Hemolymph ammonia and urea and nitrogenous excretions of *Scylla serrata* at different temperature and salinity levels

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ABSTRACT: Hemolymph ammonia and urea levels and nitrogenous excretions of *Scylla serrata* (Forskal) (202 ± 18 g) were measured when the crabs were exposed to 9 different temperature and salinity regimes (15, 25, 35°C combined with 10, 25 and 40‰ S). Hemolymph ammonia increased with a decrease in salinity, whereas hemolymph urea increased with an increase in salinity. Nitrogenous excretion increased with temperature. Excretion of ammonia-N, organic-N, nitrite-N, and nitrate-N and total nitrogen excretion ($\mu g\ g^{-1}\ h^{-1}$) increased with a decrease in salinity, whereas urea-N excretion increased with an increase in salinity. At 25°C, ammonia-N excretion accounted for 82.0, 79.2 and 56.2% of total nitrogen excreted by the crabs at 10, 25 and 40‰ S, whereas urea-N excretion accounted for 4.3, 9.1 and 30.1% respectively. It is concluded that the *S. serrata* at 40‰ S shifts its excretory pattern from ammoniotelism to ureotelism.

KEY WORDS: *Scylla serrata* · Temperature · Salinity · Hemolymph · Ammonia · Urea · Nitrogenous excretion · Metabolism

INTRODUCTION

Serrated crabs, also known as mud crabs, *Scylla serrata* (Forskal), generally inhabit intertidal flats and mangroves throughout the Indo-West Pacific region. Adult crabs inhabit pools and puddles in the littoral zone and migrate into estuaries after mating. Their eggs and resultant zoeae are released in full strength seawater. As soon as they metamorphose to juveniles, they move inshore again. Crabs which are found in the littoral zone are likely to encounter variable salinity levels (Hill et al. 1982, Davenport & Wong 1987).

In any aquatic system, temperature and salinity are considered to be the major factors that limit the distribution of invertebrates. *Scylla serrata* larvae can tolerate temperatures down to 5°C, but they are inactive below 10°C (Hill 1974). The larvae show considerable mortality at salinity levels below 17.5‰, however the juveniles survive at salinity levels in the range 2 to

60‰. Davenport & Wong (1987) reported that young crabs (carapace length 60 to 100 mm) survived for 4 d when they had been transferred from 35‰ to salinity in the range 2 to 32‰. The suitable level of salinity and temperature for the larvae is in the range 25 to 30‰, and 26 to 30°C, respectively (Chen & Cheng 1980).

In decapod crustaceans, ammonia, amino acids and urea are the 3 main end-products of nitrogen metabolism and are released mainly through the gill epithelium (Regnault 1987). Among nitrogenous wastes, ammonia-N (un-ionized plus ionized ammonia as nitrogen), which is the least expensive with regard to energy expenditure, makes up 86% of total excreted nitrogen in the shore crab *Carcinus maenas* (Needham 1957).

The effects of temperature and salinity on nitrogen excretion have been reported for *Carcinus maenas* (Haberfield et al. 1975, Spaargaren 1982, 1985, Harris & Andrews 1985). In order to understand how the nitrogenous excretion of crabs is affected under different temperature and salinity levels, we examined hemolymph ammonia and urea and nitrogenous excre-

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tions of *Scylla serrata* at different temperature and salinity levels in the laboratory.

MATERIALS AND METHODS

Seawater (34‰ S) pumped from the Keelung coast (Taiwan) adjacent to the National Taiwan Ocean University was adjusted to 40‰ with concentrated seawater and to 25 and 10‰ with municipal water that had been dechlorinated with sodium thiosulfate, then filtered through a gravel and sand bed by air-lift and aerated for 3 d before use.

Scylla serrata collected from a crab farm in Iilan, Taiwan, were shipped to our laboratory and divided into 9 groups held in 25‰ seawater at 25°C for 1 wk. Salinity was then adjusted, a decrease or increase of 2 to 3‰ per day with dechlorinated municipal water or concentrated seawater, until salinity levels of 10, 25 and 40‰ were reached. Two thirds of the crabs at each salinity level were then transferred into another 6 tanks with expected final temperatures of 15 or 35°C, and one third of crabs remained at 25°C. The temperature was adjusted, a decrease or increase of 2 to 4°C per day, until the temperature levels of 15 and 35°C were reached. The animals were then acclimated to each test solution for 1 wk prior to the experiment. During the acclimation, the crabs were fed clam *Meretrix lusoria* twice a day, but were not fed 1 d prior to the experiment.

There were 9 test solutions (15, 25 and 40‰ combined with 15, 25 and 35°C), and each treatment was conducted in 5 replicates with 1 crab in each replicate. Circular tanks (20 l capacity) were filled with 10 l of each test solution. Crabs at the intermolt stage were used for the study. The molt cycle stage was determined by the observation of cuticle at the edge of the flattened dactyl segment (paddle) in the fifth walking leg (Freeman et al. 1987). The total number of crabs used was 45. The average wet weight of crabs fasted for 1 d prior to the experiment was 202 ± 18 g, respectively, and no significant difference in weight among treatments was observed.

The crabs were exposed individually from each acclimated test solution to each new test solution (10 l), with measurements of ammonia-N (Solorzano 1969), urea-N (McCarthy 1970), nitrite-N (Bendschneider & Robinson 1952), nitrate-N (Wood et al. 1967) and total nitrogen (Solorzano & Sharp 1980) at the be-

ginning of the experiment and 24 h later. Concentration of organic-N was calculated based on the differences among total nitrogen, ammonia-N, urea-N, nitrite-N and nitrate-N. During the experiment, mean water DO (dissolved oxygen) and pH (mean ± SD) were 6.58 ± 1.13 mg l^{-1} and 8.13 ± 0.18, respectively, with no significant difference among the treatments.

The differences in concentrations of ammonia-N, urea-N, nitrite-N, nitrate-N, organic-N and total nitrogen between the beginning and end of the experiment were recorded. Weight specific ammonia-N excretion, urea-N excretion, nitrite-N excretion, nitrate-N excretion, organic-N excretion and total nitrogen excretion (µg g^{-1} h^{-1}) were calculated by multiplying water volume of each tank, and dividing by wet body weight and time lapse.
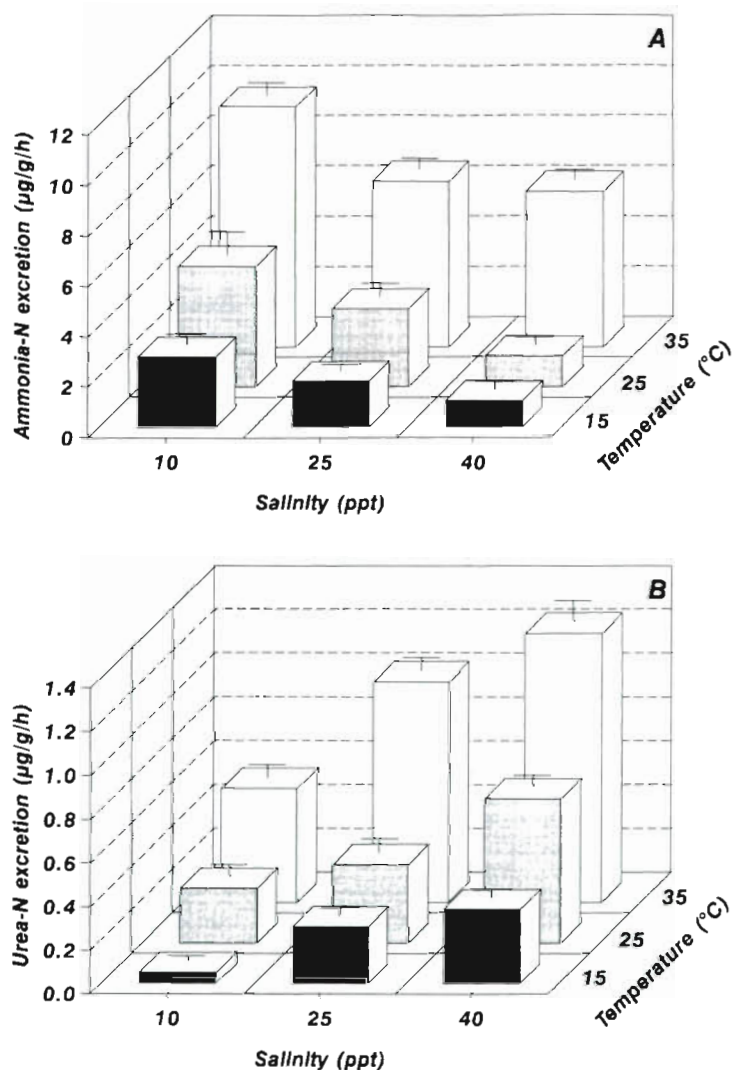

Fig. 1. *Scylla serrata*. Mean ± SE (A) ammonia-N excretion and (B) urea-N excretion of crabs maintained at different salinities and temperatures after 24 h. n = 5

After 24 h exposure to each test solution, hemolymph samples were taken individually by inserting a syringe (25-gauge × 1") into the body cavity via the proximal arthrodial membrane at the base of the right second walking leg. Hemolymph ammonia-N and hemolymph urea-N were measured colorimetrically with the methods of glutamate dehydrogenase (Sigma Procedure, No. 170) and diacetylmonoxime (Sigma Procedure, No. 535), respectively, as reported by Chen & Cheng (1995).

All data were subjected to 1-way and 2-way analyses 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 differences between treatments (Duncan 1955). The linear relationships among ammonia-N excretion, urea-N excretion, organic-N excretion, nitrite-N excretion, nitrate-N excretion, total nitrogen excretion, hemolymph ammonia-N, hemolymph urea-N, temperature and salinity were tested using the General Linear Model Procedure and Regression Procedure, version 6.03, SAS (Statistical Analysis System) computer software (SAS 1988). All statistical significance tests were at the p < 0.05 level.

RESULTS

Nitrogenous excretion of crabs increased significantly with an increase in temperature at all salinity levels. Ammonia-N excretion decreased with an increase in salinity (Fig. 1A), whereas urea-N excretion increased significantly with an increase in salinity at all 3 temperature levels (Fig 1B).

At 15°C, organic-N excretion of crabs decreased with an increase in salinity, whereas at 25 and 35°C, no significant difference in organic-N excretion was observed among the 3 salinity levels (Fig. 2A). Total nitrogen excretion (Fig. 2B), nitrite-N excretion (Fig. 3A) and nitrate-N excretion (Fig. 3B) increased significantly with a decrease in salinity.

Statistical analysis indicated that there was a significant effect of temperature on all nitrogenous excretions and a significant effect of salinity on all nitrogenous excretions except organic-N excretion. There was also a significant interaction between the effects of temperature and salinity on all nitrogenous excretions except organic-N excretion (Table 1). The relationship between ammonia-N excretion (ANE), urea-N excre-
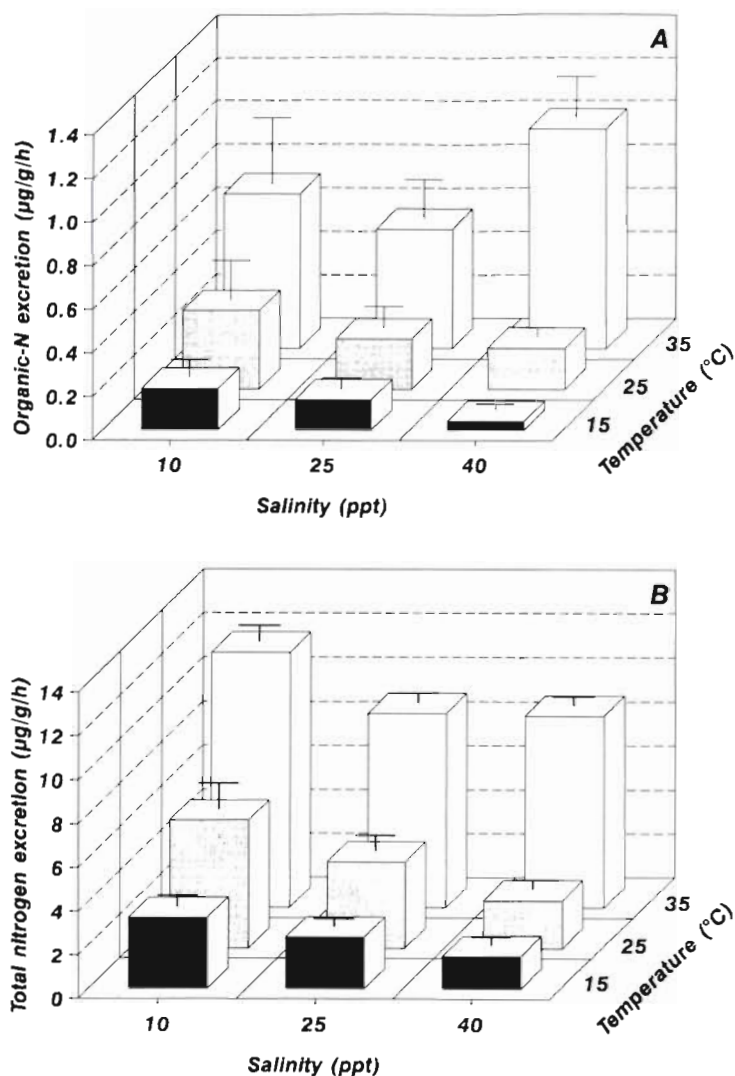

Fig. 2. *Scylla serrata*. Mean ± SE (A) organic-N excretion and (B) total nitrogen excretion of crabs maintained at different salinities and temperatures after 24 h. n = 5

tion (UNE), organic-N excretion (ONE), nitrite-N excretion (NINE), nitrate-N excretion (NANE), total nitrogen excretion (TNE), temperature (T), salinity (S) and the interaction between temperature and salinity (TS) is as follows:

$$ANE = -2.1378 + 0.3452\,T - 0.0304\,S - 0.0027\,TS$$
$$(R^2 = 0.8278)$$

$$UNE = -0.3233 + 0.0179\,T - 0.0019\,S + 0.0007\,TS$$
$$(R^2 = 0.8954)$$

$$ONE = -0.4180 + 0.0316\,T$$
$$(R^2 = 0.4283)$$

$$NINE = -0.2208 + 0.0253\,T + 0.0019\,S - 0.0004\,TS$$
$$(R^2 = 0.8635)$$

$$NANE = -0.1435 + 0.0144\,T + 0.0025\,S - 0.0003\,TS$$
$$(R^2 = 0.8925)$$

$$\text{TNE} = -2.7590 + 0.4154\,T - 0.0471\,S - 0.0019\,TS$$
$$(R^2 = 0.8267)$$

The percentage of each nitrogenous excretion to total excreted nitrogen is given in Table 2. At 25°C, ammonia-N excretion accounted for 82.02, 79.24 and 56.16% of total nitrogen, whereas urea-N excretion accounted for 4.28, 9.11 and 30.14 of total nitrogen excreted by the crabs at 10, 25 and 40‰, respectively.

At 25 and 40‰, hemolymph ammonia-N increased with a decrease in temperature. At 25 and 35°C, hemolymph ammonia-N was lowest at 40‰ (Fig. 4A). For the 3 temperature levels, hemolymph urea-N increased with salinity (Fig. 4B).

Statistical analysis indicated that there were significant effects of temperature and salinity on hemolymph ammonia-N and urea-N, and that there was a significant interaction between the effects of temperature and salinity on the hemolymph ammonia-N and urea-N (Table 3). The relationship between hemolymph ammonia-N (HAN), urea-N (HUN), T, S and TS is as follows:

$$\text{HAN} = 6.9551 + 0.0981\,T + 0.0391\,S - 0.0061\,TS$$
$$(R^2 = 0.5456)$$

$$\text{HUN} = -31.7550 + 1.3190\,T + 3.9140\,S - 0.0990\,TS$$
$$(R^2 = 0.7148)$$

DISCUSSION

Regnault (1987) reported that in decapod crustaceans, nitrogen was excreted mainly as ammonia that accounted for 60 to 70% of total excreted nitrogen, amino acids that accounted for 10% of total excreted nitrogen, and small amounts of urea and uric acids. Ammonia-N excretion is considered an indicator which reflects protein and energy utilization in crustaceans under various stressful environments (Nelson et al. 1977). An increase in ammonia excretion with increasing temperature has been observed in the shore crab *Carcinus maenas* (Needham 1957).

At low salinity (10‰), an increase in ammonia-N excretion suggests that there is an increasing catabolism of amino acids, as was documented in *Carcinus maenas* by Haberfield et al. (1975). The catabolism of amino acids is considered to be involved in osmotic regulation when animals are subjected to low salinity. Free amino acids (FAA) have been considered to play an important role in the regulation of intracellular osmolality of crustaceans in acclimation to hypoosmotic or hyperosmotic media (Boone & Claybrook 1977, Gilles & Pequeux 1983) At higher salinity levels, an increase in tissue FAA has been recorded in *C. maenas* (Siebers et al. 1972), in *Penaeus monodon* (Fang et al. 1992) and in *P. japonicus* (Dalla Via 1986). FAA may contribute more than 40 to 60% of the intracellular osmolality (Robertson 1961, Smith & Dall 1991). Studying northern brown shrimp *P. aztecus*, Bishop & Burton (1993) documented that taurine dominates the amino acid pool at low salinity, while proline dominates it at higher salinity. Unfortunately, we measured FAA in neither tissue nor hemolymph in the present study.

The excretion of ammonia-N, urea-N and organic-N has been found to account for, respectively, 61, 23 and 16% of total nitrogen excreted for green tiger prawn *Penaeus*
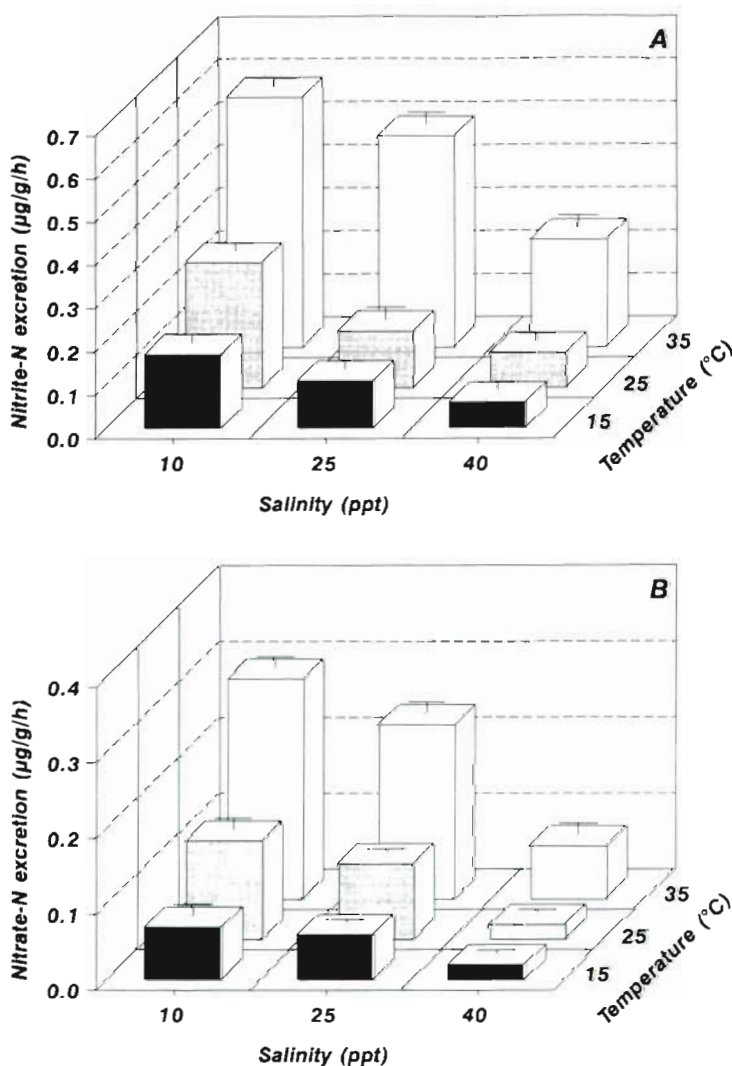

Fig. 3. *Scylla serrata*. Mean ± SE (A) nitrite-N excretion and (B) nitrate-N excretion of crabs maintained at different salinities and temperatures after 24 h. n = 5

Table 1 *Scylla serrata*. ANOVA table of ammonia-N excretion (ANE), urea-N excretion (UNE), nitrite-N excretion (NINE), nitrate-N excretion (NANE), organic-N excretion (ONE) and total nitrogen excretion (TNE) of crabs maintained at different salinities (S) and temperatures (T) for 24 h

Source	df	SS	MS	F	p > F
ANE					
Model	8	273.341	34.167	48.76	0.0001
T	2	208.496	104.248	148.76	0.0001
S	2	57.515	28.757	41.04	0.0001
TS	4	7.329	1.832	2.61	0.0549
Error	30	21.024	0.700		
Total	38	294.365			
UNE					
Model	8	4.860	0.607	73.58	0.0001
T	2	3.180	1.590	192.60	0.0001
S	2	1.406	0.703	85.19	0.0001
TS	4	0.273	0.068	8.27	0.0001
Error	30	0.247	0.008		
Total	38	5.108			
NINE					
Model	8	1.106	0.138	167.01	0.0001
T	2	0.740	0.370	446.95	0.0001
S	2	0.295	0.147	178.49	0.0001
TS	4	0.070	0.017	21.29	0.0001
Error	30	0.024	0.008		
Total	38	1.131			
NANE					
Model	8	0.295	0.037	131.02	0.0001
T	2	0.149	0.075	265.71	0.0001
S	2	0.116	0.058	205.78	0.0001
TS	4	0.029	0.007	26.29	0.0001
Error	30	0.008	0.002		
Total	38	0.304			
ONE					
Model	8	3.264	0.408	5.27	0.0004
T	2	2.693	1.346	17.38	0.0001
S	2	0.101	0.050	0.66	0.5257
TS	4	0.468	0.117	1.51	0.2236
Error	30	2.324	0.077		
Total	38	5.589			
TNE					
Model	8	425.468	53.183	49.10	0.0001
T	2	364.615	182.307	168.29	0.0001
S	2	53.848	26.924	24.85	0.0001
TS	4	7.004	1.751	1.62	0.1958
Error	30	32.498	1.083		
Total	38	457.966			

Table 2. *Scylla serrata*. The percentage of ammonia-N excretion (ANE), urea-N excretion (UNE), nitrite-N excretion (NINE), nitrate-N excretion (NANE), and organic-N excretion (ONE) from nitrogenous excretions of crabs maintained at different salinities (S) and temperatures (T)

T (°C)	S (‰)	ANE	UNE	NINE	NANE	ONE
15	10	85.58	1.53	2.14	5.21	5.54
	25	76.05	10.92	2.52	4.62	5.89
	40	70.00	22.67	1.33	4.00	2.00
25	10	82.02	4.28	2.23	4.97	6.50
	25	79.24	9.11	2.53	3.29	5.83
	40	56.16	30.14	0.91	3.65	9.14
35	10	82.03	4.47	2.49	4.99	6.02
	25	74.27	11.40	2.60	5.53	6.20
	40	70.59	14.07	0.80	2.86	11.68

and intrinsic factors, such as molt cycle, nutritional level and neuroendrocrine control (Regnault 1987, Chen et al. 1994). Since the crabs used in the present study were at the intermolt stage and had been fasted for 1 d, they were considered to be at the same nutritional level.

In crustaceans, metabolic pathways involved in nitrogen excretion are catabolism of amino acid (including certain amides), deamination of purine nucleotides with the formation of ammonia, uricolysis and the ornithine-urea cycle with the formation of urea, and degradation of nucleic acid with the formation of uric acid (Claybrook 1983, Regnault 1987). The ornithine-urea cycle initiates with the fixation of metabolic product of NH_4^+ and HCO_3^- which is supplied from CO_2 through carbonic anhydrase. The higher ammonia-N excretion at low salinity (10‰) suggests that catabolism of amino acid is required to reduce

Table 3. *Scylla serrata*. ANOVA table of hemolymph ammonia-N (HAN) and hemolymph urea-N (HUN) of crabs maintained at different salinities (S) and temperatures (T) after 24 h

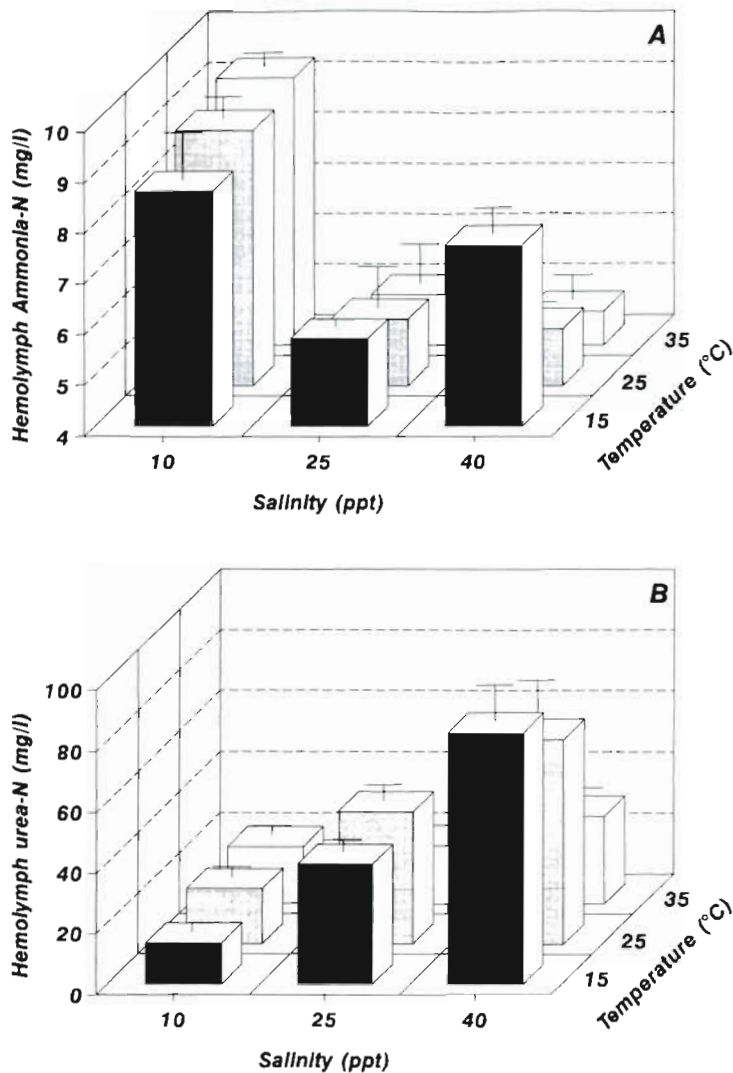| Source	df	SS	MS	F	p > F
HAN					
Model	8	125.523	15.690	13.65	0.0001
T	2	6.865	3.432	2.99	0.0657
S	2	103.879	51.939	45.18	0.0001
TS	4	14.778	3.694	3.21	0.0261
Error	30	34.487	1.149		
Total	38	160.010			
HUN					
Model	8	20071.115	2508.889	11.26	0.0001
T	2	3974.501	1987.250	8.92	0.0009
S	2	12319.739	6159.869	27.66	0.0001
TS	4	3776.874	944.218	4.24	0.0077
Error	30	6681.647	222.721		
Total	38	26752.762			

semisulcatus at 40.5‰, and to account for 57, 2 and 22% of total nitrogen excreted for *Penaeus monodon* at 30‰ (Wajsbrot et al. 1989, Chen et al. 1994). In the present study, higher urea-N excretion with a concomitant higher hemolymph urea-N at 40‰ suggests that urea is formed. The composition of nitrogenous excretion of *Scylla serrata* is greatly affected by different salinity levels.

In crustaceans, nitrogenous excretion is affected by both extrinsic factors, such as temperature and salinity,

Fig. 4. *Scylla serrata*. Mean ± SE (A) hemolymph ammonia-N and (B) hemolymph urea-N of crabs maintained at different salinities and temperatures after 24 h. n = 5

osmolality. At higher salinity (40‰), *Scylla serrata* increased its urea excretion, which suggests that urea was synthesized and subsequently released for hyper-regulation. A shift to increased excretion of urea at higher salinities has also been described in the crayfish *Orconectes rusticus* by Sharma (1968).

It is norteworthy that there is a small contribution from nitrite-N and nitrate-N excretion. The proportion of nitrite-N excretion was highest at 25‰ among the 3 salinity levels tested. The proportion of nitrate-N excretion was high at 25‰ at 35°C. After exposing *Carcinus maenas* to various salinity levels, Spaargaren (1985) reported that nitrate excretion was highest in brackish water and decreased at both higher and lower salinity levels and suggested that nitrate forma-

tion may serve in the detoxication of ammonia and the maintenance of electroneutrality inside the crabs. Nitrite and nitrate are considered to be formed from the oxidation of nitric oxide (NO), which is produced by the conversion of L-arginine to NO and citrulline in the presence of NO synthetase in the arginine pathway (Tayeh & Marletta 1989, Hrabak et al. 1992). For the final step of ornithine-urea, arginine is hydrolyzed by arginase and produces urea and ornithine (Regnault 1987).

Hemolymph ammonia-N has been reported to be 4.62 to 5.46 mg l^{-1} in blue crab *Callinectes sapidus* (Mangum et al. 1976, Cameron & Batterton 1978), 3.50 to 13.23 mg l^{-1} in *Carcinus maenas* (Binns 1969, Harris & Andrews 1985), and 37.42 mg l^{-1} in land crab *Cardisoma carnifex* (Wood & Boutilier 1985). Hemolymph urea-N levels between 8.4 and 56 mg l^{-1} have been reported in *Carcinus maenas* (Binns 1969), and a level of 3.18 mg l^{-1} in *Cardisoma carnifex* (Wood & Boutilier 1985). In the present study, hemolymph ammonia-N levels between 4.66 and 9.27 mg l^{-1} and hemolymph urea-N levels between 13.41 and 82.82 mg l^{-1} were found for *Scylla serrata* and were dependent on salinity level.

In conclusion, the present study indicates that both temperature and salinity affect the nitrogen metabolism of *Scylla serrata*. In low salinity (10‰), there is catabolism of amino acid and formation of ammonia to reduce osmolality. However, at high salinity (40‰), there is an occurrence of urea synthesis and a shift of the nitrogen excretory pattern from ammoniotelism to ureotelism for hyper-regulation. Further research is needed to clarify the activities of enzymes like arginase, carbamoyl phosphate synthetase, and carbonic anhydrase involved in urea synthesis of crabs under different salinity conditions.

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This article was submitted to the editor