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Effect of microalgae concentration on larval survival, development and growth of an Australian strain of giant freshwater prawn *Macrobrachium rosenbergii*

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ABSTRACT

The present study investigated the effects of microalgae *Nannochloropsis* sp. addition and concentration on larval survival, development and growth of an Australian strain of *M. rosenbergii* (lineage II). Newly hatched larvae were reared to postlarval (PL) stage under the condition of no algae addition ('clear water') and four *Nannochloropsis* concentrations of 2.5, 6.25, 12.5 and 25×10^5 cells/ml. All treatments were in quadruplicate and each replicate had 30 larvae stocked in a 5L vessel. Larvae were fed 3 *Artemia*/ml throughout with 100% water exchange daily. The results showed that larval survival to PL at the two higher algae concentrations of 12.5 and 25×10^5 cells/ml (70.8 and 63.3%, respectively) were significantly higher ($P<0.05$) than those of lower algae concentrations of 2.5 and 6.25×10^5 cells/ml and the 'clear water' treatments (26.7, 35.0 and 30.0%, respectively). Meanwhile, the fastest mean development to PL (30.6 days) registered at the highest algal density was 14 days shorter than that of the 'clear water' treatment (44.3 days). Larval development at the two higher algal densities were significantly shorter than that of the 'clear water' treatment and larval development of the highest algal density was further significantly faster than those of the two lower algal densities (40.1 and 40.0 days) ($P<0.05$). The mean dry weights of newly settled PL of the two high algal density treatments were also significantly heavier ($P<0.05$) than those of the lowest algal density and the 'clear water' treatments. The results have shown that the addition of *Nannochloropsis* sp. at appropriate levels substantially improved performance of larval culture of the Australian strain of *M. rosenbergii*, suggesting that the Australian native strain has a promising potential for aquacultural development.

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, has long been part of the diet of the peoples of Asia and the Pacific who greatly value its flavour. The extensive farming of *M. rosenbergii* employing various traditional methods has a long history in Southeast Asia where it occurs naturally (Ling, 1969). The development of modern culture techniques for the species in the 1960's and 70's has led to the introduction of the species to many countries and farming of the species is now wide spread throughout the world wherever appropriate climate and freshwater impoundments exist (New, 2002, 2005).

The natural distribution of *M. rosenbergii* extends from Pakistan across to Southeast Asia, south to Papua New Guinea, northern Australia (De Bruyn et al., 2004a) and in some Indian and Pacific Ocean islands (Mather and De Bruyn, 2003). In Australia, the giant freshwater prawn is widely distributed throughout the tropical northern regions and endemic to the west of the Great Dividing Range (Short, 2004). Several attempts in the past to grow local *M. rosenbergii* commercially in the country failed, reporting various problems, including low hatchery survival, excessive cannibalism, lack of technical expertise and infrastructure to consistently produce postlarvae (Cantrelle, 1988) as well as diseases that have affected commercial operations (Bergin, 1986; Owens and Evans, 1989). As a consequence of such failed attempts and strict government regulations that prohibit introduction of foreign species/strains to Australia for aquaculture, commercial freshwater prawn farming is currently non-existent in the country.

The worldwide upsurge in *Macrobrachium* culture in the past decade has prompted research interest again in Australia, particularly on the problematical hatchery phase. These new efforts were further encouraged by recent reports, which through the analyses of 16sRNA, divided natural populations of *M. rosenbergii* into 'eastern' and 'western' forms, demarcated by Huxley's Line, a biogeographic barrier running between Borneo and Sumatra extending north into the Philippines (De Bruyn et al., 2004a). Based on this finding, *M. rosenbergii* in Australia belong to the 'eastern' form, differing from the widely cultured 'western' form of mainly Malaysian origin (De Bruyn et al., 2004a). Further analysis of mitochondrial DNA revealed that within Australia, wild stocks of *M. rosenbergii* can be categorized into four genealogically distinguished lineages, i.e. Western Australia

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(lineage I), Gulf of Carpentaria/Northern Territory (lineage II), Irian Jaya (lineage III) and Papua New Guinea/North east Cape York (lineage IV) (De Bruyn et al., 2004b).

In line with these new findings, in the attempts to assess the aquaculture potential of various Australian native strains of *M. rosenbergii*, wild broodstock of Lineage II were sourced from the Flinders River system, North Queensland and larval culture trials were carried out based on techniques derived from the 'western form' (New, 2002). As both the 'clear water' and 'green water' methods have been used for larval culture of the 'western form' of *M. rosenbergii* and no clear verdict has been made as to which was superior (New, 2002), both methods were trialled during several larval culture runs for the Australian strain of *M. rosenbergii* in identical tanks to compare their relative merits. Interestingly, contrary to the current trend of commercial hatcheries overseas opting for the 'clear water' method (New, 2002), results from our trials suggested that the 'green water' method consistently produced significantly better results for the Australian strain of *M. rosenbergii*. Anecdotal observations further suggested that larval performance was linked to the density of microalgae added. Hence, the present study was designed to quantitatively assess the effects of algae addition on larval survival, development and growth of the Australian strain of *M. rosenbergii*.

2. Materials and methods

2.1. Source of broodstock and larvae

Broodstock prawns were collected from the Flinders River system and its tributaries, near the Gulf of Carpentaria (latitude 17°52.522; longitude 140°46.837), North Queensland, Australia. The prawns were transported overnight in 200 L black plastic drums with aeration to the Marine and Aquaculture Research Facility Unit (MARFU), James Cook University (JCU), Townsville, Queensland. Upon arrival at MARFU, broodstock prawns were held in recirculating 2500 L tanks at a female to male ration of 4–5:1. The prawns were fed daily in excess on shrimp, mussel, squid and formulated feed (36% crude protein; 6% crude fat and 3% fibre) designed for black tiger prawn *Penaeus monodon* (Ridley Aqua-feed, Australia). Samples of these wild prawns were sent to Queensland University of Technology (QUT), Brisbane for identification and were confirmed as Lineage II from rivers flowing into the Gulf of Carpentaria (De Bruyn et al., 2004b).

The development of ovaries and spawning of the female prawns were monitored closely. Berried females were transferred to 5–8‰ brackish water for incubation and embryonic development monitored to predict the date of hatching. On the day of hatching, broodstock females were removed from the hatching tank after larvae had hatched and salinity in the tank was increased to 12‰. Newly hatched larvae were held for 1 h at 12‰ before being collected and transferred to experimental containers for the experiment.

2.2. Experimental design and setup

Five larval culture treatments were setup with concentrations of green microalgae *Nannochloropsis* sp. set at five levels of 0, 2.5, 6.25, 12.5 and 25×10^5 cells/ ml. All treatments were in quadruplicate where each replicate consisted of a 5 L round clear plastic container (diameter=215 mm; depth=177 mm) stocked with 30 randomly selected newly hatched larvae in 12±1‰. Replicates of various treatments were organised in a random block design inside water baths set at 30.0±1.5 °C. Each container was covered with a clear plastic lid to prevent larvae jumping out during the late larval stages and gentle aeration was provided to each container via a fine-tipped glass pipette inserted through the lid. Photoperiod was set at 14.5 L: 9.5D throughout the experiment and larvae were cultured from the day of hatching (day 0) until they either reached postlarval stage (PL) or death in all replicates.

Throughout the experiment, larvae were fed a ration of 3 *Artemia*/ml with 100% water exchange carried out daily. Water was exchanged in the morning where any mortality was recorded and the surviving larvae of each replicate were transferred to an identical container with freshly prepared food (3 *Artemia*/ml and designated concentration of *Nannochloropsis*) and 12‰ water. The water salinity was pre-adjusted by diluting natural seawater of 33–36‰ with dechlorinated tap water using a refractometer (Shibuya Salinometer S-10). Water quality parameters, including ammonia, nitrite, nitrate, pH and DO, were measured weekly. Over the period of the experiment, the ammonia, nitrite and nitrate ranged from 0–0.3, 0–0.1 and 0–10 mg/L, respectively, while pH fluctuated between 7.4 to 7.9 and DO between 5.7 to 6.3 mg/L.

Microalgae *Nannochloropsis* sp. was mass cultured in 3000 L tanks at JCU's algal culture facility using a commercially available fertiliser (AQUASOL, Yates Ltd, New South Wales, Australia). The *Nannochloropsis* cultures were generally re-inoculated every 7–10 days. During the experiment, a stock solution was prepared daily by selecting a *Nannochloropsis* sp. culture at its exponential phase of growth and diluted to 12‰ by mixing with dechlorinated freshwater in a 20 L container with strong aeration. Samples were then taken from the stock solution and the algal density counted using a hemocytometer under a high power microscope. The *Nannochloropsis* concentration of the stock solution was estimated by averaging the concentrations of five 1 ml samples. The volume of stock solution required to prepare a desired concentration of microalgae for each treatment was calculated using the equation:

$$C_1V_1 = C_2V_2$$

where C_1 was the designated algal concentration for a particular treatment and V_1 was the total volume required for daily water exchange of the treatment; C_2 was the algal density of the stock solution and V_2 was the volume of the stock solution required to prepare the designated algal concentration for the particular treatment. The required volume of the stock solution was subsequently measured and diluted with 12‰ brackish water to prepare the total volumes required for daily water exchange. Meanwhile, *Artemia* cysts (INVE Inc, Thailand) were hatched daily in 18‰ and newly hatched nauplii were harvested early morning on the following day and their density counted before being fed to the larvae directly without enrichment.

Every 3 days, 16 larvae (4 from each replicate) were randomly sampled from each treatment and their developmental stage identified under a microscope according to Uno and Kwon (1969). The larvae were placed in a small pool of water during the stage identification and returned to the original culture promptly after staging. Such a process has been shown previously not to lead to larval mortality when operated properly. Once postlarvae were found in a replicate during the daily check, they were removed from the cultures and euthanized by quick freezing. The larvae were then measured for their carapace length (mm) using a microscope equipped with a camera (Leica). They were subsequently dried individually in a 60 °C oven for 24 h before being weighed for dry weight using a Cahn C-33 microbalance (0.001 mg).

2.3. Data analysis

Based on results of larval staging, larval stage index (LSI) was calculated according to Manzi et al. (1977) and Mallasen and Valenti (2006):

$$\mathrm{LSI} = (\sum S_i \times n_i)/N$$

Where S_i=larval stage (i=1–11; representing each larval stage); n_i= number of larvae in stage S_i; N=total number of larvae examined. The survival, mean development time from hatching to PL, mean carapace