excretion rate in shrimp exposed to 5.10, 10.11 and 20.04 mg l^{-1} after 1 h increased to 723.4, 460.9 and 245.3 % of that in controls, respectively. (Fig. 1).

Analysis of variance indicated that there was a significant effect of ambient ammonia-N concentration and exposure time on the ammonia-N excretion rate ($F = 561.90$, df = 3,99, $p < 0.05$ and $F = 993.18$, df = 4,99, $p < 0.05$, respectively), and that there was a significant interaction between the effects of ambient ammonia-N concentration and exposure time on ammonia-N excretion rate ($F = 125.66$, df = 12,99, $p < 0.05$). The relationship between ammonia-N excretion rate (*ANER*), ambient ammonia-N (C) and exposure time (t) is as follows: $ANER = 0.1719 - 0.0038C - 0.0074t + 0.00005Ct$ ($R^2 = 0.9890$).

In controls, hemolymph ammonia-N ranged from 2.67 to 2.79 mg l^{-1} with a mean (± SD) of 2.71 ± 0.04 mg l^{-1}. In comparison, for those shrimp in the 20.04 mg l^{-1} ammonia-N treatment, hemolymph ammonia-N increased from 3.09 mg l^{-1} after 1 h to 16.51 mg l^{-1} after 24 h (Table 4). Shrimp exposed to increased concentrations of ambient ammonia-N had significantly higher concentrations of hemolymph ammonia-N after 8 h.

Major changes in hemolymph ammonia-N occurred between 8 and 16 h for all treatments except the 0.03 mg l^{-1} ammonia-N treatment. Hemolymph ammonia-N in shrimp exposed to 10.11 and 20.04 mg l^{-1} ammonia-N after 16 h increased to 441.7 and 535.8 % of that in controls, respectively (Fig. 2).

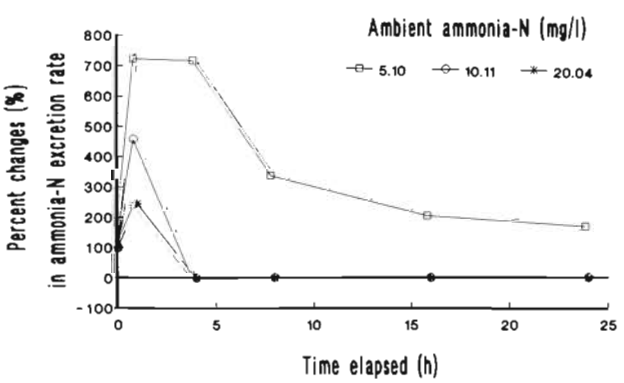

Fig. 1. *Penaeus chinensis*. Percent change (%) in ammonia-N excretion rate of shrimp exposed to different concentrations of ambient ammonia-N after 1, 4, 8, 16 and 24 h as compared to that in control shrimp (0.03 mg l^{-1} solution)

Table 4. *Penaeus chinensis*. Mean (± SE) hemolymph ammonia-N in shrimp exposed to different concentrations of ammonia-N over 24 h (5 replicates for each test solution and time period.) Data in the same column followed by different letters are significantly different ($p < 0.05$).

Initial ammonia-N conc. (mg l^{-1})	Hemolymph ammonia-N (mg l^{-1}) after:					
	0 h	1 h	4 h	8 h	16 h	24 h
0.03	2.73 (0.08)	2.79 b (0.04)	2.69 b (0.04)	2.67 d (0.04)	2.70 d (0.03)	2.70 d (0.05)
5.10		2.83 b (0.03)	2.71 b (0.04)	3.01 c (0.05)	3.96 c (0.04)	6.38 c (0.07)
10.11		2.87 b (0.02)	6.97 a (0.06)	7.18 b (0.04)	11.97 b (0.23)	14.19 b (0.20)
20.04		3.09 a (0.08)	7.08 a (0.12)	8.42 a (0.12)	14.52 a (0.09)	16.51 a (0.18)

Analysis of variance indicated that there was a significant effect of ambient ammonia-N concentration and exposure time on the hemolymph ammonia-N of shrimp (F = 6476.48, df = 3,99, $p < 0.05$, and F = 3271.98, df = 4,99, $p < 0.05$, respectively), and there was a significant interaction between the effects of ambient ammonia-N concentration and exposure time on hemolymph ammonia-N of shrimp (F = 715.10, df = 12,99, $p < 0.05$). The relationship between hemolymph ammonia-N (HAN), ambient ammonia-N (C) and exposure time (t) is as follows: $HAN = 2.4679 + 0.0733C + 0.0360t + 0.0296Ct$ ($R^2 = 0.9981$).

In controls, hemolymph protein ranged from 100 to 105 mg ml^{-1} with a mean (± SD) of 103 ± 2 mg ml^{-1}. In comparison, for specimens exposed to the 20.04 mg l^{-1} ammonia-N seawater, levels of hemolymph protein decreased to 61 mg ml^{-1} and then to 48 mg ml^{-1} after 4 and 24 h of exposure, respectively (Table 5). Shrimp exposed to increasing concentrations of ambient ammonia-N had significantly less protein in the hemolymph after 4 h.

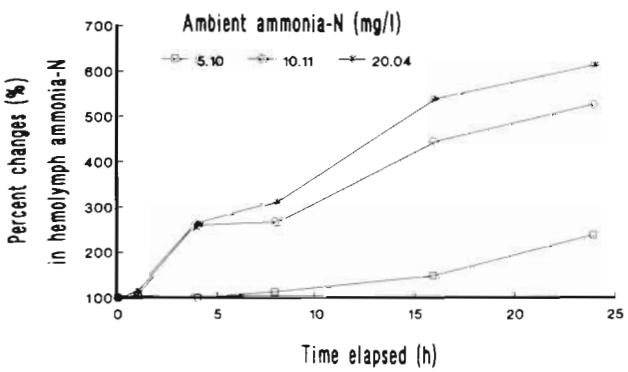

Fig. 2. *Penaeus chinensis*. Percent change (%) in hemolymph ammonia-N of shrimp exposed to different concentrations of ambient ammonia-N after 1, 4, 8, 16 and 24 h as compared to that in control shrimp (0.03 mg l^{-1} solution)

Major changes in hemolymph protein occurred between 1 to 4 h for all treatments except the 0.03 mg l^{-1} ammonia-N treatment. Hemolymph protein in shrimp exposed to 5.10, 10.11 and 20.04 mg l^{-1} ammonia-N after 4 h decreased to 84.4, 69.9 and 59.2 % of that in controls, respectively (Fig. 3).

Analysis of variance indicated that there was a significant effect of ambient ammonia-N concentration and exposure time on the hemolymph protein of shrimp (F = 253.86, df = 3,99, $p < 0.05$ and F = 72.38, df = 4,99, $p < 0.05$, respectively), and that there was a significant interaction between the effects of ambient ammonia-N concentration and exposure time on hemolymph protein of shrimp (F = 12.31, df = 12,99, $p < 0.05$). The relationship between hemolymph protein (HP), ambient ammonia-N (C) and exposure time (t) is as follows: $HP = 102.8217 - 1.2067C - 0.1315t - 0.0747Ct$ ($R^2 = 0.9374$).

Discussion. The effects of extrinsic factors such as water temperature and salinity on ammonia excretion were documented for shore crab *Carcinus maenas* (Haberfield et al. 1975), for Kuruma shrimp *Penaeus japonicus* (Spaargaren et al. 1982), for tiger shrimp *Penaeus monodon* (Lei et al. 1989) and for spot prawn *Pandalus platyceros* (Quarmby 1985). Increased ambient noise was also reported to affect ammonia excretion of sand shrimp *Crangon crangon* (Regnault & Lagardere 1983). Marangos et al. (1990) examined nycthemeral variation of ammonia excretion in *Penaeus japonicus* adults and postlarvae, and found that hourly specific excretion was enhanced during the dark period in adults, but found no significant difference for postlarvae between day and night. The effects of intrinsic factors such as body size and molt cycle on ammonia excretion were documented for *Penaeus chinensis* (Wu & Lou 1965) and freshwater prawn *Macrobrachium rosenbergi* (Stern & Cohen 1982).

Mangum et al. (1976) reported that addition of am-

Table 5. *Penaeus chinensis*. Mean (± SE) hemolymph protein in shrimp exposed to different concentrations of ammonia-N over 24 h (5 replicates for each test solution and time period). Data in the same column followed by different letters are significantly different ($p < 0.05$)

Initial ammonia-N conc. (mg l^{-1})	Hemolymph protein (mg ml^{-1}) after:					
	0 h	1 h	4 h	8 h	16 h	24 h
0.03	104 (2)	105 a (1)	105 a (1)	104 a (1)	100 a (2)	101 a (1)
5.10		104 ab (1)	88 b (5)	92 b (3)	88 b (3)	88 b (3)
10.11		99 b (2)	72 c (3)	69 c (2)	71 c (1)	71 c (1)
20.04		100 b (2)	61 d (2)	59 d (3)	51 d (4)	48 d (4)

monia to ambient medium reduced the ammonia excretion rate of blue crab *Callinectus sapidus*. However, Regnault (1987) reported that the ammonia excretion rate of *Crangon crangon* was not influenced by ammonia-N concentrations ranging from 28 to 1218 mg l^{-1}. The present study indicates that ammonia-N excetion of *Panaeus chinensis* increased as ambient ammonia-N increased in the range of 0.03 to 5.10 mg l^{-1}, but decreased in the range of 5.10 to 20.04 mg l^{-1}.

Studying *Panaeus japonicus* subadults, Chen & Kou (1991) found that shrimp exposed to increasing concentrations of ambient ammonia-N had significantly higher levels of hemolymph ammonia-N after 2 h, and suggested that once NH_3 diffuses into the hemolymph, the relative proportions of NH_3 and NH_4^+ composing the ambient ammonia-N readjust and thus NH_3 diffuses continously in the hemolymph.

Young-Lai et al. (1991) reported that a decrease of hemolymph osmolality in the American lobster *Hormarus americanus* following exposure to ammonia

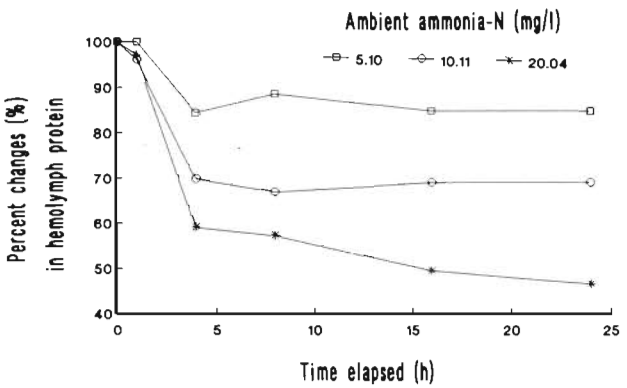

Fig. 3. *Penaeus chinensis*. Percent change (%) in hemolymph protein of shrimp exposed to different concentrations of ambient ammonia-N after 1, 4, 8, 16 and 24 h as compared to that in control shrimp (0.03 mg l^{-1} solution)

at 150 mg l^{-1} was caused by lower concentrations of sodium in the hemolymph. Armstrong et al. (1978) demonstrated that high levels of ambient NH_4^+ are associated with inhibition of sodium absorption and an increase of NH_4^+ uptake in *Macrobrachium rosenbergii*.

Protein levels in the hemolymph were 100 mg ml^{-1} in *Penaeus japonicus* (Boucard et al. 1985), and 103 mg ml^{-1} in *P. chinensis* in the present study. Studying shore crabs *Carcinus maenas*, Haberfield et al. (1975) observed an increase in the catabolism of amino acids that resulted in excretion of nitrogen, mainly as ammonia, as medium osmolality was decreased. The decrease in hemolymph protein of *P. chinensis* with increased ambient ammonia-N in the range of 0.03 to 20.04 mg l^{-1} may be due to an increase in catabolism of protein to adjust osmoregulation. Unfortunately, hemolymph osmolality was not monitored in the present study.

In crustaceans, the possible mechanism of ammonia excretion is passive NH_3 and/or NH_4^+ efflux, and ion exchange of NH_4^+ for Na^+ (Regnault 1987). The active Na^+/NH_4^+ exchange requires a specific carrier enzyme called Na^+,K^+-ATPase (Towle et al. 1976). Chen & Nan (1992) demonstrated that *Penaeus chinensis* exposed to 5.043 mg l^{-1} had higher total ATPase and Na^+,K^+-ATPase activities in the gill than those exposed to 0.037 mg l^{-1} after 4 h. However, total ATPase and Na^+,K^+-ATPase activities of shrimp exposed to 10.106 and 20.093 mg l^{-1} ammonia-N were significantly lower than those exposed to 5.043 and 0.037 mg l^{-1} ammonia-N after 8 h. In the present study, the fact that ambient ammonia-N at 10.11 mg l^{-1} caused net ammonia-N uptake, increased hemolymph ammonia-N and decreased hemolymph protein of *P. chinensis* after 4 h supported a reverse movement of Na^+ and NH_4^+. Further research is needed to monitor free amino acid and electrolytes in hemolymph of penaeid exposed to ambient ammonia.

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