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EFFECT OF ROTIFER DENSITY ON SURVIVAL AND DEVELOPMENT OF MUD CRAB *SCYLLA SERRATA* (FORSKÅL) LARVAE

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Abstract: An experiment of mud crab, *Scylla serrata* with single factor design was set up at the Marine and Aquaculture Research Unit of James Cook University, Queensland, Australia for 26 days in order to improve the hatchery technique, i.e development and survival of larvae using different rotifer densities. The mud crab larvae were reared at 0, 5, 10, 20, 40, and 60 ml^{-1} rotifer densities. Survival and development increased with the increase of rotifer density. At higher rotifer density situation, the highest 35-40% survival of Z-I stage was achieved at 40-60 ml l^{-1}. Higher density beyond 40 ml l^{-1} resulted in higher mortality which needs further investigation. Moreover, rotifer was found as suitable diet for Z-I and Z-II larvae. From Z-III onwards, rotifers were found not suitable for the larval survival and development of mud crab larvae.

Key words: *Scylla serrata,* zoea, megalopa, larval development, rotifer, live feed

Introduction

The species of the genus *Scylla*, commonly known as mud crab or mangrove crab are commercially important crustacean (brachyuran) species found in tropical areas of brackish and saltwater estuaries or mangrove forest of many Indo-Pacific countries. Because of their large size, high meat yield, and delicate flavour, mud crabs are considered as a commercially important seafood delicacy throughout the World. Four commercially important crab species under the genus *Scylla* are *S. serrata, S. olivacea*, *S. paramamosain*, and *S. tranquibarica*. Among them, *S. serrata* is the biggest, fastest growing and widely distributed species (Fortes, 1999; Keenan, 1999). There is a growing interest in farming of mudcrab throughout the World. Recently, aquaculture production of mud crab contributed a large proportion of the world's crab production (Anon, 1999). However, aquaculture production relies mainly on juveniles captured from the natural environment (Fortes, 1999; Keenan, 1999), which is not reliable and sustainable. Since farming of mudcrab has been expanding unpredictably very rapidly and seed supply form the natural habitat is inconsistent, it is important to develop the reliable and practical method for hatchery seed production for sustainable growth of the industry.

Larval rearing of mud crab is a bottleneck in mud crab aquaculture. Hatchery techniques of mudcrab have been extensively studied by the scientists for several decades (Baylon and Failaman, 1999; Baylon *et al.,* 2001; Brick, 1974; Heasman and Fielder, 1983; Hill, 1974; Mann *et*

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al., 1999*,* 2001; Marichamy and Rajapackiam, 1992; Quinitio *et al.,* 1999; Zainoddin, 1992). In spite of some success in artificial breeding, survivals were reported to be low and inconsistent (Mann *et al.,* 1999; Baylon and Failaman, 1999; Quinitio *et al*., 1999; Fortes, 1999) to become commercially viable (Keenan, 1999). Moreover, available literatures on larval rearing of mud crab are by no means extensive. More research for technological improvement is required to achieve high survival and commercially viable production. High mortalities of larvae were mainly attributed to inappropriate food and feeding density, salinity and light requirement, stocking density and type of substrate, high sensitivity of zoea to water turbulence and sudden change in temperature (Baylon and Failaman, 1999). Thus, successful mud crab larval rearing requires knowledge of key biological and physical factors. Suitable culture environment favors higher survival and faster development. Feeding management is one of the factors that needs to be addressed to improve larval performance. Food density must also be tailored to the needs and consumption of the larvae at different larval stages so that food is not wasted, larvae are not underfed and rearing water is not fouled. The usefulness of live food to larvae at particular stage also needs to be examined.

Feeding schemes of mud crab *S. serrata* larval culture using different live feeds were studied by several researchers (Baylon and Failaman, 1999; Brick, 1974; Heasman and Fielder, 1983; Marichamy and Rajapackiam, 1992; Williams *et al*., 1999; Zainoddin, 1992). They used mostly *Artemia*, rotifer, and copepods as live feeds. In these studies rotifers were used either as a combination diet with other live feeds or as a single diet for comparative study. No study was conducted where rotifers were solely used as live feed. The attributes which makes rotifer more suitable than *Artemia* and copepods in mud crab larvae culture includes slow swimming speed, planktonic nature, small size, high digestibility, tolerance of high density, high production rate, and ability to influence nutritional value (Daintith, 1991). Therefore, this study was conducted to compare larval survival and development from zoea to megalopa of *Scylla serrata* using rotifers at different densities.

Materials and Methods

Source of larvae: The experiment was carried out at the Marine and Aquaculture Research Facility Unit (MARFU) of James Cook University, Townsville city, Queensland, Australia for 26 days. Spawners of *S. serrata* with carapace length of at least 140 mm were collected using baited traps from local estuaries. Species was identified following Keenan *et al*. (1998). Male and female crabs were identified observing abdominal flaps. The crabs were disinfected with 100 ppm formalin overnight and reared in two 5000 l circular tanks until females became ovigerous. Seawater was supplied at a rate of about 15 l min^{-1} through re-circulating system of the research facility. Salinity and temperature in the tanks ranged at 28 - 36 ppt, and 26 – 29 °C respectively. The spawners were fed with squid, mussel, and shrimp or fish meat about 5 to 8% of their body weight once a day in the evening and the uneaten feed was removed in the morning. After extruding eggs into the abdomen, ovigerous females were disinfected in 50-80 ppm formalin for 6 hrs and then transferred to a 300 l indoor tank for egg incubation and hatching. Filtered seawater treated with UV (ultra violet) was supplied through re-circulating system approximately at a rate 1.5 l min^{-1}. Salinity and temperature were maintained at 32 - 36 ppt, and 26 - 29 °C respectively. Egg incubation was required up to 14 days and the berried crabs were not fed during this period in order to reduce the presence of particulate and dissolved organic matter in the tank. Eggs were sampled regularly to check the embryonic development and to predict the hatching time. One or two days prior to hatching, the berried crabs were once again disinfected with 50 ppm formalin for 6 hrs and at the same time the incubation tank was cleaned and used as hatching tank. Hatching normally occurred in the early morning. In the larval rearing tanks seawater was pumped from the storage tank filtered through three cartridges (10, 5 and 1 µm) and UV filters. Salinity of water for the experiments was maintained at 32-35 ppt. Seawater was also treated with 10 ppm Streptomycin.

Feed preparation: Unicellular yellow green microalgae *Nannocloropsis occulata* was batch cultured in five 1000 l flat bottom oval tanks under natural sunlight. The pathogen free stock culture was collected from the Microalgae Culture Laboratory, James Cook University. Tanks were filled with filtered seawater (32-35 ppt salinity) up to about 80% of their capacity and treated with 10 ppm chlorine for 24 hrs. After 24 hrs of vigorous aeration, the tank was inoculated with 200 l of algae (20% of the tank volume) and fertilized with 20-30 g of soluble plant fertilizer ('Aquasol', Hortico Australia Pvt. Ltd). The algal culture turned dark green in 4-7 days and became suitable for rotifer food or used as starter for the next batch of culture. Pathogen free SS-strain of rotifer *Brachionus rotundiformis*, maximum 150 µm lorica length, was cultured outdoor in three 150 l flat-bottom round tanks using *N. occulata* as feed. The stock was collected from Marine and Aquaculture Research Facility Unit (MARFU) of James Cook University. Rotifer was maintained at a density of 400 ml^{-1} primarily by keeping the culture as clean as possible. Filter screens were provided in the culture tanks to accumulate dead algae and detritus. The rotifer culture tanks were cleaned daily by draining 50 to 100 % of the tanks' volume through a 60 µm sieve and the rotifers were washed with seawater to flush out faeces, bacteria and ciliates before they were returned to the tank. The rotifer tank was also cleaned before the rotifers were returned and fed with algae. Every 7 days, the cultures was filtered and transferred to a neighboring tank to allow sequential cleaning of the tanks. When required, rotifers ware siphoned from the tank into a 50 l flow through container fitted with a 63 µm mesh screen to keep them always submerged in water. This method helped from preventing damage of rotifers during harvest. Harvested rotifers were washed with fresh water and seawater following filtration through a 300 µm mesh screen to remove any debris. Three replicates of 1 ml clean rotifer samples were counted under a dissecting microscope before being fed to the mud crab larvae.

Experimental design and data collection: A single factor experimental design was used in this study to investigate the effects of rotifer densities on larval survival and development. Thirty vigorously swimming and positively phototactic newly hatched zoea were reared up to the megalopa stage in 500 ml glass beakers using three replicates of six rotifer density (0, 5, 10, 20, 40, and 60 ml^{-1}). All culture vessels (glass beakers) were disinfected through chlorination. All the replicates were placed in the water bath, where temperature was maintained between 26 to 30 °C using an immersion heater (Rena Cal 300W). No aeration was provided. A photoperiod of L:D=16:8 was used through out experiment using fluorescent lamps at an intensity of approximately 4000 lax. Larvae were fed according to the protocol provided by Zeng and Li (1999). Every morning, larvae were transferred using a large bore pipette to new culture vessels filled with new seawater and fresh food. At this time survival, mortality, and molts were recorded. Zoeal stages were identified based on characteristics provided by Ong (1964). Experiments were terminated when all larvae either died or metamorphosed to the megalopa stage. Larvae were considered dead when they became opaque, or when no movement of any appendage was observed.

Data analysis: The cumulative survival rate (%) of a particular larval stage was calculated as the number of larvae molted to the next stage divided by the total larval number of a replicate at the beginning of the experiment. Larval development was expressed as the mean intermolt duration (day) and as the mean cumulative development time (day) of a larval stage. Cumulative survival data were arcsin transformed Cumulative development time (day) was log transformed. One way analysis of variance was performed at 5% level of significance to compare survival and development data among the treatments. When the difference was found significant, then Tukey's honestly significant test (HSD) was conducted to find out which treatments were different. All statistical analyses were performed using SPSS for Windows, version 10.0 (Microsoft Inc.).

Results

Cumulative survival rates (%) at zoeal stages were given in the Table 1. No larva survived at 0 ml^{-1} and 5 ml^{-1} rotifer density. At 10 ml^{-1} density, a few larvae molted to Z-I stage. Where as at 20 ml^{-1} larvae could survive until Z-III stage. At 40 and 60 ml^{-1} density larvae could pass easily all five zoeal stages and molted to megalopa. The results showed that suitable rotifer density for larval rearing of *S. serrata* during Z-I and Z-II stage should be ranged from 40 to 60 ml^{-1}. Higher mortality at stages Z-III, Z-IV and Z-V indicated that though rotifer was sufficient as live feed during Z-I and Z-II stage but in the later stages it was not suitable food for their survival.

There was delay in molting to the next stage where the rotifer density was low (Table 2). At 60 ml^{-1} density, mean cumulative development time of Z-I, Z-II, Z-III, Z-IV, and Z-V were comparatively shorter than the larvae reared at 40 ml^{-1} rotifer density. Apart from development time, duration in Z-I and Z-II at 60 ml^{-1} were also shorter than that of at 40 ml^{-1}. Unexpectedly, at 10 ml^{-1} and 20 ml^{-1} treatments, some Z-I managed to survive without molting for maximum 17 days and 15 days after hatching.

Table 1. Cumulative survival rates (%) of *Scylla serrata* larvae reared at different rotifer densities.

Zoeal stages	Rotifer densities (individual ml^{-1})					
	0	5	10	20	40	60
Z-I	-	-	7.8	21.1	40.0	37.8
Z-II	-	-	-	10.0	30	27.8
Z-III	-	-	-	4.4	16.7	18.9
Z-IV	-	-	-	0	8.9	12.2
Z-V	-	-	-	0	3.3	1.1

The result of the experiment also showed that rotifer was not good enough for larval metamorphosis to megalopa stages (Table 3). At 40 and 60 ml^{-1} density a number of megalopa were molted to first crablet stage, but the number was minimum, only 1-2%. Mean cumulative development time of the megalopa to first crablet stage was shorter at 60 ml^{-1} (29 days) than at 40 ml^{-1} (32 days). Duration in megalopa stage was similar (about 7-8 days) in both 40 and 60 ml^{-1} rotifer densities.

Table 2. Intermolt duration and cumulative development time (mean and range) of *S. serrata* larvae fed at different rotifer densities.

Zoeal stages		Rotifer densities (individual ml^{-1})					
		0	5	10	20	40	60
Z I	Duration (day) (range)	-	-	11.5±2.2 (3-17)	6.6±1.6 (3-15)	5.1±0.2 (4-8)	4.7±0.4 (3-8)
Z II	Duration(day) Cum. Develop. (range)	-	-	-	9.6 16.2±2 (9-20)	4.7 9.7±0.8 (7-19)	3.4 8.2±0.5 (7-10)
Z III	Duration(day) Cum. Develop. (range)	-	-	-	5.3 21.5±4.8 (16-26)	6.5 16.2±1.0 (14-18)	6.9 15.1±0.2 (12-18)
Z IV	Duration(day) Cum. Develop. (range)	-	-	-	-	4.4 20.6±2.1 (18-24)	4.4 19.1±1.6 (17-24)
Z V	Duration(day) Cum. Develop. (range)	-	-	-	-	3.8 24.3 (24-25)	2.5 22.0

Discussion

The survival rate of *Scylla serrata* larvae in this experiment was shown to increase steadily with density of rotifers. The highest survival of Z-I and Z-II was observed at 40 ml^{-1} rotifer density. Similar result was reported for *S. paramamosain* (Li *et al*., 1999; Zeng and Li, 1999). Feeding behavior of *S. serrata* may explain the higher survival at higher rotifer densities since prey concentration has been reported as one of the most important factors in rearing decapod crustaceans (Minagawa and Murano, 1993). It was observed that early larvae of mud crab capture food by chance during their tail flipping behaviour. When they flip their tail, they come into physical contact with prey, and then the larvae hold the food item by their forked tail and pass to the mouth parts for consumption. Higher food density would increase the chance for larvae to encounter and capture preys, thus enhancing survival and development. Moreover, increasing physical contact between food items and larvae as density increased may also stimulate larvae to increase their tail flipping frequency (Heasman and Fielder, 1983). However, when rotifer density was increased up to 200 ml^{-1}, larval survival was not significantly increased (Zeng and Li, 1999). This was probably due to lower nutritional value of rotifer compared to requirement of mud crab larvae for further growth and development. The experiment also showed slight decrease in survival at 60 ml^{-1} treatments which was possibly due to water quality deterioration caused by excess rotifers. This assumption needs to be confirmed with water quality experiments using higher density rotifers (for example >80 ml^{-1} or 100 ml^{-1}). However, this study suggested a suitable range of rotifer density at 40-60 ml^{-1} during zoea stage of mud crab larval rearing. Moreover, it was also found that at lower densities such as 10 ml^{-1} and 20 ml^{-1} treatments, some Z-I exhibited unusually prolonged survival without molting. Anger (2001) suggested that sometimes,

Table 3. Survival and development of mud crab megalopa reared in different rotifer density.

	Rotifer densities (individual					
	0	5	10	20	40	60
Survival (%)	-	-	-	-	2.22	1.11
Duration (day)	-	-	-	-	7.67	7
Cum. Develop. (range)	-	-	-	-	32	29

during intermolt stage larvae may undergo a period of development arrest. The reasons behind such incident are still unclear.

The results also showed that rotifer might be good enough for the survival and development of Z-I and Z-II stages but not for the later stages. At higher density treatments (40 and 60 ml^{-1}) about 40% Z-I and 30% Z-II survived but at the same rotifer density survival of Z-III, Z-IV and Z-V were about 17%, 10% and 2% respectively. Similar results were reported for *S. serrata* (Baylon and Failaman, 1999) and for *S. paramamosain* (Zeng and Li, 1999) in which rotifers were found to be suitable for Z-I and Z-II and *Artemia* for Z-III onwards. This result once again indicates the lower nutritional value of the rotifers which may not be good as food for Z-III larvae and beyond. This assumption needs to be tested through measuring nutritional value of zoea fed rotifers and *Artemia*. If our hypothesis is proved to be true, there will be no significant difference in dry weight, carbon, nitrogen, hydrogen content of Z-I and Z-II fed with rotifer and *Artemia* and if not true there will be significant difference in dry weight, carbon, nitrogen, hydrogen content between rotifer and *Artemia* fed Z-III larvae and onwards stages. Therefore, larvae that are fed with rotifer alone will show slow growth and longer time to metamorphose to next stage. This assumption was also supported by the results of this experiment which showed that larvae fed exclusively rotifers was not enough to promote metamorphosis up to megalopa stage. In the treatments where larvae were fed only rotifers, mere 0.9% of the newly hatched larvae were able to reach megalopa stage and the mean development time was about 23 days. Whereas, Baylon and Failaman (1999) used a combination diet of *Artemia* and rotifer, and reported higher survival (69%) up to Z-V stage, higher metamorphosis (56%) and the shorter time to produce megalopa (17 days from hatching). Similar results were also reported by Li *et al.* (1999). Thus, rotifers were not a complete diet for all larval stages and if fed with rotifer alone larvae may encounter mass mortality and their molting may be delayed.

This experiment identified Z-III as a critical stage among larval stage, because, at this stage, the gastric mill of the digestive system starts to develop and the number and size of the hepatopancreas start to increase (Li *et al.,* 1999). Larvae require high energy to maintain this development and the energy demand continues to metamorphosis up to megalopa stage. Rotifer alone may not be sufficient to meet this energy demand. Histochemical observations suggested that accumulation of glycogen, lipid and protein in larval alimentary tract reached their highest levels at Z-V and the megalopa stage, but generally showed a significant increase at Z-III (Li *et al.,* 1999). However, detailed histological and histochemical studies of the digestive system throughout the larval development need to be conducted.

Conclusion

Rotifer was found as suitable live feed only for Z-I and Z-II stages at an optimum density of 40-60 ml^{-1}. However, it was found not suitable for larval development and survival beyond Z-III stages. This study recognizes the need for further research to assess development and survival of larvae beyond Z-III stages fed with *Artemia*, determine nutritional value of rotifer and *Artemia*, study histology and histochemistry of the mudcrab larvae in order to device a full proof feeding protocol of mud crab larvae.

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