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The effects of supplemental dietary cholesterol on growth, development and survival of mud crab, *Scylla serrata*, megalopa fed semi-purified diets

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Abstract

The effects of varying levels of dietary cholesterol on growth, development time and survival of mud crab, *Scylla serrata* megalopa were investigated using semi-purified microbound diets (MBD). Five iso-energetic diets containing different level of cholesterol ranging from 0.14% to 1% of dry weight of the diet were tested. Fifteen megalopa were reared individually for each dietary treatment, and development time and survival were recorded on a daily basis. More than 25% of megalopa from all treatments were able to metamorphose into the first crab stage, suggesting that the endogenous level of cholesterol in the basal diet (0.14%) was sufficient to support development of the megalopa stage of this species. Widest mean carapace width (3.53 ± 0.08 mm) and highest mean dry weight (2.11 ± 0.22 mg) were recorded for juveniles that molted from megalopa fed live *Artemia*, whereas no megalopa in the unfed control treatment metamorphosed into crabs. The average development time from megalopa to the juvenile crab stage varied between the treatments, where megalopa fed live *Artemia* or MBD containing 0.2%, 0.4% or 0.8% total cholesterol showed the most synchronized molting (between 8.0 and 9.9 days). Longest development time was recorded for the megalopa fed diets containing 0.14% or 1% total cholesterol (both 11 days). Highest survival (74.3%) was recorded for the megalopa fed a diet containing 0.8% cholesterol. The results of this study are valuable in research to develop formulated diets for mud crab larvae as a replacement for live food in hatchery culture.

Keywords: Mud crab; *Scylla serrata*; Megalopa; Cholesterol requirement; Microbound diet

1. Introduction

The demand for mud crabs of the genus *Scylla* has increased rapidly over the last decade, providing great potential for the development of the mud crab aquaculture industry. However, a major bottleneck to such development is a lack of reliable hatchery protocols which has resulted in seeds–stock shortages (Ruscoe et al., 2004). As a consequence, today's mud crab farms rely almost exclusively on juveniles caught in the wild (Djunaidah et al., 2003). Development of more effective and efficient hatchery techniques and more reliable production of juveniles is therefore considered critically important for sustainable growth of the industry.

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Hatchery culture of *Scylla serrata* depends heavily on live food organisms such as rotifers and *Artemia* sp., both of which are expensive and time consuming to culture (Dainteath and Quin, 1991) and inconsistent in terms of nutritional quality (Sorgeloos et al., 1986; Tucker, 1992; Southgate, 2003). On this basis, a number of recent studies have investigated the development of formulated microbound diet (MBD) particles as a food for *S. serrata* larvae (Genodepa et al., 2004a,b; Holme et al., 2006). These studies have shown that MBD are readily ingested by *S. serrata* larvae and may support similar rates of growth and survival of megalopa as *Artemia* nauplii. These findings have provided the impetus for research to develop a nutritionally suitable, high quality microbound diet which will be an essential step towards more effective mud crab production.

Cholesterol is an important sterol, serving as a precursor for many physiologically active compounds such as sex and molting hormones, adrenal corticoids, bile acids and vitamin D (Sheen, 2000). Most animals can synthesize sterols from acetate, but crustaceans, like other arthropods, are incapable of *de novo* production of sterols (Sheen et al., 1994; Teshima and Kanazawa, 1971). Dietary cholesterol is therefore considered essential for good growth and high survival in crustaceans (Sheen, 2000), and the quantitative and qualitative requirements for cholesterol in formulated diets for crustaceans have been studied since the early 1970's. Examples of the estimated cholesterol requirements of crustaceans range from 0.1% to 1.4% for juvenile *P. japonicus* (Shudo et al., 1971), from 0% and 0.12%–0.5% for adult and juvenile marine lobster (*Homarus* sp.), respectively (Castell and Covey, 1976) and 0.23–0.42% for the white shrimp *Litopenaeus vannamei* (Duerr and Walsh, 1996). Limited information is available on the cholesterol requirements of mud crabs, however, in experiments with *S. serrata* juveniles, significantly higher weight gain was observed for crabs fed diets containing 0.5% and 0.79% dietary cholesterol (Sheen, 2000). No crabs fed the diet without dietary cholesterol survived, and the diet containing cholesterol levels higher than 1.12% had an adverse effect on juvenile mud crab growth.

High mortality is observed when *S. serrata* megalopa molt to the first crab stage, a phenomenon commonly referred to as 'molting-death-syndrome'. This mortality seems to be a result of the inability of the larvae to completely shed their exoskeleton during molting (Genodepa et al., 2004b). Although the cause of the problem is not fully understood, it is believed to be associated with inappropriate nutrition (Hamasaki et al., 2002; Williams et al., 1999). Limited work has been conducted on the nutritional requirements of megalopa of *S. serrata*, but recent studies have suggested that total replacement of the live food with MBD particles is possible for the megalopa stage (Genodepa et al., 2004a,b; Holme et al., 2006). These findings indicate that great potential lies in the use of MBD as a tool for further investigation of the nutritional requirements for the *S. serrata* larvae. Such research will be essential of development of an MBD suitable for commercial production, which again will be an important step towards more cost-effective and reliable hatchery production of mud crabs.

The aim of this study was to determine the dietary cholesterol requirement for megalopa of *S. serrata*. Five different levels of cholesterol were formulated into a basal MBD, and survival, dry weight, carapace width and development time in each treatment were used to evaluate the optimum dietary cholesterol level.

2. Materials and methods

2.1. Source of larvae

Mature *S. serrata* females were collected in estuarine areas around Townsville, North Queensland, Australia. The crabs were disinfected in formalin (100 mg L^{-1}) for 6 h (Mann et al., 1999) before being placed into 1000 L outdoor tanks provided with sandy bottoms and shelter. The tanks were run as a flow-through re-circulating system supplied with UV radiated and filtered seawater. Salinity and water temperature were maintained at 28–36‰ and 25–29 °C, respectively, and the broodstock were fed a diet of prawns, mussels and squid once daily at a rate of 5–8% 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. Indoor tank water was filtered down to 1 μm and UV irradiated, with salinity and water temperature at 32–36‰ and 26–29 °C, respectively. Females were not fed during the 10–14 day long egg incubation period, and the tanks were siphoned every morning to remove feces and discarded eggs.

Newly hatched, photopositive zoea I were attracted to the surface using a strong light source, and the larvae were transferred to flat-bottomed 300 L indoor tanks at a density of 100–120 larvae L^{-1}. The water was treated with antibiotics (10–15 mg L^{-1} streptomycin sulphate) just before stocking and salinity and water temperature were maintained at 22–25‰ and 28 °C, respectively. As the larvae grew, salinity was gradually increased to 25–28 ‰ for the megalopa (Genodepa, 2003).

Newly stocked larvae were fed rotifers (*Brachionus* sp.) at a density of 40–60 individuals mL^{-1} the first day of culture, and this density was maintained by daily