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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)

Nicholas Romano *, Chaoshu Zeng

School of Marine and Tropical Biology, James Cook University, Townsville, Qld 4811, Australia

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Abstract

The current study was conducted to determine the LC_{50} 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 LC_{50} value of 95.35 mg/L ammonia-N (6.81 mg/L NH_3-N) or 6.80 mmol/L total ammonia-N (0.486 mmol/L NH_3-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 Ca^{2+} 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 Ca^{2+} levels. These physiological responses may explain the high ammonia tolerance of early *S. serrata* juveniles.

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; Triño 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 (NH_3) and ionised form (NH_4^+), 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 NH_4^+ 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^+/NH_4^+ transport which exchanges Na^+ inwards and NH_4^+ outwards to

* Corresponding author. Tel.: +61 07 47256482; fax: +61 07 47814585.
E-mail address: Nicholas.Romano@jcu.edu.au (N. Romano).

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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, CO_2 accumulation can lead to haemolymph acidosis (Henry and Wheatly, 1992; Varley and Greenaway, 1992) while increases in external NH_4^+ levels or haemolymph HCO_3^- levels can lead to haemolymph alkalosis (Campbell, 1973; Taylor and Whiteley, 1989; Wilson and Taylor, 1992; Rebelo 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 (Rebelo 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 *Nannochlorposis* 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* (Ridely) 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 NH_4Cl) 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 (NH_4Cl) (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 LC_{50} values, mortality observations were made at 12-h intervals for 96 h. Death was assumed when no