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Effect of nitrite on larval development of giant river prawn *Macrobrachium rosenbergii*

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Abstract

The effects of ambient nitrite concentrations on larval development of giant river prawn *Macrobrachium rosenbergii* were evaluated. The trials were conducted in two phases: phase 1, larvae from stages I through VIII and phase 2, larvae from stage VIII until post-larvae. In both phases larvae were kept in water with nitrite (NO_2–N) concentrations of 0, 2, 4, 8 and 16 mg/L. Oxygen consumption was analyzed for larvae in stage II at nitrite concentrations of 0, 4, and 8 mg/L. Survival, weight gain, larval stage index and metamorphosis rate decreased linearly with increasing ambient nitrite concentration. However, there was no significant difference between larvae subjected to 0 and 2 mg/L NO_2–N. In phase 1, there was total mortality at 16 mg/L NO_2–N, while in phase 2 larval development stopped at stage X in this treatment. The oxygen consumption in stage II increased significantly at NO_2–N concentration from 0 to 4 mg/L, but there was no difference between 4 and 8 mg/L NO_2–N. In conclusion, increasing ambient nitrite up to 16 mg/L NO_2–N delays larval development, reduces larval growth rate and causes mortality, whereas no significant effect occurs for levels below 2 mg/L NO_2–N. However, the establishment of a general safe level of nitrite to *M. rosenbergii* hatchery may be difficult due to the great variability in larvae individual sensitivity.
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Keywords: Freshwater prawn; Larvae; *Macrobrachium*; Nitrite

1. Introduction

Freshwater prawn culture has expanded considerably in recent years, especially in Asian countries (New, 2005). Global production of giant river prawn, *Macrobrachium rosenbergii*, increased from 17,129 to 180,221 tonnes between 1993 and 2003 (FAO, 2005). This rapid increase may be mainly due to the dramatic developments in culture technologies, and the great environmental sustainability of freshwater prawn farming (Valenti and Tidwell, 2006).

M. rosenbergii larviculture is commonly conducted in intensive systems with high stocking density. In this situation, larvae might be exposed to high nitrite and ammonia concentration (Tomasso, 1994). Recirculating hatchery systems have been used to maintain low ammonia and nitrite levels by means of nitrification (Valenti and Daniels, 2000). This is a biological process in which ammonia (NH_4^+) from the animal excrements and the organic residues are oxidized into nitrite (NO_2^-)

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and sequentially into nitrate (NO_3^-). Generally, nitrite shows at low levels (<0.5 mg/L NO_2–N) because it is converted into nitrate immediately. However, upon the establishment of nitrification in biological filters, or during imbalance in the process nitrite levels may rise in water (Russo and Thurston, 1991; Valenti and Daniels, 2000; Jensen, 2003). Levels as high as 20 mg/L NO_2–N may be attained in imbalance recirculating systems (Forster, 1974; Masser et al., 1999). Although there are no papers reporting high mortality of *M. rosenbergii* larvae due to nitrite level, it is a potential toxicant in recirculating systems (Grosell and Jensen, 1999; Parra and Yúfera, 1999). Therefore, establishing safe levels of nitrite for *M. rosenbergii* larvae is very important.

The toxicity of nitrite in crustaceans is barely understood (Boyd and Tucker, 1998) and recent research has been concentrated on penaeid shrimps (Cheng and Chen, 1999, 2000, 2002; Chen and Cheng, 2000; Sowers et al., 2004). The mechanisms of nitrite action and tolerance limits in caridean prawns are almost unknown. Chen and Lee (1997a,b) studied nitrite effect on ion regulation, acid-base balance and respiration of *M. rosenbergii* adults, while Armstrong et al. (1976) and Wickins (1976) carried out the toxicity tests in larvae and post-larvae of *M. rosenbergii*, respectively. Armstrong et al. (1976) have shown that the highest concentration in which no larval mortality occurred during the first 24 h was 9.7 mg/L NO_2–N. Several authors (e. g. Correia et al., 2000; New, 2002) recommend nitrite levels (NO_2–N) below 0.1 mg/L for *M. rosenbergii* larviculture. Although this has been effective for most situations, it is a conservative empirical value because there are no conclusive data focusing on the effects of nitrite on larval development of this prawn. Therefore, the objective of this study was to evaluate the effects of ambient nitrite concentrations on *M. rosenbergii* larval development. These results may contribute to the determination of safe levels of nitrite for hatcheries.

2. Materials and methods

2.1. Larval development test

The effects of nitrite concentrations on survival, weight gain and larval development were evaluated. Larvae were placed in beakers filled with 300 mL of test solution, and provided with aeration. The beakers were placed in a water bath at 30 °C inside black trays in order to minimize light reflections and to prevent larvae crowding around luminous points, due to phototactic response.

Nitrite concentrations of 0, 2, 4, 8 and 16 mg NO_2–N\L were tested, and each treatment was conducted in six replicates. This range was assigned because preliminary trials suggested that there was no significant effect at concentrations below 3 mg/L NO_2–N. Concentrated nitrite solution was prepared by dissolving sodium nitrite (4.93 g/L $NaNO_2$) and then, it was diluted with 12‰ brackish water to prepare the experimental concentrations. Brackish water was prepared by mixing 35‰ natural seawater with distilled water.

The test was divided in two phases. Phase 1 lasted from stage I zoea until most of the larvae reached stage VIII (13 days). Phase 2, from stage VIII zoea until most of the larvae reached post-larval stage (15 days).

Larvae were obtained from a single prawn female. In phase 1, 15 newly hatched larvae (approximately 0.030 mg dry weight) were carefully rinsed with test solution and transferred to each beaker. Larvae were fed exclusively *Artemia* nauplii (about 80 nauplii/larvae/day). Simultaneously, the leftover larvae (from the same offspring) were reared in a 120 L cylindrical tank equipped with heating and a biological filter. Water salinity, temperature, and pH were kept at around 12‰, 30 °C, and 8, respectively. Inorganic nitrogen in the water was kept at low levels (total ammonia<0.01 mg/L; nitrite<0.01 mg/L; nitrate<3.0 mg/L). In phase 2, 15 larvae from stage VIII (approximately 0.590 mg dry weight) were randomly sampled from the larviculture tank, rinsed with test solution, and placed into each beaker. Larvae were fed *Artemia* nauplii (about 80 nauplii/larvae/day) and supplementary diet once daily. Supplementary diet were prepared using chicken eggs, fish and mollusk flesh, dried milk, wheat flour, vitamins and minerals (see Mallasen and Valenti, 1998 for feed composition). Food residues were siphoned 3 h after feeding.

Test solutions in the beakers were completely replaced every 24 h with new solution prepared each day, at which time dead larvae were removed and counted. Nitrite analyses confirmed the similarity between the actual and nominal concentrations. Water quality was monitored periodically. Temperature was measured daily using a mercury thermometer; dissolved oxygen was measured with a YSI Model 55 polarographic oxygen meter and pH was measured using a YSI Model 63 pH meter every three days. Nitrite and ammonia levels were determined three times a week before water replacement, according to the methods of Hach reagent kit and Solorzano (1972), respectively, using a Hach DR-2000 spectrophotometer.

At the end of each phase, the surviving animals were counted, observed under Leica Model MZ6 stereomicroscope to determine larval stage (according to Uno and

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