Acute toxicity of ammonia and its effects on the haemolymph osmolality, ammonia-N, pH and ionic composition of early juvenile mud crabs, *Scylla serrata* (Forskål)

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**Abstract**

The current study was conducted to determine the LC50 value of ammonia-N as well as the effects of acute exposure to elevated ammonia on the haemolymph osmolality, ionic composition, ammonia-N and pH levels of early juvenile mud crabs, *Scylla serrata*. The results show that early *S. serrata* juveniles have a high 96-h LC50 value of 95.35 mg/L ammonia-N (6.81 mg/L NH3-N) or 6.80 mmol/L total ammonia-N (0.486 mmol/L NH3-N). Following a 96-h exposure, the haemolymph osmolality and K+ levels of the surviving crabs remained unaltered (*p* > 0.05) at all ammonia-N concentrations, while the haemolymph Na+ and Ca2+ were significantly lower (*p* < 0.05) for the crabs exposed to 5.710 and 7.138 mmol/L ammonia-N. While the haemolymph ammonia-N levels of the crabs significantly increased (*p* < 0.01) with increasing external ammonia-N concentrations, the haemolymph ammonia-N of the crabs remained below the external ammonia-N concentrations. The haemolymph pH of the crabs significantly increased between 0.714 and 4.283 mmol/L total ammonia-N. However, at 5.710 mmol/L total ammonia-N the haemolymph pH dropped and was not significantly different (*p* > 0.05) from that of the control crabs which coincided with significantly lower (*p* < 0.05) haemolymph Na+ and Ca2+ levels. These physiological responses may explain the high ammonia tolerance of early *S. serrata* juveniles.

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**Keywords:** Ammonia toxicity; Haemolymph osmolality; Haemolymph ionic composition; Haemolymph pH; Haemolymph ammonia; Mud crab juveniles; *Scylla serrata*

1. Introduction

The mud crab, *Scylla serrata*, commonly inhabits estuarine systems and is native throughout the Indo-Pacific region (Hill et al., 1982; Hyland et al., 1984). Their harvests support a commercial fishery industry, however, to meet increasing demands they are currently cultured in a wide range of aquaculture systems including pens, ponds and recirculating systems (Keenan, 1999; Trího et al., 1999). In closed aquaculture systems, ammonia levels are often one of the most important limiting factors as rapid accumulation of ammonia can occur due to excessive feeding, stress and/or interrupted biofiltration (Timmons et al., 2002). As the ammonia tolerance is often species-specific (Nan and Chen, 1991; Chen and Lin, 1992; Rebelo et al., 1999; Lin and Chen, 2001; Romano and Zeng, 2007), determining the ammonia tolerance of a targeted cultured species can benefit both the aquaculture and fisheries industry. Of the two forms of ammonia that exist in water, the un-ionised form (NH3) and ionised form (NH4+), the un-ionised form is considered more toxic to aquatic animals as it can easily diffuse across gill membranes (Armstrong et al., 1978; Evans and Cameron, 1986).

At unphysiologically high external ammonia levels, aquatic crustaceans may actively excrete ammonia across a concentration gradient (Weihrauch et al., 2004). While this process is not completely understood, and may vary with different species and conditions, it is believed to occur on the gills via ouabain-sensitive Na+/K+-ATPase, where NH4+ substitutes for K+, leading to reduced haemolymph K+ levels (reviewed by Weihrauch et al., 2004). Moreover, other transport mechanisms has suggested to be involved, specifically an apical amiloride-sensitive Na+/NH4+ transport which exchanges Na+ inwards and NH4+ outwards to
the media (Pressley et al., 1981; Lucu, 1989; Weihrauch et al., 2004). However, at excessively high ammonia-N levels, an interruption of this mechanism can occur leading to an impairment of osmo-ionoregulation (Young-Lai et al., 1991; Chen and Chen, 1996; Harris et al., 2001).

The ability to regulate acid–base balance is necessary to maintain/optimise enzymatic function and membrane stability in aquatic animals (Campbell, 1973; Henry and Wheatly, 1992; Pavasovic et al., 2004). However, disturbances or changes to acid–base regulation may be attributed to numerous factors, with varying effects, which include temperature, respiratory/metabolic processes and haemolymph ionic composition changes (Henry and Wheatly, 1992; Varley and Greenaway, 1992; Siebers et al., 1994; Whiteley et al., 2001). For example, CO2 accumulation can lead to haemolymph acidosis (Henry and Wheatly, 1992; Varley and Greenaway, 1992) while increases in external NH4+ levels or haemolymph HCO3− levels can lead to haemolymph alkalosis (Campbell, 1973; Taylor and Whiteley, 1989; Wilson and Taylor, 1992; Rebele et al., 1999).

Understanding and determining the species-specific ammonia tolerance and physiological responses are important to aquaculture management, conservation and toxicology. However, while the ammonia-N tolerance through larvae ontogenetic development of S. serrata has been studied (Neil et al., 2005), currently there is no published information on the ammonia-N tolerance, or the osmo-ionoregulatory response to elevated ammonia-N exposure, for the mud crab S. serrata juveniles or adults. Furthermore, limited information is available concerning the effects of elevated ammonia-N levels on the haemolymph pH of crustaceans (Rebele et al., 2000).

The aim of the current study was to determine the ammonia tolerance of early juvenile mud crabs, Scylla serrata. In addition, to better understand the underlying physiological mechanisms, the haemolymph osmolality, ionic composition, ammonia-N and pH levels of the crabs were also measured following their 96-h exposure to elevated ammonia-N levels.

2. Materials and methods

2.1. Source of crabs

The larviculture of Scylla serrata (Forskål) was performed according to Holme et al. (2006). Briefly, the broodstock crabs were collected from the estuary areas of Townsville, North Queensland, Australia, and kept in outdoor recirculating systems until spawned. When a spawning female was found, the buried female was transferred to a round indoor 300-L tank and kept individually until hatching. On the day of hatching, the larvae were stocked at approximately 100–120 individuals/L and fed rotifers (Branchionus sp.) at approximately 40–60 individuals/mL. Daily additions of the microalgae Nanochloropsis sp. were made to maintain the rotifer density. On the second day from the Zoea II stage, newly hatched Artemia sp. nauplii were daily added and from the Zoea IV stage onwards, a mixture of Artemia nauplii and enriched Artemia (INVE; AAA) metanauplii were fed to the larvae until their settlement to the first crab stage (C1).

Three days after the majority megalopae metamorphosis to the C1 stage, all settled crabs were transferred to outdoor recirculating 1000-L oval tanks. All tanks were underneath a shed area and connected to the same water source. The salinity and temperature of the water were 30±2‰ and 28±2 °C, respectively. Numerous hides, consisting of PVC pipes, rocks, coral and mesh, were provided to reduce cannibalism. The crabs were daily fed a formulated crumble food (43% protein; 6% fat; 3% fibre), designed for the tiger prawn Penaeus monodon (Ridley) to satiation, and every second day supplemented with frozen diced mussel meat. Crabs that reached the C3 stage were transferred and kept individually in containers (diameter 16 cm × height 19 cm) in order to prevent cannibalism and track molting. Each container had numerous 3.75 mm holes to facilitate adequate water exchanges. When the C5 stage was reached, the crabs were transferred indoors for the experiment. Prior to commencing the experiment, the crabs were blotted dry using a tissue and placed on zeroed scale (Adventurer Pro digital scale; 0.001 g) in a small container of water to obtain their wet weights.

2.2. Experimental design and set-up

A total of 180 crabs (mean mass = 0.373±0.024 g) were used for the 96-h acute ammonia toxicity experiment. Each crab was individually kept within a 5-L container (height=20 cm; diameter=21 cm), filled with 3.5-L seawater containing the desired ammonia-N concentration. All containers were bathed within six 1000-L oval tanks, in a random block design, and the water temperature was maintained at 28±0.5 °C through submersible heaters and air conditioning. The water used throughout the experiment was natural source seawater (5 μm filtered and UV sterilised), and in all cases, the ammonia, nitrite and nitrate level were of 0.01±0.00 mg/L. The seawater was pre-adjusted to 30‰ through the addition of de-chlorinated freshwater and pre-adjusted to a pH of 8.10 through the addition of sodium hydroxide (NaOH) pellets. A total of 8 ammonia-N treatments (10, 20, 40, 60, 80, 100, 120 and 140 mg/L or 0.714, 1.428, 2.855, 4.283, 5.710, 7.138, 8.565 and 9.993 mmol/L nitrogen in the form of NH4Cl) and a control (no ammonia-N added) were set up. For each ammonia-N treatment and control a total of 20 crabs were individually placed in each 5-L container and therefore each crab acted as a replicate. Stock solutions of ammonia, in the form of ammonium chloride (NH4Cl) (Ajax Finchem, analytical reagent) were made daily according to Chen and Kou (1993) and diluted to the desired concentration. All containers received a daily 100% water exchange according to the “static renewal method” described by the American Public Health Association (1985). At the 24th, 48th and 72nd hour of the experiment, each crab was fed with the crumble tiger prawn pellets for 1 h prior to water exchanges. However, the crabs were then starved for 24-h prior to haemolymph sampling at approximately the 96th hour according to Lignot et al. (2000). The photoperiod was L:D=14 h:10 h with a light intensity between 132 and 170 lx as measured by a lux meter (TPS, MC-88 Light meter, Australia).

To calculate the LC50 values, mortality observations were made at 12-h intervals for 96 h. Death was assumed when no
movement occurred when mechanically stimulated with a glass rod.

To confirm the actual ammonia-N concentrations used, 3 samples from each test solution, were taken on the first and last day of the experiment, and stored at −15 °C for a maximum of 7 days. The ammonia samples levels were determined using a salicylate method (Krom, 1980) and measured on a colorimeter (HACH 10200; USA) after appropriate dilutions.

### 2.3. Haemolymph sampling and analysis

At the end of the 96-h experiment all surviving crabs at the intermolt stage, as determined according to Chen and Chia (1997), were measured for haemolymph osmolality, total Na⁺, K⁺, Ca²⁺, as well as total ammonia-N and pH levels. To obtain the haemolymph a syringe was inserted through the proximal arthropodial membrane at the base of the right second walking leg of the crabs. To determine haemolymph osmolality, an aliquot of obtained haemolymph (50 µL) was immediately analysed on an Osmomat 030 cryoscopic osmometer (Gonotec, UK). To determine total haemolymph Na⁺, K⁺ and Ca²⁺ levels the haemolymph (20 µL) was immediately diluted with 2 mL of distilled water and analysed on flame photometer (Sherwood 410, UK). For the haemolymph ammonia-N measurements, 4 haemolymph samples from the crabs exposed to each ammonia-N concentration, as well as the control, were determined using a salicylate method (Krom, 1980) as above after appropriate dilutions. The haemolymph pH levels were measured at 28 °C using a pH digital meter (WP-80; TPS, Australia) equipped with a Microelectrodes Inc. micro-pH electrode (MI-710, US) after a two-point calibration with precision buffers. Haemolymph data were not obtained for the crabs exposed to 8.565 and 9.993 mmol/L (or 120 and 140 mg/L) total ammonia-N as a complete mortality occurred within the 96-h experimental duration. Furthermore, due to a combination of fewer surviving crabs and limited amount of obtained haemolymph, no data were obtained for the haemolymph pH of the crabs exposed to 7.138 mmol/L (or 100 mg/L) total ammonia-N.

### 2.4. Data analysis

The LC50 (median lethal concentration) values of total ammonia-N, NH₃-N, and the chi-square goodness of fit were computed using SAS program PROC PROBIT (SAS Institute Inc., 1990) after estimated lines were satisfactory. The un-ionised form of ammonia (NH₃-N) was calculated according to Whitfield (1974) based on a salinity of 30‰, temperature of 28 °C and a pH of 8.10.

The LC50 values, their 95% confidence intervals of total ammonia-N, NH₃-N, and the chi-square goodness of fit were computed using SAS program PROC PROBIT (SAS Institute Inc., 1990) after estimated lines were satisfactory. The un-ionised form of ammonia (NH₃-N) was calculated according to Whitfield (1974) based on a salinity of 30‰, temperature of 28 °C and a pH of 8.10.

To determine significant differences in the haemolymph osmolality, total Na⁺, K⁺, Ca²⁺, total ammonia-N and pH levels of the crabs a one-way ANOVA was used, after confirmation of normality and homogeneity of data. If the homogeneity of variance was violated, a log transformation of the data was performed prior to further analysis. If any significant differences were detected (p<0.05), differences among treatments were identified using Tukey’s HSD test. To determine the relationship between haemolymph total ammonia-N and the external total ammonia-N concentrations a logistic regression was used (Zar, 1999).

### 3. Results

#### 3.1. Ammonia toxicity

No mortalities were observed during the 96-h experimental period in the control, 0.714, 1.428, 2.855 or 4.283 mmol/L total ammonia-N. Meanwhile, all crabs died within a 36-h when exposed to 9.993 mmol/L total ammonia-N. A Chi-square analysis indicated that ammonia-N toxicity significantly increases (p<0.01) with duration of exposure. The 96-h LC50 value (and the 95% confidence interval) of total ammonia-N was 6.80 mmol/L (7.13–6.52) or 95.35 mg/L (99.85–91.35) (Fig. 1). The 96-h LC50 value of un-ionised ammonia-N (NH₃-N) (and the 95% confidence interval) was 0.486 mmol/L (0.51–0.47) or 6.81 mg/L (7.13–6.53) (Table 1).
3.2. Haemolymph osmolality and haemolymph Na\(^+\), K\(^+\) and Ca\(^{2+}\) concentrations

The results show that the surviving crabs in all ammonia-N concentrations and control exhibited hyper-osmoregulation at a salinity of 30‰, as the haemolymph osmolality of the crabs were substantially higher than that of external seawater of 30‰ (Table 2). Furthermore, compared to the control crabs, the haemolymph osmolality was not significantly altered (\(p > 0.05\)) by elevated ammonia-N exposure. The haemolymph K\(^+\) levels remained higher than the external media and were not significantly (\(p > 0.05\)) altered by exposure to elevated ammonia-N levels when compared to the control (Table 2). However, at an exposure to 5.710 and 7.138 mmol/L total ammonia-N, both the haemolymph Na\(^+\) and Ca\(^{2+}\) levels of the crabs were significantly lower (\(p < 0.05\)) when compared to those from the control. The haemolymph Na\(^+\) were below the external media when the crabs were exposed to 4.283 mmol/L or higher, while the haemolymph Ca\(^{2+}\) was below the external media when the crabs were exposed to 0.714 mmol/L or higher (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Ammonia-N concentrations (mmol/L)</th>
<th>Osmolality (mosM/kg), Sodium (mmol/L), Potassium (mmol/L), Calcium (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.714 mmol/L)</td>
<td>968.73±8.85 a, 415.66±2.93 a, 11.66±0.28 a, 11.00±0.23 a</td>
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<tr>
<td>1.428 mmol/L (20 mg/L)</td>
<td>967.85±10.01 a, 407.22±2.56 ab, 11.40±0.30 a, 10.70±0.15 ab</td>
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<tr>
<td>2.855 mmol/L (40 mg/L)</td>
<td>965.92±7.96 a, 407.12±5.10 ab, 11.44±0.33 a, 10.77±0.22 ab</td>
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<tr>
<td>4.283 mmol/L (60 mg/L)</td>
<td>960.71±6.69 a, 397.90±4.41 ab, 11.33±0.40 a, 10.44±0.17 ab</td>
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<tr>
<td>5.710 mmol/L (80 mg/L)</td>
<td>979.09±8.55 a, 397.11±5.05 b, 11.70±0.26 a, 10.00±0.25 b</td>
</tr>
<tr>
<td>7.138 mmol/L (100 mg/L)</td>
<td>951.50±8.04 a, 392.25±3.19 b, 11.50±0.12 a, 10.00±0.40 b</td>
</tr>
</tbody>
</table>

* Haemolymph obtained at a temperature of 28 °C and salinity of 30‰.
* Within columns, different letters indicate significant (\(p < 0.05\)) differences.

### 3.3. Haemolymph ammonia and pH levels

A significantly positive relationship (\(p < 0.01; r^2 = 0.791\)) was detected between haemolymph total ammonia-N and the external media (\(Y = 0.658(X) - 0.241; Y = \text{haemolymph total ammonia-N}; X = \text{external total ammonia-N level}\)). However, at all total ammonia-N concentrations tested, the surviving crabs had haemolymph total ammonia-N levels below that of the external media (Fig. 2).

The mean haemolymph pH from the control crabs was 7.09±0.036, which was lower than the external media of 8.10. The haemolymph pH values of the crabs exposed to all total ammonia-N concentrations were also lower than the external media. With the exception of the crabs exposed to 5.710 mmol/L total ammonia-N, the haemolymph pH significantly increased as the external ammonia-N concentrations increased. However, at a total ammonia-N concentration of 5.710 mmol/L, the haemolymph pH of the crabs sharply dropped and was not significantly different (\(p > 0.05\)) from those of the control (Fig. 3).

### 4. Discussion

In comparison to other crustaceans, particularly penaeid prawns, early *S. serrata* juveniles have a substantially higher tolerance to ammonia-N. This is especially valid considering the high NH\(_3\)–N tolerance, as NH\(_3\) is more easily diffusible across the gills, and therefore more toxic than NH\(_4\)\(^+\) (Table 3).
The “safe” level, considered not only non-lethal, but allows the animal to thrive, is derived by multiplying the 96-h LC50 value with an empirical factor of 0.1 (Sprague, 1971). Based on the results of current experiment, the “safe” level for early S. serrata juveniles was 0.68 mmol/L (or 9.53 mg/L) total ammonia-N and a “safe” level of 0.049 mmol/L (or 0.68 mg/L) NH3-N. As the “safe” level for most other crustaceans reported so far are generally lower than 0.36 mmol/L (or 5.0 mg/L) total ammonia-N and 0.018 mmol/L (or 0.25 mg/L) NH3-N, the high ammonia tolerance of early S. serrata juveniles may be due to an ecological adaptation described by Weihrauch et al. (1999). Weihrauch et al. (1999) have suggested that benthic crustacean species that commonly bury in the sediment for extended periods will experience heightened levels of localised ammonia-N. Therefore, to cope with such levels, an adaptive response is required to transport the haemolymph ammonia against a concentration gradient (i.e. to the environment) (Weihrauch et al., 1999). Indeed, early juvenile mud crabs, S. serrata, are well known to bury themselves in the sediment for long durations (Hill et al., 1982; Hyland et al., 1984). However, interestingly, both the total ammonia-N and NH3-N tolerance of early S. serrata juveniles is over twofold higher than similar-sized early blue swimmer crab, Portunus pelagicus, juveniles (Romano and Zeng, 2007). This may reflect the different habitats of these two species, as P. pelagicus, also known as sand crabs, predominately inhabit sandy shores/embayments in clearer waters (Kangas, 2000), while S. serrata are more abundant on muddy shores/mangrove flats with highly organic, and often anoxic, sediments (Hill et al., 1982; Hyland et al., 1984). It should be noted, however, that as S. serrata commonly inhabit estuarine systems, the ammonia-N tolerance may vary depending on the salinity as demonstrated with the red-tailed prawn Penaeus penicillatus (Chen and Lin, 1991), the fleshy prawn Penaeus chinensis (Chen and Lin, 1992), the burrowing crab Chasmagnathus granulata (Rebelo et al., 1999), the Pacific white shrimp Litopenaeus vannamei (Lin and Chen, 2001) and the green tiger prawn Penaeus semisulcatus (Kir and Kumlu, 2006). The results of the current study demonstrate that early S. serrata juveniles have an ability to maintain their haemolymph total ammonia-N levels substantially lower that of the external environment. This adaptive response has been demonstrated in various decapod crustaceans including the American clawed lobster Homarus americanus (Young-Lai et al., 1991), the tiger shrimp Penaeus monodon (Chen and Kou, 1993), the

<table>
<thead>
<tr>
<th>Species</th>
<th>Life stage</th>
<th>Weight (g)</th>
<th>Salinity (%)</th>
<th>Ammonia-N (mg/L)</th>
<th>NH3-N (mg/L)</th>
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<td>219 at 20 °C</td>
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<td>Present study</td>
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* NH3-N values were not calculated in study, although the NH3-N range presented is calculated based on a pH of 7.00 used in the study and on a speculative temperature range of 20–30 °C.
Norwegian lobster *Nephrops norvegicus* (Schmitt and Uglow, 1997), *C. granulata* (Rebelo et al., 1999), the Dungen crab *Cancer pagurus*, the common shore crab *Carcinus maenas*, the Chinese mitten crab *Eriocheir sinensis* (Weihrauch et al., 1999) and the freshwater crayfish *Pacifastacus leniusculus* (Harris et al., 2001). Such adaptive responses may be accomplished through either internal ammonia detoxification (e.g. conversion of ammonia to glutamate or urea) (Schmitt and Uglow, 1997; Rebelo et al., 1999) or by active ammonia excretion (reviewed by Weihrauch et al., 2004). While both ammonia detoxification to urea and active ammonia excretion have been previously demonstrated with *S. serrata* adults, active ammonia excretion predominates (79.24% of total nitrogen excretion) at a hypo-osmotic condition of 25% (Chen and Chia, 1996). Based on the haemolymph ionic composition and pH levels obtained in this study at the hypo-osmotic conditions, a similar mechanism is likely functional at the early juvenile stages as well.

While active ammonia-N excretion is currently not completely understood, and this process has been demonstrated to vary with species and environmental condition, it is believed to occur via Na⁺/K⁺-ATPase where K⁺ can be substituted for NH₄⁺, leading to reduced intracellular K⁺ ions and increased Na⁺ influx (reviewed by Weihrauch et al., 2004). However, a second mechanism of an apically located Na⁺/NH₄⁺ transport has been proposed for *Carcinus sapidus* (Pressley et al., 1981) and *Carcinus maenas* (Lucu, 1989) leading to increased Na⁺ uptake. In contrast, in other crustaceans, including *H. americanus* (Young-Lai et al., 1991), *P. japonicus* (Chen and Chen, 1996) and *P. leniusculus* (Harris et al., 2001), exposure to elevated ammonia-N levels was shown to cause a significant reduction in haemolymph Na⁺ levels. It is believed that this phenomenon was the result of an ammonia-N induced Na⁺/NH₄⁺ impairment or a depolarisation of the cell membrane, thereby reducing Na⁺ influx (Young-Lai et al., 1991; Chen and Chen, 1996; Harris et al., 2001). Such reduced haemolymph Na⁺ levels were directly linked to reduced haemolymph osmolality in *H. americanus* (Young-Lai et al., 1991) and *P. japonicus* (Chen and Chen, 1996).

Similarly, while the haemolymph Na⁺ levels of early *S.serrata* juveniles significantly decreased when total ammonia-N levels reached 5.710 and 7.138 mmol/L (or 80 and 100 mg/L), which may suggest an impairment of the apically located Na⁺/NH₄⁺ transport (Young-Lai et al., 1991; Chen and Chen, 1996; Harris et al., 2001), the osmolality and K⁺ ions were not significantly altered. This indicates that other haemolymph ions vary with species and environmental condition, it is believed to completely understood, and this process has been demonstrated to occur via apically located Cl⁻/HCO₃⁻ transport (Péqueux, 1995).

Uninterrupted active ammonia excretion in early *S. serrata* juveniles between 1.428 and 4.285 mmol/L (or 20 to 60 mg/L) total ammonia-N likely explains the haemolymph pH levels obtained in this study.

Acid–base regulation in crustaceans may be attributed to various factors, including temperature, respiratory/metabolic processes and haemolymph ionic composition changes (Henry and Wheatley, 1992; Varley and Greenaway, 1992; Siebers et al., 1994; Whiteley et al., 2001). In the current study, the haemolymph pH of the crabs from the control, and from all ammonia-N treatments, was substantially lower than that of the external media level of 8.10. This indicates a strong ability of the juvenile crabs for acid–base regulation (Siebers et al., 1994), which has been linked with their strong osmoregulatory abilities (Varley and Greenaway, 1992). However, at an exposure between 1.428 and 4.285 mmol/L (or 20 to 60 mg/L) total ammonia-N, the haemolymph pH of the crabs concomitantly increased with increasing ammonia-N concentrations. Interestingly, this occurred without any significant changes in the haemolymph osmolality or total Na⁺, K⁺, Ca²⁺ levels. Exposure to elevated NH₄Cl-N levels had reportedly led to haemolymph alkalosis in the saltwater adapted rainbow trout *Oncorhynchus mykiss* (Wilson and Taylor, 1992) and the authors suggested this was the result of an apical Na⁺/NH₄⁺ exchange. As it has been previously demonstrated that the majority of nitrogen excretion by *S. serrata* occurs via active ammonia-N transport (Chen and Chia, 1996), the observed haemolymph alkalosis in early *S. serrata* juveniles may also be attributed to an Na⁺/NH₄⁺ exchanger (Wilson and Taylor, 1992) and/or V-type H⁺-ATPase activity (Weihrauch et al., 2002). Such a model(s) suggests that as NH₃ diffuse across the gills and into the haemolymph, NH₃ is protonated to NH₂ via the removal of haemolymph H⁺, and is subsequently excreted into the environment (Campbell, 1973). Such a conversion process, and resultant elimination of H⁺ and production of OH⁻, may explain the increased haemolymph pH levels of these early juvenile *S. serrata* crabs when exposed to between 1.428 and 4.285 mmol/L (or 20 to 60 mg/L) total ammonia-N.

However, at a higher ammonia-N level of 5.710 mmol/L (or 80 mg/L) total ammonia-N, the haemolymph pH levels significantly decreased which coincided with significantly reduced haemolymph Na⁺ and Ca²⁺ levels. A reduction in haemolymph pH levels may be accomplished by the significantly reduced haemolymph Ca²⁺ levels (Taylor and Whiteley, 1989; Rebelo et al., 1999), increased exchange of haemolymph HCO₃⁻ onwards by apically located Cl⁻/HCO₃⁻ transport (Henry and Wheatley, 1992) which may explain the unaltered haemolymph osmolality and/or haemolymph CO₂ accumulation (Varley and Greenaway, 1992). The latter can be caused by ammonia-N induced anterior gill damage, including epithelial thickening and lamellae collapse, as observed in *C. granulata* adults (Rebelo et al., 2000) and *P. pelagicus* early juveniles (Romano and Zeng, 2007). Further investigation is warranted to determine the exact cause(s).

In conclusion, the high ammonia tolerance of early *Scylla serrata* juveniles, in comparison to other crustacean species, is likely the result of various compensatory responses including their ability to maintain the haemolymph total ammonia-N levels considerably lower than the external media, uninterrupted osmoregulation following elevated ammonia-N levels and strong ability for acid–base regulation.

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References


