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Nitrogen excretion and changes of hemocyanin, protein and free amino acid levels in the hemolymph of *Penaeus monodon* exposed to different concentrations of ambient ammonia-N at different salinity levels

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ABSTRACT: *Penaeus monodon* (22.064 ± 2.263 g) were exposed individually at 10, 20 and 30‰ salinity to 0 (control), 1, 5, 10 and 20 mg l^{-1} ammonia-N for 24 h. Changes in hemocyanin, protein and free amino acid levels, and whole shrimp ammonia-N excretion, urea-N excretion and dissolved organic N (DON) excretion were determined. Ammonia-N excretion decreased with increased salinity and with increased ambient ammonia-N. Ammonia-N accounted for 56.87, 70.43 and 78.13 % of total nitrogen excreted by *P. monodon* in 30, 20 and 10‰, respectively. DON increased to 67.26, 78.79 and 86.1% of total nitrogen excreted by the shrimp exposed to 20 mg l^{-1} ammonia-N in 30, 20 and 10‰, respectively. Net ammonia-N uptake occurred as shrimp were exposed to ambient ammonia-N greater than 5 mg l^{-1} at all salinity levels tested. Hemocyanin and protein levels decreased, whereas hemolymph ammonia, urea, total free amino acid levels and taurine level increased, with increased ambient ammonia-N. Following exposure to ambient ammonia-N, *P. monodon* changed its excretory pattern, accumulation of hemolymph ammonia, and catabolism of hemocyanin and protein to balance osmoregulation.

KEY WORDS: *Penaeus monodon* · Nitrogen excretion · Hemocyanin · Protein · Free amino acid · Ammonia · Urea · Accumulation · Salinity · Metabolism

INTRODUCTION

The tiger shrimp *Penaeus monodon* is widely distributed throughout the Indo-Pacific region. This species is of commercial importance. It is an omnivore with a rapid growth and tolerates a wide range of salinities and temperatures. The level of salinity suitable for growth of *P. monodon* is in the range 10 to 30‰ (Liao & Murai 1986). Effects of salinity on oxygen consumption and ammonia-N (un-ionized plus ionized ammonia as nitrogen) excretion were reported by Lei et al. (1989).

Ammonia, the end product of protein catabolism, accounts for more than half of the nitrogenous waste released by decapod crustaceans (Regnault 1987). Most ammonia is excreted via the gill epithelium, through diffusion of NH_3 (un-ionized ammonia) and/or NH_4^+ (ionized ammonia), and Na^+/NH_4^+ exchanges (Kormanik & Cameron 1981, Pequeux & Gilles 1981, Pressley et al. 1981).

Elevated environmental ammonia has been reported to affect growth and molting (Chen & Kou 1992), oxygen consumption and ammonia-N excretion (Chen & Lin 1992), and Na^+, K^+-ATPase activities of penaeids (Chen & Nan 1992). Ammonia has also been reported to affect osmoregulatory capacity and Na^+ concentration in the hemolymph of American lobster *Homarus americanus* (Young-Lai et al. 1991).

The respiratory pigment hemocyanin represents 60 to 95% of the total protein in the hemolymph of crustaceans (Djangmah 1970, Jeuniaux 1971).

Changes in osmotic concentration are considered to induce drastic changes in protein and amino acid composition in the tissues. Hemocyanin was reported to increase in shore crab *Carcinus maenas* under hypo-osmotic stress (Boone & Schoffeniels 1979). Hemolymph protein was affected by salinity in Chinese crab *Eriocheir sinensis* (Gilles 1977) and by ambient ammonia in fleshy prawn *Penaeus chinensis* (Chen et al. 1993).

The concentration of FAA (free amino acid) in crustaceans is generally several times higher than that in vertebrates. Nitrogen metabolism and the role of FAA in the regulation of intracellular osmolarity and volume regulation have been widely studied and reviewed by Claybrook (1983) and Gilles & Pequeux (1983). Several studies have reported an increase of ammonia excretion and a spontaneous decrease of tissue FAA when crustaceans are exposed to hypo-osmotic conditions (Haberfield et al. 1975, Mangum et al. 1976). Effects of salinity on FAA composition have been examined for Kuruma shrimp *Penaeus japonicus* by Dalla Via (1986) and for *P. monodon* by Fang et al. (1992). However, nothing is known of the effects of ammonia on hemocyanin and FAA levels and electrolytes in the hemolymph of penaeids.

Using *Penaeus chinensis* juveniles, Chen & Lin (1992) reported that ammonia-N excretion increased when increased ambient ammonia-N was in the range 0.036 to 0.942 mg l^{-1}, and decreased when increased ambient ammonia-N was in the range 0.942 to 9.921 mg l^{-1}, in 30‰ seawater. We hypothesize that shrimp in high concentrations of ambient ammonia-N may disturb their nitrogen metabolism and shift to urea excretion, as do some teleosts (Olson & Fromm 1971). The purpose of the present study was to determine hemocyanin, hemolymph protein and FAA levels, and ammonia-N, urea-N and dissolved organic N excretions, of *P. monodon* exposed to different concentrations of ambient ammonia-N at different salinities.

MATERIALS AND METHODS

Penaeus monodon obtained from a shrimp farm in Ilan, Taiwan, were kept in 3 circular tanks with seawater at 30‰ for 1 wk, and then acclimated to 30, 20 and 10‰ for another 1 wk in our laboratory. The salinity was adjusted by a decrease of 2 to 3‰ per day with fresh water until salinities of 30, 20 and 10‰ were reached. Shrimp were fed daily with commercial shrimp food (39% crude protein) manufactured by Tairoun Products Co. (Taipei, Taiwan). They were starved for 2 d prior to the experiment.

Ammonia test solutions consisting of concentrations of 0 (control), 1, 5, 10 and 20 mg l^{-1} ammonia-N were prepared with 30, 20 and 10‰ according to the procedure reported previously (Chen & Nan 1992). The concentrations of ammonia-N are sublethal, based on a short-term toxicity test which showed no mortality of *Penaeus monodon* (4.87 ± 1.40 g) exposed to 40 mg l^{-1} ammonia-N in 20‰ after 24 h (Chen et al. 1990).

Shrimp were taken from the holding tank and transferred individually to a 20 l circular plastic tank containing 10 l of each test solution. Individual tanks were aerated by means of an air blower with an aeration stone attached. After 24 h exposure, shrimp were examined for molt stage by examining the uropoda, in which partial retraction of the epidermis can be distinguished (Wassenberg & Hill 1984). Only shrimp in the intermolt stage were used for the study. There were 15 treatments, each treatment being conducted with 5 individuals. The total number of shrimp used was 75.

Shrimp used ranged from 19.43 to 25.68 g with an average weight of 22.06 ± 2.26 g (mean ± SD). Each tank was covered with a plastic cap to prevent escape. The experiment started at 09:00 h and lasted for 24 h under a photoperiod of 12 h light : 12 h dark with measuremenis of ambient ammonia-N (Solorzano 1969), urea-N (McCarthy 1970), total dissolved nitrogen (Solorzano & Sharp 1980), nitrite-N (Bendschneider & Robinson 1952) and nitrate-N (Wood et al. 1967) at the beginning and end of the experiment. Analyses of these water parameters were finished in 3 d. Dissolved organic N (DON) was calculated based on the difference between total dissolved nitrogen, urea-N, ammonia-N, nitrite-N and nitrate-N. The differences in concentrations of ammonia-N, urea-N, DON, nitrite-N and nitrate-N of each test solution at the end and the beginning of the experiment were converted to ammonia-N excretion, urea-N excretion, DON excretion, nitrite-N excretion and nitrate-N excretion (µg g^{-1} h^{-1}). During the experimental period, mean water temperature, dissolved oxygen and pH (mean ± SD) were 25.5 ± 0.5 °C, 7.34 ± 0.16 mg l^{-1} and 8.12 ± 0.03, respectively.

After 24 h exposure to each test solution, shrimp were sampled for hemolymph hemocyanin, protein and FAA levels. All the experiments were completed in 15 d. Sampling and preparation of hemolymph for analyses of hemocyanin and protein was undertaken as follows. Hemolymph (100 µl) was taken by carefully inserting a 1.0 ml disposable syringe, fitted with a 22 gauge, 0.50 × 25 mm needle which was rinsed with buffer (0.10 M sodium citrate, 0.25 M sodium chloride, pH 7.5), into the abdomen. The hemolymph samples were removed individually using the syringe from the pericardial cavity through the intersegmental membrane between the cephalothorax and the abdominal segment. The sampled hemolymph was placed in a 1.5 ml Eppendorf tube containing 900 µl distilled

water, and then mixed immediately with type 37600 mixer (Barnstead/Thermolyne Company, Dubuque, IA, USA). Using this method, the hemolymph did not clot and was also dissolved equally by the addition of water. Since the reported concentrations of hemolymph protein of penaeids range from 62 to 103 mg ml^{-1} (Bursey & Lane 1971, Balazs et al. 1974, Chen & Cheng 1993, Chen et al. 1993), dilution of hemolymph sample is necessary. This dilution technique has been used successfully for determinations of hemocyanin and protein in a number of decapod crustaceans (Senkbeil & Wriston 1981, Hagerman 1983, 1986, Ferraris et al. 1986, Hagerman & Baden 1988, Chen & Cheng 1993, Chen et al. 1993). To check if dilution affected the measured concentrations of hemocyanin and protein, the hemolymph samples were allowed to coagulate at 4 °C, and then centrifuged at 10 000 × *g* for 10 min. Concentrations of hemocyanin and protein were examined using (1) hemolymph diluted with distilled water, (2) hemolymph diluted with buffer and (3) serum. No significant difference was found among the 3 treatments. Measurements of hemocyanin and protein were therefore carried out using diluted hemolymph with distilled water.

Hemolymph protein was determined with a Bio-Rad Protein Assay Kit No. 500-0006 (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin (molecular weight 66 000 daltons) as a standard according to a method derived from Bradford (1976). For measurement of hemocyanin, 100 µl hemolymph was immediately diluted with 900 µl distilled water in a 10 mm quartz cuvette and the absorbance was measured at 335 nm using a Hitachi U-2000 spectrophotometer (Hitachi Ltd, Tokyo, Japan). The hemocyanin concentration was calculated using an extinction coefficient (E^{mM}_{1cm})of 17.26, calculated from $E^{1\%}_{1cm}$ = 2.83 (Nickerson & Van Holde 1971) on the basis of a functional subunit of 74 000 (Antonini & Brunori 1974). The ratio of hemocyanin to protein was calculated by dividing the concentration of hemocyanin (mmol l^{-1}) by that of protein (mmol l^{-1} converted from mg ml^{-1} to mmol l^{-1} by dividing by 66).

For measurement of FAA level, 0.5 ml hemolymph was sampled from each individual exposed to each test solution. An equal volume of 5% sulfosalicylic acid was added to precipitate protein, and centrifuged at 13 300 × *g* for 15 min with a Model Sigma 4K10 centrifuge (Sigma Laborzentrifugen GmbH, Osterode, Germany). A volume of 200 µl of supernatant was diluted with 1 ml of 0.02 N HCl, then 80 µl of the mixture was injected into a Model L-8500 High Speed Amino Acid Analyzer connected with a Model D-2850 Chromato-Integrater (Hitachi Ltd, Tokyo, Japan) for analysis of FAA. Ammonia and urea concentrations were recorded at the same time by the analyzer.

Two-way analysis of variance was used for statistical analysis (Steel & Torrie 1980). The linear relationships among ambient ammonia-N, salinity, and the variables ammonia-N excretion, urea-N excretion, DON excretion, nitrite-N excretion, nitrate-N excretion, hemocyanin, hemolymph protein, the ratio of hemocyanin/protein, FAA, hemolymph ammonia and hemolymph urea were tested using the General Linear Model Procedure and Regression Procedure, version 6.03 of the SAS (Statistical Analysis System) computer software (SAS 1988). All statistical significance tests were at the $p < 0.05$ level.

RESULTS

In the control solution (0) at 10, 20 and 30 %, ammonia-N excretion varied from 10.7 to 14.8, 6.1 to 10.3 and 4.5 to 6.7 µg g^{-1} h^{-1}, respectively, with averages of 12.5, 8.8 and 5.5 µg g^{-1} h^{-1}. In comparison, for shrimp exposed to 5 mg l^{-1} ammonia-N or higher at all salinity levels, ammonia-N excretion was inhibited and net ammonia-N uptake occurred. Ammonia-N uptake of shrimp increased when increased ambient ammonia-N was in the range 5 to 20 mg l^{-1} at all salinity levels tested (Fig. 1A).

Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on ammonia-N excretion, and that there was a significant interaction between the effects of ambient ammonia-N and salinity on ammonia-N excretion. The relationship between ammonia-N excretion (*ANE*), and ambient ammonia-N (*C*), salinity (*S*) and the interaction between ambient ammonia-N and salinity (*CS*) is as follows: $ANE = 5.6049 - 0.9145C - 0.1311S - 0.0013CS$ ($R^2 = 0.66$).

In the control solution at 10 and 20‰, urea-N excretion of shrimp varied from 0.09 to 0.13 and 0.18 to 0.30 µg g^{-1} h^{-1}, respectively, with averages of 0.12 and 0.27 µg g^{-1} h^{-1}. Urea-N excretion of shrimp increased with increased concentrations of ambient ammonia-N at all 3 salinity levels. Urea-N excretion of shrimp exposed to 20 mg l^{-1} ammonia-N at 30‰ was 23.2 µg g^{-1} h^{-1}, significantly higher than that of shrimp exposed to the same ambient ammonia-N at 10 and 20‰ (Fig. 1B).

Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on urea-N excretion, and that there was a significant interaction between the effects of ambient ammonia-N and salinity on urea-N excretion. The relationship between urea-N excretion (*UNE*) and *C*, *S* and *CS* is as follows: $UNE = -1.0531 + 0.6651C + 0.0612S + 0.0135CS$ ($R^2 = 0.89$).

Dissolved organic N excretion increased when increased ambient ammonia-N was in the range 0 to

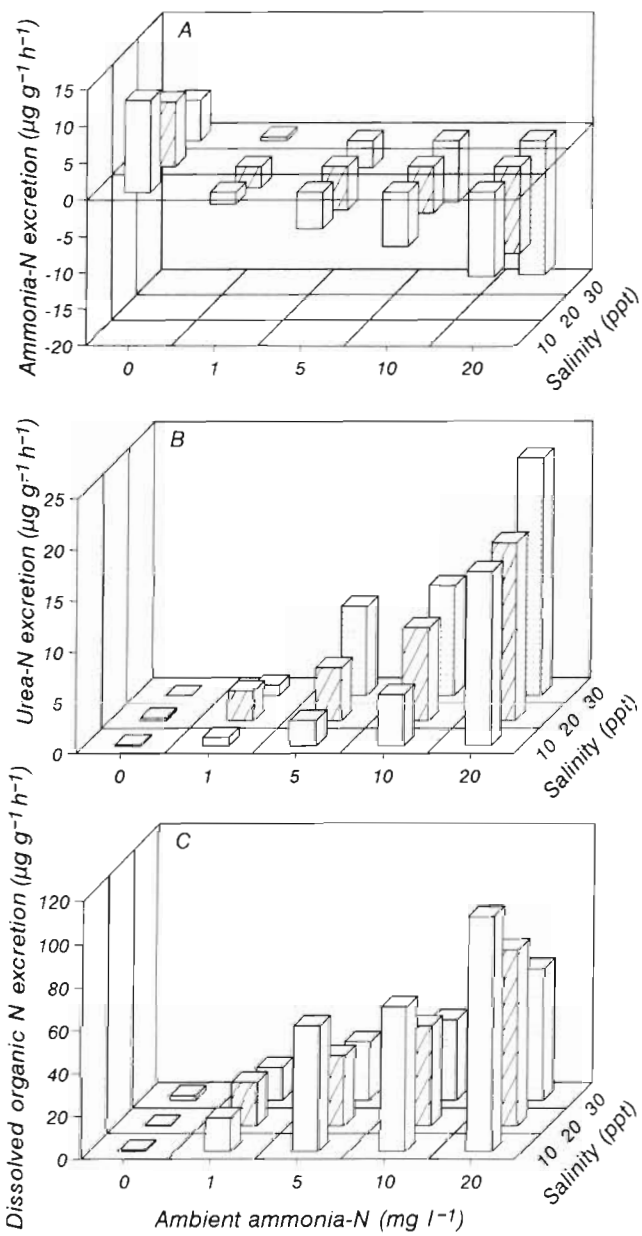

Fig. 1. *Penaeus monodon*. Mean (A) ammonia-N excretion, (B) urea-N excretion and (C) dissolved organic N excretion of shrimp exposed to different concentrations of ambient ammonia-N at different salinity levels after 24 h

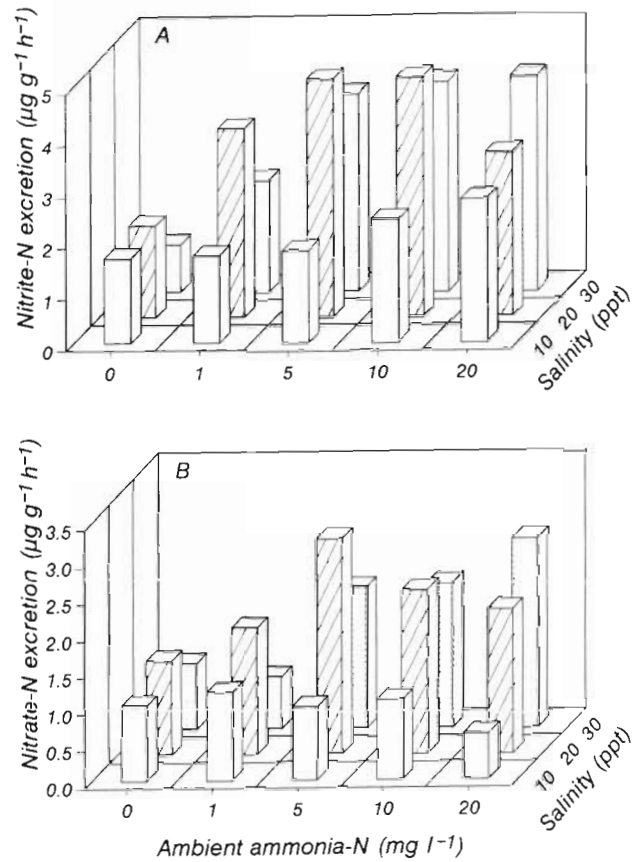

Fig. 2. *Penaeus monodon*. Mean (A) nitrite-N excretion and (B) nitrate-N excretion of shrimp exposed to different concentrations of ambient ammonia-N at different salinity levels after 24 h

20 mg l^{-1} at 3 salinity levels (Fig. 1C). Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on DON excretion, and that there was a significant interaction between the effects of ambient ammonia-N and salinity on DON excretion. The relationship between DON excretion (*DONE*) and *C*, *S* and *CS* is as follows: $DONE = 15.7667 + 6.0606C - 0.1696S - 0.1195CS$ ($R^2 = 0.83$).

Nitrite-N excretion increased when increased concentrations of ambient ammonia-N were in the range 0 to 20 mg l^{-1} at 3 salinity levels (Fig. 2A). Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on nitrite-N excretion, and that there was a significant interaction between the effects of ambient ammonia-N and salinity on nitrite-N excretion. The relationship between nitrite-N excretion (*NINE*) and *C*, *S* and *CS* is as follows: $NINE = 1.9516 - 0.0034C + 0.0215S + 0.0037CS$ ($R^2 = 0.24$).

No significant effect of ambient ammonia-N on nitrate-N excretion was found, nor was there a significant interaction between the effects of ambient ammonia-N and salinity on nitrate-N excretion (Fig. 2B).

Percentages of ammonia-N excretion, urea-N excretion, DON excretion, nitrite-N excretion and nitrate-N excretion are given in Table 1. It indicates that ammonia-N excretion represented 56.87, 70.43 and 78.13% of total nitrogen excreted by *Penaeus monodon* in 30, 20 and 10‰, respectively. Urea-N excretion and DON excretion increased with increased ambient

ammonia-N. In 20 mg l^{-1} ammonia-N, urea-N constituted 25.38, 16.74 and 11.15% of total nitrogen excreted, whereas DON constituted 67.26, 78.79 and 86.15% of total nitrogen excreted by *P. monodon* in 30, 20 and 10‰, respectively.

Hemocyanin (Fig. 3A) and protein (Fig. 3B) in the hemolymph of shrimp decreased with increased ambient ammonia-N at all salinities. Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on the hemocyanin and hemolymph protein, and that there was a significant interaction between the effects of ambient ammonia-N and salinity on hemocyanin. However no significant interaction between the effects of ambient ammonia-N and salinity on hemolymph protein was observed. The relationships between hemocyanin (*HC*) and *C*, *S* and *CS*, and between hemolymph protein (*HP*) and *C* and *S*, are as follows: $HC = 1.0247 - 0.0194C - 0.0039S + 0.0003CS$ ($R^2 = 0.56$), $HP = 125.0963 - 0.8590C - 1.2124S$ ($R^2 = 0.38$).

The ratio of hemocyanin to protein in the hemolymph of shrimp decreased with increasing ambient ammonia-N at 3 salinity levels (Fig. 3C). Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on the hemocyanin/protein ratio. However, no significant interaction between the effects of ambient ammonia-N and salinity on the hemocyanin/protein ratio was observed. The relationship between the hemocyanin/protein ratio (*RHP*) and *C* and *S* is as follows: $RHP = 50.1311 - 0.4062C + 0.6820S$ ($R^2 = 0.47$).

Shrimp exposed to ambient ammonia-N at 1 mg l^{-1} decreased levels of hemocyanin more than protein. Hemocyanin of shrimp exposed to 20 mg l^{-1} at 10, 20 and 30‰ was 63.2, 75.7 and 76.2% of that of control shrimp, while hemolymph protein of shrimp exposed to 20 mg l^{-1} at 10, 20 and 30‰ was 78.7, 84.1 and 88.3% of that of control shrimp.

Total free amino acid (TFAA) level increased with increased salinity level and ambient ammonia-N (Fig. 4A). Analysis of variance indicated that there was a significant effect of ambient ammonia and salinity on the TFAA. However, there was no significant interaction between the effects of ammonia-N and salinity on TFAA in the hemolymph. The relationship between TFAA, and *C* and *S* is as follows: $TFAA = 3665.3688 + 79.9472C + 54.2113S$ ($R^2 = 0.46$).

Analysis of variance indicated that there was a significant effect of salinity on essential free amino acid (EFAA). However, no significant effect of ambient ammonia-N on EFAA was observed, nor was any significant interaction observed between the effects of ammonia-N and salinity on EFAA in the hemolymph of shrimp (Fig. 4B).

No significant effect of ambient ammonia-N and salinity on nonessential free amino acid (NFAA) was observed, nor was any significant interaction observed between the effects of ambient ammonia-N and salinity on NFAA in the hemolymph of shrimp (Fig. 4C).

Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on hemolymph ammonia, and that there was a significant interaction between the effects of ambient ammonia-N and salinity on hemolymph ammonia (Fig. 5A). The relationship between hemolymph ammonia (*HA*) and

Table 1. *Penaeus monodon*. Percentage of nitrogenous excretion of shrimp exposed to different concentrations of ambient ammonia-N at 3 salinity levels for 24 h. *ANE*: ammonia-N excretion; *NINE*: nitrite-N excretion; *NANE*: nitrate-N excretion; *UNE*: urea-N excretion; *DONE*: dissolved organic N excretion

Salinity (%)	Nitrogen excretion	Nitrogen excretion (%) at ambient ammonia-N concentration of:				
		0 mg l^{-1}	1 mg l^{-1}	5 mg l^{-1}	10 mg l^{-1}	20 mg l^{-1}
30	*ANE*	56.87	2.47	–	–	–
	NINE	9.59	11.01	9.21	7.45	4.55
	NANE	9.23	3.59	4.59	3.61	2.81
	UNE	2.13	5.19	20.92	19.78	25.38
	DONE	22.18	77.74	65.28	69.16	67.26
20	*ANE*	70.43	–	–	–	–
	NINE	14.49	12.78	10.12	7.40	3.05
	NANE	10.31	6.01	6.39	3.55	1.42
	UNE	2.19	10.24	11.44	14.59	16.74
	DONE	2.58	70.97	72.05	74.46	78.79
10	*ANE*	78.13	–	–	–	–
	NINE	10.34	8.80	2.83	3.17	2.21
	NANE	6.41	6.19	1.56	1.44	0.49
	UNE	0.72	4.31	3.98	6.69	11.15
	DONE	4.40	80.70	91.63	88.70	86.15

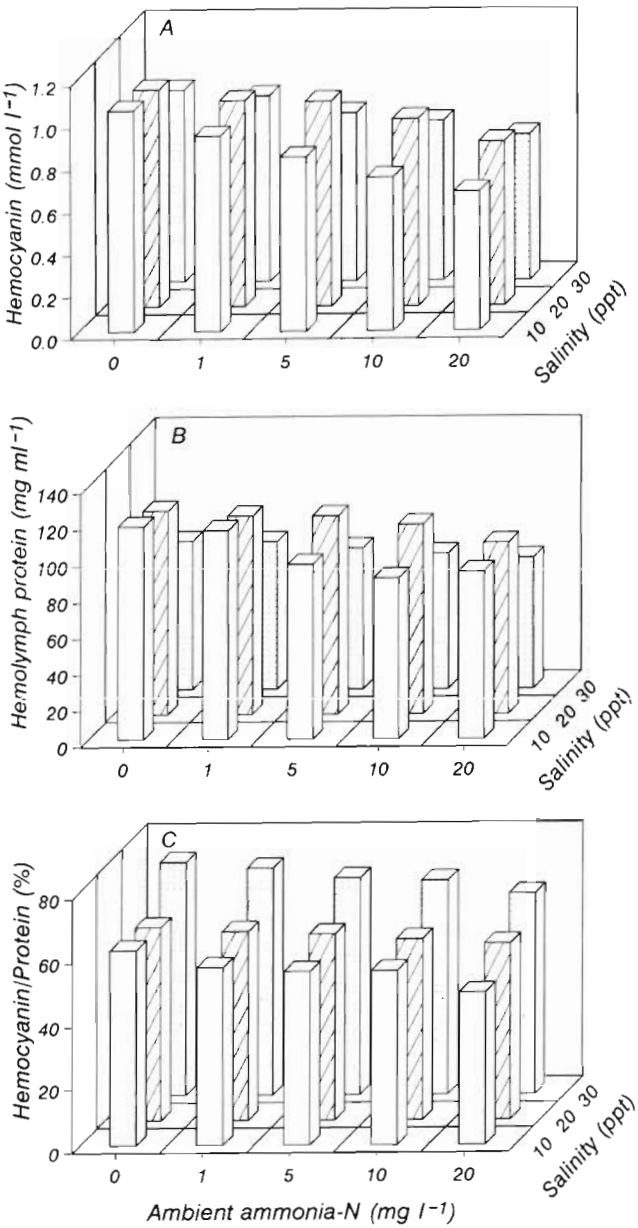

Fig. 3. *Penaeus monodon*. Mean (A) hemocyanin, (B) hemolymph protein and (C) the ratio of hemocyanin/protein of shrimp exposed to different concentrations of ambient ammonia-N at different salinity levels after 24 h

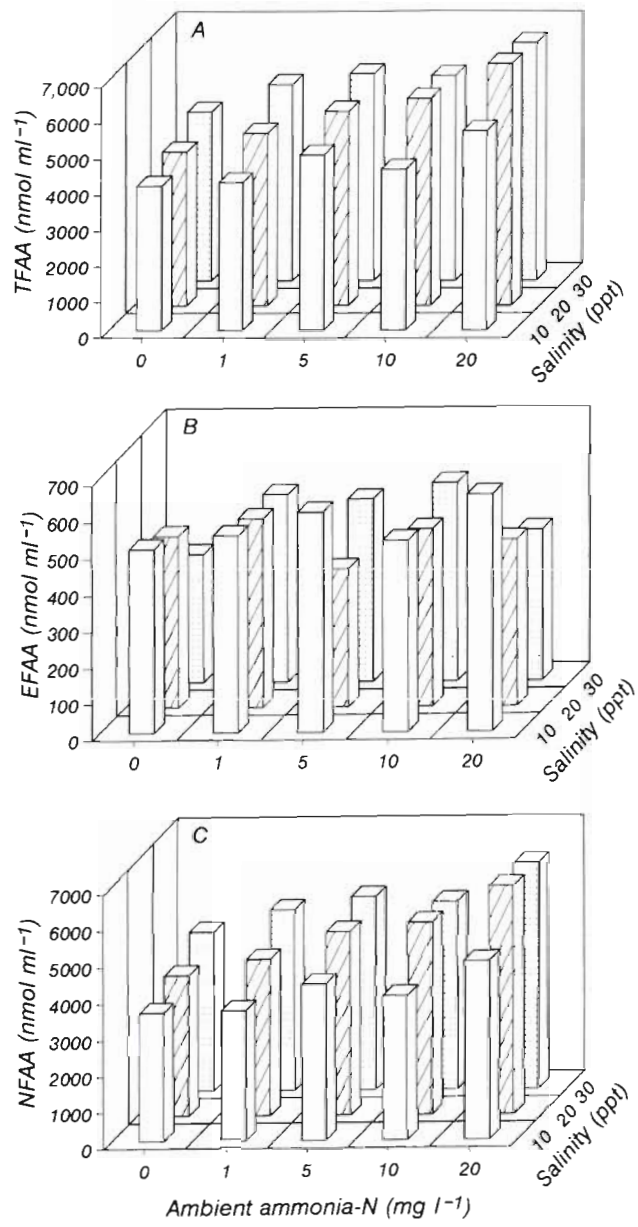

Fig. 4. *Penaeus monodon*. Mean (A) total free amino acid, (B) essential free amino acid and (C) nonessential free amino acid in the hemolymph of shrimp exposed to different concentrations of ambient ammonia-N at different salinity levels after 24 h

C, S and CS is as follows: $HA = 390.6486 + 6.7646C - 0.8274S + 1.5948CS$ ($R^2 = 0.69$).

Analysis of variance indicated that there was a significant effect of ambient ammonia-N and salinity on hemolymph urea. However, no significant interaction between the effects of ambient ammonia-N and salinity on hemolymph urea was observed. The relationship between hemolymph urea (HU) and C and S is as follows: $HU = -147.2772 + 19.6697C + 58.8944S$ ($R^2 = 0.49$).

DISCUSSION

Concentration of ammonia in an organism's blood, in which ammonia enters via diffusion from ambient water or metabolic production, is a principal feature for assessing physiological function. In crustaceans, the normal blood ammonia level varies from 0.033 to 0.95 mmol l^{-1} among species (Binns 1969, Mangum et al. 1976, Harris & Andrews 1985, Hagerman et al. 1990, Chen & Kou 1991). The present study showed

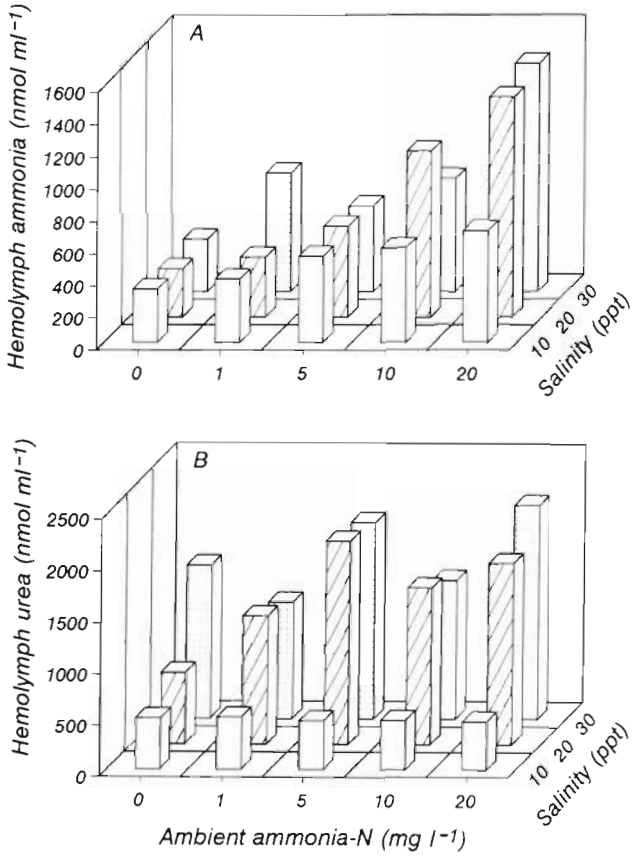

Fig. 5. *Penaeus monodon*. Mean (A) ammonia and (B) urea in the hemolymph of shrimp exposed to different concentrations of ammonia-N at different salinity levels after 24 h

hemolymph ammonia levels of 0.336, 0.302 and 0.328 mmol l^{-1} in 10, 20 and 30‰, respectively.

Effects of salinity on ammonia excretion have been documented for *Carcinus maenas* (Haberfield et al. 1975), for *Penaeus chinensis* (Chen & Lin 1992), for *P. japonicus* (Spaargaren et al. 1982, Chen & Lai 1993), for *P. monodon* (Lei et al. 1989) and for spot shrimp *Pandalus palatyceros* (Quarmby 1985). Increase of ammonia-N excretion occurred in decreased salinity from 30 to 10‰ in both *P. chinensis* and *P. japonicus* (Chen & Lin 1992, Chen & Lai 1993).

Three pathways account for the loss of metabolic ammonia in fish and crustaceans: (1) diffusion of NH_3 from blood to water, (2) exchange transport of NH_4 with Na^+ and (3) conversion to a less toxic compound like urea (Campbell 1973). Diffusion of NH_3 is the principal route of excretion because blood concentrations are usually much higher than the ambient water concentration (Kinne 1976).

Regnault (1987) reported that the ammonia excretion of common shrimp *Crangon crangon* was not influenced by ammonia-N concentrations ranging from 28 to 1218 mg l^{-1}. The present study indicated that ammonia-N excretion of *Penaeus monodon* increased as ambient ammonia-N increased in the range 0 to 5 mg l^{-1}, but decreased in the range 5 to 20 mg l^{-1}, and that net ammonia-N uptake occurred at ambient ammonia-N greater than 5 mg l^{-1} after 24 h exposure. Similar results were obtained by Chen & Lin (1992) for *P. chinensis* juveniles (0.54 ± 0.11 g) which displayed a net ammonia-N uptake at ambient ammonia-N of 5.087 mg l^{-1}. Studying *P. japonicus* (13.91 ± 0.65 g), Chen & Kou (1991) reported that shrimp exposed to increased concentrations of ambient ammonia-N had significantly greater levels of hemolymph ammonia. They suggested that once NH_3 diffuses from water into the hemolymph, the relative proportions of NH_3 and NH_4^+ constituting the ambient ammonia-N readjust and thus NH_3 diffuses continuously in the hemolymph.

Chen et al. (1993) reported that hemolymph levels of ammonia-N in *Penaeus chinensis* increased when shrimp were exposed to increased ambient ammonia-N. After 4 h, the hemolymph ammonia-N level in shrimp exposed to 0.03 mg l^{-1} ambient ammonia-N was 2.69 mg l^{-1}, whereas the hemolymph ammonia-N level in shrimp exposed to 10.11 mg l^{-1} ammonia-N was 6.97 mg l^{-1}. The present study indicated that, during 24 h exposure to ammonia-N increased from 0 to 20 mg l^{-1} in 30‰, hemolymph ammonia rose from 328 to 1412 µmol l^{-1}, hemolymph urea rose from 1492 to 2079 µmol l^{-1} with a concomitant decrease in ammonia-N excretion from 8 to −18 µg g^{-1} h^{-1}. There were concomitant increases in DON excretion from 2.2 to 61.4 µg g^{-1} h^{-1} and urea-N excretion from 0.2 to 23.2 µg g^{-1} h^{-1}. This indicated that shrimp exposed to different concentrations of ammonia-N changed their excretory pattern.

It is noteworthy that high ambient ammonia caused shrimp to increase nitrite-N excretion. Jensen (1990) reported that hemolymph nitrite of freshwater crayfish *Astacus astacus* exposed to 0.8 mmol l^{-1} nitrite reached 8 to 10 mmol l^{-1} within 2 d. Chen & Chen (1992) reported that the concentration of hemolymph nitrite-N of *Penaeus monodon* (15.87 ± 0.69 g) was 0.140 µg ml^{-1}. We propose that some of the ammonia entering the hemolymph may be converted to nitrite inside the animal, then coupled with hemocyanin and subsequently released under ammonia exposure.

The present study indicated that a small amount of nitrate-N was excreted by *Penaeus monodon*, and that nitrate-N excretion seemed to increase with increased ambient ammonia-N. Nitrate-N excretion of *P. monodon* exposed to 5 mg l^{-1} ammonia-N in 20‰ was higher than when exposed to the same concentration of ammonia-N at 10 and 30‰. Spaargaren (1985) exposed *Carcinus maenas* to various salinity levels,

and found that nitrate excretion was highest in brackish water and decreased at both higher and lower salinity levels. He suggested that nitrate formation may serve in the detoxication of ammonia and the maintenance of electroneutrality inside the crab.

Ammonia makes up 70 to 87% of total excreted nitrogen in marine amphipods (Dresel & Moyle 1950), 60 to 100% in the copepods *Calanus helgolandicus* (Corner & Newell 1967) and *Tigriopus brevicornis* (Harris 1973), 72% in spiny lobster *Fasus edwardsi* (Binns & Peterson 1969), 86% in *Carcinus maenas* (Needham 1957) and 95% in Atlantic ditch shrimp *Palaemontes varians* (Snow & Williams 1971) and common shrimp *Crangon crangon* (Regnault 1983). Regnault (1987) reported that in decapod crustaceans, ammonia and amino acid accounted for 60 to 70 and 10% respectively of total nitrogen excreted. In the present study, ammonia-N accounted for 56.87, 70.43 and 78.13% of total nitrogen excreted by *Penaeus monodon* at 30, 20 and 10‰, respectively. DON, which is considered to be mainly free amino acids, accounted for 22.18, 2.58% and 4.40% of total nitrogen excreted by the shrimp at 30, 20 and 10‰, respectively. However, when *P. monodon* was exposed to 5 mg l^{-1} ammonia-N at 30, 20 and 10‰, ammonia-N excretion was inhibited, and urea-N excretion and DON excretion increased to 20.92, 11.44 and 3.98% and to 65.28, 72.05 and 91.63% of total nitrogen, respectively. Our study reveals that the contribution of nitrogenous excretion in penaeids differs under various environmental conditions.

Hemolymph protein is reported for penaeids as 74 to 78 mg ml^{-1} in southern pink shrimp *Penaeus duorarum* (Bursey & Lane 1971), 94 mg ml^{-1} in aloha prawn *P. marginatus* (Balazs et al. 1974), 103 mg ml^{-1} in *P. chinensis* (Chen et al. 1993) and 82, 112 and 112 mg ml^{-1} in *P. monodon* at 30, 20 and 10‰ (present study). The respiratory pigment hemocyanin accounts for 76 to 80% of hemolymph protein in *P. japonicus* (Chen & Cheng 1993). Haberfield et al. (1975) observed an increase in the catabolism of amino acids that resulted in excretion of nitrogen, mainly as ammonia with decreased medium osmolarity. The fact that *P. monodon* exposed to ambient ammonia-N greater than 20 mg l^{-1} had reduced hemocyanin and protein concentrations suggested an increase in catabolism of hemocyanin and protein to adjust osmoregulation. This physiological response affected the hemocyanin level more than the protein level. Hemocyanin played an important role in the catabolism of hemolymph protein of the penaeid under ammonia stress.

Tissue FAA of *Penaeus japonicus* was 546.81 and 1120.33 µmol g^{-1} in 10 and 40‰ (Dalla Via 1986), and that of *P. monodon* was 185, 320 and 365 µmol g^{-1} in 15, 30 and 45‰, respectively (Fang et al. 1992). Hemolymph FAA was 3.32 µmol ml^{-1} in speckled shrimp, *Metapenaeus monoceros* (Rajulu & Kulasekarapandian 1972), 5.80 µmol ml^{-1} in *Homarus gammarus* (Camien et al. 1951), 3.3, 6.3 and 2.8 µmol ml^{-1} in *P. monodon* in 15, 30 and 45‰ (Fang et al. 1992), and 2.717, 2.749 and 2.541 µmol ml^{-1} in *P. monodon* in 10, 20 and 30‰ (present study). An increase in the FAA and ammonia level, and a decrease of hemocyanin and protein in the hemolymph of shrimp exposed to ammonia-N at 5 mg l^{-1}, explain the occurrence of further catabolism of amino acid to produce ammonia.

Chen & Chen (unpubl.) found that the contribution of electrolytes in the hemolymph osmolarity decreased from 95.1 to 84.5% for *Penaeus japonicus* subjected to 20 mg l^{-1} ammonia-N. Despite the major part of hemolymph osmolarity of penaeid being regulated by inorganic ions (Fang et al. 1992), a significant increase of taurine in the present study implied that it was kept as osmolite. Taurine is well known to be strongly concentrated in the intracellular component, with a hemolymph concentration of 0.5 µmol ml^{-1} in a number of decapods (Charmantier et al. 1976). Taurine, which was 0.442, 0.398 and 0.429 µmol ml^{-1} in 10, 20 and 30‰, increased to 0.689, 0.530 and 0.540 µmol ml^{-1} at 20 mg l^{-1} ammonia-N in 10, 20 and 30‰, respectively. The comparatively high levels of taurine and ammonia suggested that they played an important role in hemolymph osmoregulation of penaeids under ammonia stress.

Armstrong et al. (1978) demonstrated that ambient NH_4^+ results in the inhibition of sodium absorption and an increase of NH_4^+ uptake of giant freshwater prawn *Macrobrachium rosenbergii*. Young-Lai et al. (1991) reported that a decrease of hemolymph osmolarity in *Homarus americanus* following exposure to ammonia at 150 mg l^{-1} was caused by lower concentrations of sodium in the hemolymph. It is expected that the same responses may also exist in the hemolymph of *Penaeus monodon* when they are exposed to high ambient ammonia-N. Unfortunately, no determination was made for osmolarity and electrolytes in the hemolymph in the present study.

Using *Penaeus chinensis*, Chen & Nan (1992) documented that ammonia-N excretion was inhibited and Na^+, K^+-ATPase activity decreased as shrimp were exposed to ambient ammonia-N at 10 and 20 mg l^{-1}. In the present study, ambient ammonia-N at 5 mg l^{-1} caused an increase of hemolymph ammonia and urea, and decreases of hemocyanin and protein with a concomitant increase of free amino acids after 24 h exposure, suggesting that *P. monodon* exposed to ambient ammonia-N affects its nitrogen metabolism, and decomposes its hemocyanin and protein into free amino acids and urea to balance osmoregulation

(Lasserre 1976). Accumulated ammonia-N in the hemolymph may also destroy the biosynthesis function of protein in hepatopancreas (Senkbeil & Wriston 1981). Further research is needed to determine the electrolytes in hemolymph, and the enzyme activities involved in nitrogen metabolism of a penaeid under ammonia stress.

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