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Accumulation of ammonia in the hemolymph of *Penaeus japonicus* exposed to ambient ammonia

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ABSTRACT: *Penaeus japonicus* (14.12 ± 0.74 cm, 13.91 ± 0.65 g) were exposed individually in 34 ppt seawater to 5, 10, 50 and 100 mg l^{-1} ammonia-N (un-ionized plus ionized ammonia as nitrogen) at pH 8.2: additional trials were run using 100 mg l^{-1} ammonia-N at pH 6.3, 7.2, 8.2 and 9.0. Background hemolymph ammonia values were 4.45 ± 0.44 mg l^{-1} and 3.00 ± 0.32 mg l^{-1} ammonia-N, respectively, for shrimp feeding normally and shrimp starved for 2 d. Shrimp exposed to 5, 10, 50 mg l^{-1} and controls survived 16 h trial exposures. No significant difference ($p > 0.05$) in hemolymph ammonia was observed between controls and shrimp exposed to 5 mg l^{-1} for 16 h. However, hemolymph ammonia of shrimp exposed to 10, 50 and 100 mg l^{-1} was significantly higher ($p < 0.05$) after 2 h exposure than for controls. Hemolymph ammonia of shrimp exposed to 100 mg l^{-1} ammonia-N at pH 9.0 was significantly higher ($p < 0.05$) than of those exposed to the same concentration at pH 6.3, 7.2 and 8.2. Shrimp exposed to 100 mg l^{-1} ammonia-N at pH 9.0 and 8.2 accumulated maximal concentrations of ammonia in 30 min and 12 h, respectively. Those exposed to 50 mg l^{-1} ammonia-N at pH 8.2 reached a maximum after 16 h. Both NH_3 (un-ionized ammonia) and NH_4^+ affected ammonia accumulation and caused death; shrimp which had accumulated ammonia-N in the hemolymph to a level of 20 mg l^{-1} were weakened and eventually died.

INTRODUCTION

Ammonia constitutes more than half the nitrogenous wastes excreted by crustaceans (Hartenstein 1970, Kinne 1976, Regnault 1987). Ammonia exists in aqueous solution in both un-ionized (NH_3) and ionized (NH_4^+) forms. The proportion of NH_3 to NH_4^+ in water increases primarily with increases in water temperature and pH, and decreases in salinity (Trussel 1972, Whitfield 1978). Un-ionized ammonia is thought to be lipophilic and easily diffusible across respiratory membranes (Kormanik & Cameron 1981); by contrast NH_4^+ is lipophobic and penetrates membranes less readily (Armstrong et al. 1978).

Penaeus japonicus is one of the most common penaeid shrimp species currently being cultured in Japan and Taiwan. Culture of this species has expanded rapidly since 1987 (Liao & Chien 1990), because of mass mortalities among *P. monodon* (Lightner et al. 1987) and resistance of *P. japonicus* to MBV (monodon baculovirus) disease (Fukuda et al. 1988). Ammonia, one of the most common toxic wastes in an intensive system, may have detrimental effects on cultured animals even with frequent water exchange (Colt & Armstrong 1981, Chen et al. 1989a).

The median lethal concentration (LC_{50}) of ammonia to *Penaeus japonicus* has been reported by Chen et al. (1989b) for larvae, and by Kou & Chen (1991) for juveniles. The EC_{50} (concentration that reduces growth by 50 % of the controls) of ammonia on *P. japonicus* (0.5 to 1.5 g) was reported by Wickins (1976). Accumulation of ammonia in the water may retard shrimp growth and in extreme cases cause death (Wickins 1976, Armstrong et al. 1978, Chen & Lin 1991). This paper describes ammonia accumulation in the hemolymph of *P. japonicus* exposed to different levels of ammonia in the laboratory.

MATERIALS AND METHODS

Penaeus japonicus were obtained from private grow-out farms, acclimated in the laboratory for 3 d and fed commercial shrimp feed at a rate of 5 % of body weight once a day. For 2 d prior to the experiment, the shrimp were not fed. The specimens used had a mean total length of 14.12 ± 0.74 cm (± SD) and weighed 13.91 ± 0.65 g (mean ± SD).

Seawater pumped from the Keelung coast (Taiwan) was filtered through a sand and gravel bed by air-

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lifting, and was aerated for 3 d before use. The chemical characteristics of the seawater were determined using the methods of Strickland & Parsons (1976), and are given in Table 1. Some of the seawater was adjusted from its initial pH of 8.2 to pH 6.3, 7.2, and 9.0 with 2 N HCl or 1 N NaOH over a period of 2 wk.

Ammonia test solutions were prepared by dissolving 382 g of ammonium chloride (Merck reagent grade) in 10 l distilled water to make 10 000 mg l^{-1} 'ammonia-N' (un-ionized plus ionized ammonia as nitrogen), and then diluted to desired concentrations with seawater Three tests were conducted. For the first and second tests, the concentrations of ammonia-N were 0 (control), 10, 50, 100 mg l^{-1} and 0 (control), 5, 10, 50 mg l^{-1}, respectively in 34 ppt seawater at pH 8.2. For the third test, concentration of ammonia-N was 100 mg l^{-1} in 34 ppt seawater at pH 6.3, 7.2, 8.2 and 9.0, respectively. Concentrations of 'NH_3-N' (un-ionized ammonia as nitrogen) and 'NH_4^+-N' (ionized ammonia as nitrogen) were calculated according to the equations of Bower & Bidwell (1978) based on 34 ppt, 25 °C and the respective pH level. Concentrations of ammonia-N, NH_3-N and NH_4^+-N for each test solution are listed in Table 2.

Only intermolt shrimps were selected for the study. The molt stage was determined by examining the uropoda in which partial retraction of the epidermis can be distinguished (Wassenberg & Hill 1984). The starved intermolt shrimp were collected randomly from the holding tanks and individually transferred to 20 l circular plastic tanks containing 10 l test solution. Each treatment was conducted in triplicate. Total numbers of shrimp used in the first, second and third tests were 132, 48 and 120, respectively. The body weight of the shrimp varied from 12.48 to 15.23 g with no significant difference ($p < 0.05$) among the 3 groups. Each tank was covered with a plastic cap to prevent escape. Water temperature was maintained at 25 ± 0.5 °C, and dissolved oxygen, measured every 3 h with a Delta Scientific 2110 DO meter (Delta Company, USA), was kept at 5.46 ± 0.60 mg l^{-1}.

Table 1. Chemical characteristics of seawater used in the study

Salinity	34 ppt
pH	8.2
Ammonia	39 ± 11 µg l^{-1} as ammonia-N
Nitrite	6 ± 4 µg l^{-1} as nitrite-N
Nitrate	45 ± 20 µg l^{-1} as nitrate-N
Phosphate	26 ± 5 µg l^{-1} as orthophosphate-P
Silica	1.0 ± 0.1 mg l^{-1} as SiO_2
Total alkalinity	102 ± 5 mg l^{-1} as $CaCO_3$
Total hardness	6120 ± 387 mg l^{-1} as $CaCO_3$
Sulfide	24.3 ± 1.8 µg l^{-1}
Ca	780 ± 100 mg l^{-1}
Mg	1040.1 ± 26.8 mg l^{-1}

Table 2. Concentrations of NH_3 and NH_4^+ of total ammonia in test solutions used in this study, calculated from ammonia-N based on 34 ppt, 25 °C and pH level

pH	Ammonia-N (mg l^{-1})	NH_3-N (mg l^{-1})	NH_4^+-N (mg l^{-1})
First and second tests			
8.2	Control	0	0
8.2	5	0.35	4.65
8.2	10	0.69	9.31
8.2	50	3.45	46.55
8.2	100	6.90	93.10
Third test			
6.3	100	0.09	99.91
7.2	100	0.74	99.26
8.2	100	6.90	93.10
9.0	100	31.87	68.13

Shrimp were sampled at the specified time intervals up to 14 or 16 h. Hemolymph samples were taken by syringe from the pericardial cavities through the ends of the rostra. Hemolymph ammonia was determined using the Sigma Diagnostic Kits Ultraviolet No. 170-uv (Sigma 1982) (based on the measurement of reductive ammonia of 2-oxoglutarate using glutamate dehydrogenase and reduced nicotinamide adenine dinucleotide; Mondzac et al. 1965). The hemolymph ammonia of shrimp held under normal feeding conditions was also determined, and found to be 4.45 ± 0.44 mg l^{-1}.

All data were subjected to 1-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 differences between treatments (Duncan 1955).

RESULTS

First and second tests

Penaeus japonicus exposed to 100 mg l^{-1} died within 14 h of exposure (Table 3). Shrimp exposed to 5, 10, 50 mg l^{-1} and their matching controls survived 16 h exposure (Table 4).

In controls, hemolymph ammonia-N ranged from 2.58 to 3.35 mg l^{-1} with an average of 3.00 ± 0.32 mg l^{-1} In comparison, for specimens in the 100 mg l^{-1} ammonia-N seawater, hemolymph ammonia-N increased from 3.13 mg l^{-1} after ¼ h to 20.53 mg l^{-1} after 12 h.

Shrimp exposed to increased levels of ambient ammonia had significantly increased hemolymph ammonia levels ($p < 0.05$) after 2 h (Table 3). However, after 1 h no significant difference ($p > 0.05$) was observed in the levels of hemolymph ammonia of shrimp exposed to 10, 50 and 100 mg l^{-1} ammonia-N

Table 3. *Penaeus japonicus*. Mean (± standard deviations) ammonia-N (mg l^{-1}) in the hemolymph at different times of exposure to different levels of ammonia-N (mg l^{-1}) after various periods. Data in the same column having different superscripts are significantly different ($p < 0.05$)

Ammonia-N (mg l^{-1})	Time elapsed (h) ½	1	1½	2	4	6	8	10	12	14	16
Control	2.59a (0.79)	2.58a (0.74)	2.82a (0.30)	3.16a (0.34)	3.13a (0.38)	3.12a (0.40)	3.27a (0.35)	3.19a (0.19)	2.63a (0.24)	3.35a (0.48)	3.10a (0.13)
10	2.71a (0.61)	2.77a (0.68)	3.36a (0.46)	3.98b (1.40)	6.66b (1.01)	6.38b (0.11)	6.83b (0.46)	6.77b (0.46)	9.90b (0.00)	10.89b (0.39)	11.33b (0.82)
50	3.10a (0.14)	2.56a (0.61)	3.99bc (0.89)	5.91c (0.74)	8.54c (0.65)	8.64c (0.56)	11.62c (1.94)	13.53c (1.00)	17.14c (0.77)	17.23c (1.95)	20.13c (1.24)
100	3.13a (0.37)	4.86a (1.03)	5.31c (0.69)	9.57d (0.77)	10.69d (1.11)	11.41d (1.02)	13.02d (0.32)	15.48d (1.15)	20.53d (0.52)	Death	

(Table 3). There was no difference between those exposed to 5 mg l^{-1} and controls after 16 h (Table 4).

Shrimp exposed to 10, 50 and 100 mg l^{-1} after 16, 16 and 12 h accumulated hemolymph ammonia-N to a maximum of 11.33, 20.13 and 20.53 mg l^{-1}, respectively (Table 3).

Third test

When *Penaeus japonicus* were exposed to increased pH levels at 100 mg l^{-1} ammonia-N, hemolymph ammonia increased (Fig. 1). After 10 min, hemolymph ammonia of shrimp exposed to 100 mg l^{-1} ammonia-N at pH 9.0 was significantly higher ($p < 0.5$) than for those exposed to the same concentration of ammonia-N at pH 8.2, 7.2 and 6.3. However, no significant difference ($p > 0.05$) in hemolymph ammonia was observed among those exposed to 100 mg l^{-1} at pH 6.3, 7.2 and 8.2 for 30 min. Shrimp exposed to 100 mg l^{-1} at pH 6.3 and 7.2 survived 14 h of exposure. However, those exposed to 100 mg l^{-1} at pH 9.0 died after exposures ranging from 30 min to 1 h (Fig. 1).

Table 4. *Penaeus japonicus*. Mean (± standard deviations) ammonia-N (mg l^{-1}) in the hemolymph at different times of exposure to different levels of ammonia-N (mg l^{-1}). Data in the same column having different superscripts are significantly different ($p < 0.05$)

Ammonia-N (mg l^{-1})	Time elapsed (h) 4	8	12	16
Control	2.94a (0.15)	3.20a (0.18)	3.04a (0.08)	3.14a (0.09)
5	3.07a (0.10)	3.15a (0.06)	3.03a (0.06)	3.13a (0.07)
10	6.09b (0.22)	7.23b (0.18)	10.13b (0.64)	10.86b (0.15)
50	8.57c (0.21)	11.76c (0.75)	17.47c (0.38)	20.45c (0.54)

Shrimp exposed to 100 mg l^{-1} at pH 6.3, 7.2, 8.2 and 9.0 for 14, 14, 12 and ½ h, respectively, accumulated ammonia-N to a maximum of 21.55, 21.41, 19.54 and 19.76 mg l^{-1} respectively in the hemolymph.

DISCUSSION

The concentration of ammonia in an organism's blood, which ammonia enters via diffusion from ambient water or metabolic production, is a principal feature for assessing physiological function. In fish, the normal level of plasma ammonia varies with species. Concentrations of ammonia in the blood of starved rainbow trout *Salmo gairdneri* and coho salmon *Oncorhynchus kisutch* were 3 and 0.26 mg l^{-1}, respectively (Fromm & Gillette 1968, Buckley et al. 1979). Reported concentrations of ammonia in the hemolymph of crustaceans range from 2 to 18 mg l^{-1} (Mangum et al. 1976, Armstrong et al. 1978).

Diffusion of NH_3 from blood to water, exchange of NH_4^+ with Na^+, and conversion to a non-toxic compound are 3 routes by which fish and crustaceans lose metabolic ammonia (Campbell 1973). Diffusion of NH_3 is a principal route of excretion because blood levels are normally much higher than ambient water concentrations (Kinne 1976). Ammonia in the blood of rainbow trout was reported to be 9 to 40 times greater than that in ambient water (Fromm & Gillette 1968).

Nelson & Kropp (1985) reported that the metabolic rates of starved *Macrobrachium lar* (a tropical freshwater prawn) were different from those for fed individuals. In this study, the hemolymph ammonia levels of normal-feeding and starved *Penaeus japonicus* were 4.45 ± 0.44 mg l^{-1} and 3.00 ± 0.32 mg l^{-1}, respectively. Thus, once the shrimp were exposed to ambient water having ammonia-N exceeding 10 mg l^{-1}, hemolymph ammonia after 2 h of exposure became a function of ambient ammonia. This suggests that when the shrimp were exposed to more than 10 mg l^{-1}

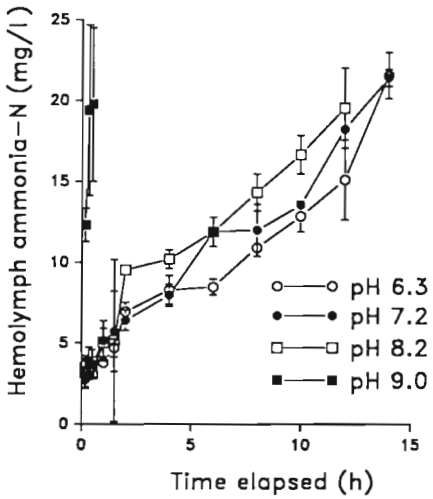

Fig. 1. *Penaeus japonicus*. Relationships between hemolymph ammonia-N (mg l^{-1}) and time elapsed (h), for shrimps exposed to 100 mg l^{-1} ammonia-N at pH 6.3, 7.2, 8.2 and 9.0 for different periods

ammonia-N, diffusion of NH_3 from hemolymph to water was reversed. Using *P. chinensis* (10.38 ± 0.30 g), Chen & Nan (unpubl.) documented that excretion of ammonia increased as ambient ammonia-N increased to 5 mg l^{-1}, but excretion of ammonia was reduced when the level of ambient ammonia-N exceeded 10 mg l^{-1}. Fromm & Gillette (1968) reported that the blood level of ammonia-N in rainbow trout rose from 40 to 70 mg l^{-1} with a concomitant decrease in ammonia excretion rate of about 43 % as ambient ammonia-N increased from 0 to 8 mg l^{-1}.

After 12 to 16 h of exposure, hemolymph ammonia of shrimp exposed to 10 mg l^{-1} ammonia-N approximated the concentration in water from which they were taken. Hemolymph ammonia of shrimp exposed to 50 and 100 mg l^{-1} ammonia-N at pH 8.2 was less than the concentrations in the water from which the shrimp were taken even after 16 and 12 h of exposure (Table 3). Likewise, hemolymph ammonia values for shrimp exposed to 100 mg l^{-1} at pH 6.3, 7.2, and 9.0 were less than the concentrations in the waters from which the shrimp were taken after 14 h, 14 h and 30 min of exposure respectively (Fig. 1).

The un-ionized ammonia (NH_3-N) content was 0.69, 3.45 and 6.90 mg l^{-1} in 10, 50 and 100 mg l^{-1} ammonia-N at pH 8.2, and 0.09, 0.74 and 31.87 mg l^{-1} in 100 mg l^{-1} ammonia-N at pH 6.3, 7.2 and 9.0 (Table 2). *Penaeus japonicus* exposed to 50 and 100 mg l^{-1} at pH 8.2 accumulated ammonia-N in the hemolymph in 2 h at concentrations of NH_3-N greater than those in the ambient waters. This fact indicates that both NH_3 and NH_4^+ affected accumulation of ammonia in the hemolymph of the shrimps. We suggest that once NH_3 diffuses into the hemolymph, the balance between NH_3 and NH_4^+ in ambient ammonia readjusts and NH_3 thus diffuses continuously in the hemolymph.

Armstrong et al. (1978) investigated the effect of ammonia on the freshwater prawn *Macrobrachium rosenbergii* at different pH values and suggested that high ambient NH_4^+ levels inhibited the normal Na^+ influx rate. Campbell (1973) suggested that NH_3 entering the blood from the ambient water or through metabolic productioin is converted to NH_4^+. The resultant release of hydroxyl ions and subsequent elevation of blood pH values significantly affected enzyme-catalyzed reactions and membrane stability. The fact that the shrimp died upon exposure to 100 mg l^{-1} at pH 8.2 and 9.0 after 14 and 1 h, respectively, suggests ammonia may affect the enzyme systems. Unfortunately, the Na^+, enzyme system, and pH levels in the hemolymph of the shrimps were not measured in this study.

Penaeus japonicus exposed to 100 mg l^{-1} at pH 9.0 accumulated 19.76 mg l^{-1} ammonia-N in the hemolymph after ½ h. The concentration of NH_3 in this solution was much higher than that in the other 3 solutions. At the higher pH levels, NH_3 levels increase; NH_3 keeps diffusing inward and death ensues when tolerable body loads are exceeded. Concentration of hemolymph ammonia could be used as an index of ammonia loading for *P. japonicus* intensive culture systems.

The NH_3-N is 0.69 mg l^{-1} in 10 mg l^{-1} ammonia-N solution at pH 8.2, and is 0.74 mg l^{-1} in 100 mg l^{-1} ammonia-N solution at pH 7.2 (Table 2); however, NH_4^+-N is 9.31 and 99.26 mg l^{-1}, respectively, i.e. greater by a factor of 10. Despite the similar levels of NH_3, hemolymph ammonia of shrimp in the 10 mg l^{-1} ammonia-N solution at pH 8.2 was lower than that of shrimp in the solution with a concentration of 100 mg l^{-1} at pH 7.2. This observation suggests that NH_4^+ plays an important role in elevation of hemolymph ammonia. Hemolymph ammonia may be attributed exclusively to diffusion of NH_3, because a new balance of NH_3 and NH_4^+ occurs when NH_3 enters the hemolymph, but may also be attributed to diffusion or penetration of NH_4^+ in addition to Na^+ exchange.

Both NH_3 and NH_4^+ could affect accumulation of ammonia in the hemolymph of *Penaeus japonicus*. Further research is needed to document the carrying capacity of hemocyanin with oxygen, its physiological significance and the respiration of the shrimp exposed to ambient ammonia.

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LITERATURE CITED

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

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

Buckley, J. A., Whitmore, C. M., Liming, D. S. (1979). Effect of prolonged exposure to ammonia on the blood and liver glycogen of coho salmon (*Oncorhinchus kisutch*). Comp. Biochem. Physiol. 63C: 297–303

Campbell, J. W. (1973). Nitrogen excretion. In: Progress, C. L. (ed.) Comparative animal physiology. W B. Saunders, Philadelphia, p. 279–316

Chen, J. C., Lin, C. Y (1991). Effect of ammonia on growth of *Penaeus penicillatus* juveniles. Comp. Biochem. Physiol. (in press)

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

Chen, J. C., Tu, C. C., Yang, W. S. (1989b). Acute toxicity of ammonia to larval *Penaeus japonicus*. J. Fish. Soc. Taiwan 16: 261–270

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

Duncan, D. E. (1955). Multiple-range and multiple F test. Biometrics 11: 1–42

Fromm, P. O., Gillette, J. R. (1968). Effects of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 26: 887–896

Fukuda, H., Momoyama, K., Sano, T (1988). First detection of monodon baculovirus in Japan. Nippon Suisan Gakkaishi 54: 45–48

Hartenstein, R. (1970). Nitrogen metabolism in non-insect arthropods. In: Campbell, J. W. (ed.) Comparative biochemistry of nitrogen metabolism – I, the invertebrates. Academic Press, New York, p. 298–372

Kinne, O. (1976). Cultivation of marine organisms: water-quality management and technology. In: Kinne, O. (ed.) Marine ecology, Vol. III, Cultivation, Part 1. Wiley, Chichester, p. 79–300

Kormanik, G., A., Cameron, J. N. (1981). Ammonia excretion in animals that breathe water: a review. Mar Biol. Lett. 2: 11–23

Kou, Y Z., Chen. J. C. (1991). Acute toxicity of ammonia to *Penaeus japonicus* juveniles. Aquacult. and Fish. Manag. 22: 259–263

Liao, I. C., Chien, Y. H. (1990). Evaluation and comparison of culture practices for *Penaeus japonicus*, *P. pencillatus* and *P. chinensis* in Taiwan. In: Main, K. L., Fulks, W. (eds.) The culture of cold-tolerant shrimp: Proceedings of an Asian-U.S. Workshop on Shrimp Culture. The Oceanic Institute, Honolulu, p. 49–63

Lightner, D. V., Hedrick, R. P., Fryer, J. L., Chen, S. N., Liao, I. C., Kou, G. H. (1987). A survey of cultured penaeid shrimp in Taiwan for viral and other important disease. Fish Pathol. 22: 127–140

Mangum, C. P., Silverthorn, S. V., Harris, J. L., Towle, D. W., Krall, A. R. (1976). The relationship between blood pH, ammonia excretion and adaption to low salinity in the crab *Callinectes sapidus*. J. exp. Zool. 195: 129–136

Mondzac, A., Ehrlich, G. E., Seegmiller, J. E. (1965). An enzymatic determination of ammonia in biological fluids. J. Lab. Clin. Med. 66: 526–531

Nelson, S. G., Kropp, R. K. (1985). Ammonia excretion and nitrogen assimilation by the tropical freshwater prawn *Macrobrachium lar* (Crustacea, Palaemonidae). Comp. Biochem. Physiol. 81A: 699–704

Regnault, M. (1987). Nitrogen excretion in marine and freshwater crustacea. Biol. Rev. 62: 1–24

Sigma (1982). The quantitative ultraviolet determination of ammonia in plasma at 340 nm. Sigma Technical Bulletin 170-uv. Sigma Chemical Co., St Louis

Steel, R. G. D., Torrie, J. H. (1980). Principles and procedures of statistics. McGraw-Hall, New York

Strickland, J. D. H., Parsons, T R. (1976). A practical handbook of seawater analysis. Bull. Fish. Res. Bd Can. 167

Trusell, R. P. (1972). The percent un-ionized ammonia in aqueous ammonia solution at different pH levels and temperatures. J. Fish. Res. Bd Can. 29: 1505–1507

Wassenberg, T. J., Hill, B. J. (1984). Moulting behaviour of the tiger prawn *Penaeus esculentus* (Haswell). Aust. J. mar Freshwat. Res. 35: 561–571

Whitfield, M. (1978). The hydrolysis of ammonium ions in sea water – experimental confirmation of predicted constants at one atmosphere pressure. J. mar. biol. Ass. U.K. 58: 781–787

Wickins, J. F. (1976). The tolerance of warm-water prawns to recirculated water. Aquaculture 9: 19–37

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