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SCIENCE DIRECT
Aquaculture 257 (2006) 482–490
Aquaculture
www.elsevier.com/locate/aqua-online

Use of microbound diets for larval culture of the mud crab, *Scylla serrata*

May-Helen Holme *, Chaoshu Zeng, Paul C. Southgate

Tropical Crustacean Aquaculture Research Group, School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811, Australia

Received 9 December 2005; received in revised form 9 March 2006; accepted 10 March 2006

Abstract

Routine commercial production of mud crab seed is currently restricted by our limited understanding of the nutritional requirements of *Scylla* spp. as well as problems commonly associated with the live foods used in mud crab hatcheries. This study investigated the use of microbound diet (MBD) particles as a food source for megalopa and zoea III stage larvae of *Scylla serrata*. In the first experiment, the nutritional value of four MBD containing dried rotifers, *Artemia* meal, fish meal or squid meal were evaluated for megalopa reared individually in 250-ml aquaria. Survival of MBD-fed megalopa to the first crab stage ranged from 46.7% to 60.0% with those fed MBD containing fish meal or squid meal showing higher survival than those fed MBD containing *Artemia* meal or dried rotifers. Larvae fed live *Artemia* showed the highest survival (80%), while unfed megalopa did not survive to the first crab stage. There were no significant differences ($P>0.05$) in the average time required for megalopa to reach the first crab stage when fed any of the four MBD. However, shortest development time was recorded for larvae fed live *Artemia*. In a second experiment, zoea III larvae were cultured communally at a density of 25 larvae l^{-1} and were fed either 100% live *Artemia* nauplii, 100% MBD or a 50%:50% combination of MBD and *Artemia*. Highest survival (66%) and development rate to the zoea IV stage were recorded for larvae fed the 50%:50% combination of MBD and *Artemia*. Some successful molts were also found among larvae fed MBD exclusively, while total mortality was observed in unfed (control) replicates. The results indicate that the experimental MBD may contain certain beneficial nutrients lacking in *Artemia* and that co-feeding the MBD with *Artemia* may enhance larval survival and development. However, they also show that total replacement of live food with the experimental MBD will result in poor survival of zoea III larvae of *S. serrata*. The results indicate great potential for the use of MBD particles as dietary components for both zoea and megalopa stages of *S. serrata.* These findings have important implications for the eventual development of cost-effective and reliable hatchery techniques for mud crabs.

Keywords: Mud crab; *Scylla serrata*; Microbound diet; Megalopae; Live food replacement; Zoea III; Co-feeding

1. Introduction

Despite considerable potential for mud crab (*Scylla* spp.) farming in the Indo-Pacific region (Cowan, 1984; Brienl and Miles, 1994; Keenan, 1999; Trino and Rodriguez, 2002), hatchery production of mud crab seed is often inconsistent with low survival (Keenan, 1999).

* Corresponding author. Tel.: +61 7 47815568; fax: +61 7 47814585.
E-mail address: mayhelen.holme@jcu.edu.au (M.-H. Holme).

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doi:10.1016/j.aquaculture.2006.03.014

Viability at hatch seems to be an ongoing problem, and mortality is further inflicted by pathogenic micro-organisms and a phenomenon known as 'molting-death syndrome' (Williams et al., 1999). Molting-death syndrome is caused by the inability of larvae to completely shed the old carapace before the new carapace hardens and is most often observed during the molt from zoea V to megalopa and from megalopa to the first crab stage (Fielder and Heasman, 1999). Why this happens is not yet fully understood, but the phenomenon is believed to result, at least partially, from inappropriate nutrition of larval stages which are generally fed 'traditional' live foods such as rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.) nauplii (Keenan, 1999). The need to provide more appropriate nutrition for *Scylla serrata* larvae and juveniles has stimulated recent research to determine nutritional requirements of both larval (Suprayudi et al., 2004) and juvenile crabs (Sheen and Wu, 1999; Sheen, 2000) and to assess the potential of microbound diets (MBD) as replacements for live foods during larval culture. Prior research has shown that particles of semi-defined MBD containing dried rotifers were readily ingested by *S. serrata* larvae (Genodepa et al., 2004a) and supported similar rates of survival and development of *S. serrata* megalopa to those fed live *Artemia* (Genodepa et al., 2004b). The experimental MBD used in previous feeding experiments contained ^{14}C-labelled rotifers (39.7% of total dry weight) as a means of estimating larval ingestion of MBD (Genodepa et al., 2004a, b). Although this diet supported good survival and development of mud crab megalopa (Genodepa et al., 2004b), live food production is both costly and time consuming. Development of a MBD without live food components could hence make hatchery production of mud crab seed a cheaper and more reliable process.

Despite the clear potential of MBD as a food source for *S. serrata* megalopa (Genodepa et al., 2004b), their potential as a food source for zoeal larvae of the same species has not previously been determined. Research with penaeid and fish larvae has shown that early larval stages often have reduced digestive capabilities compared to older larvae and that digestion is heavily reliant on enzymes obtained from live prey (Jones et al., 1997; Kolkovski, 2001). On this basis, formulated diet particles are generally fed to early larval stages in conjunction with live food organisms, a practice commonly referred to as 'co-feeding' (Kanazawa et al., 1989). This procedure is now broadly applied in the hatchery culture of many commercial species (e.g., Lauff and Hofer, 1984; Peron-Le Ruyet et al., 1993; Jones et al., 1993).

For *S. serrata* larvae, substantial change in feeding behaviour is associated with metamorphosis from the planktonic zoea stages to the clawed, more benthic megalopa stage. During the zoea stages, larvae only feed on particles suspended in the water column, whereas the megalopa are capable of feeding on larger food particles on the bottom (Genodepa, 2003). This change in feeding behaviour is an important consideration for the development of formulated diets. This paper reports on two feeding experiments assessing the potential of an experimental MBD developed in this laboratory for both zoea and megalopa larvae of *S. serrata*. The first experiment evaluated MBD containing various defined ingredients as a replacement for live food fed to *S. serrata* megalopae. The second experiment determined the suitability of MBD for either total or partial replacement (i.e., co-feeding) of live foods for zoea III larvae of *S. serrata*.

2. Materials and methods

2.1. Source of larvae

Mature *S. serrata* females were collected in baited traps in estuarine areas around Townsville, north Queensland, Australia. The crabs were disinfected in 100 mg l^{-1} formalin for 6 h, before being placed into 1000 l outdoor tanks with sand bottoms and shelter. The tanks were filled with 1 μm filtered seawater and salinity and water temperature were maintained between 28–33‰ and 26–30 °C, respectively. Broodstock crabs were fed a diet of prawns, mussels and squid once daily at a rate of approximately 5–8% of their body weight. Berried crabs were disinfected using 50–60 μl l^{-1} formalin solution for 6 h, before being transferred to 300 l indoor tanks for egg incubation and hatching. Incubation tanks were supplied with recirculating 1 μm filtered and UV irradiated seawater (recirculating at ca. 1.5 l min^{-1}), and salinity and water temperature were maintained between 33–36‰ and 26–29 °C, respectively. Females were not fed during the egg-incubation period, and the tanks were siphoned every morning to remove faeces and any eggs discarded by the females.

Mud crab larvae go through five zoeal stages before they metamorphose to become megalopa. Immediately after eggs hatching, the zoea I larvae were attracted to the water's surface using a strong light source and were transferred to flat-bottomed, 300 l indoor tanks at a density of 100–120 larvae l^{-1}. Antibiotic (10 mg l^{-1} streptomycin sulphate) was added to the culture water once only on the first day of stocking and salinity and water temperature were maintained at 20–24‰ and 26–