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Larval rearing of mud crab, *Scylla tranquebarica* (Fabricius, 1798) and feeding requirements of its zoea$_1$

G. Maheswarudu , Josileen Jose, K.R. Manmadhan Nair, M.R. Arputharaj,
A. Ramakrishnan, A.Vairamani, S.Mohan and S.Palinichamy

Visakhapatnam Regional Centre of CMFRI,Oceanview Layout, Pandurangapuram, Visakhapatnam -530 003, India
Email: maheswarudu@yahoo.com

Abstract

Feeding experiments were conducted with zooplankton to find out the suitable food for zoea$_1$ of the mud crab *Scylla tranquebarica*. Zoeae$_1$ from seven berried females, three from wild and four from rematured specimens were the source material for the present study. The veliger larvae (50-55μm) of pearl oyster and two species (*Pseudodiaptomus* spp. and *Labidocera* spp.) of pelagic copepods (500-1000 μm) were tested as feed. However, the zoea$_1$ accepted only the former. The feeding activity could be resolved into 5 stages. Significant role of the serrated caudal setae in prey abrasion into required size suitable to mandibles for mastication and the size preference of the items of diet in relation to the growth of the mandibles with the advancement of zoeal stage also have been discussed. In all the 7 feeding experiments that were conducted by providing rotifer, *Brachionus rotundiformis* (<100 μm), the zoea$_1$ advanced to zoea$_3$/zoea$_4$ or the first crab stage. The present study is a significant development in narrating the feeding behaviour of zoea$_1$.

Keywords: Larval rearing, feeding requirements, zoea$_1$, mud crab, *Scylla tranquebarica*

Introduction

The giant mud crab *Scylla tranquebarica* is in high demand both in the domestic and export markets due to its delicacy as well as size. The enormous demand has geared up the aquaculture industry to invest intensive efforts for developing a technology to ensure sustainable production from the grow-out. However, seed production in the hatchery is the prime part of development of a comprehensive technology. Though efforts were initiated earlier to perfect the protocol for larval rearing (Raja Bai Naidu, 1955; Ong, 1964) and seed production on large scale (Brick, 1974; Heasman and Fielder, 1983), a viable technology could not be perfected. Therefore, seed production posed a major hindrance for expansion of farming ventures. Of late, there are many reports on the protocol of seed production, but they are mostly restricted to laboratory conditions limited to small containers. With low survival rate the production of seed remains inconsistent. Earlier workers used *Artemia* nauplii as feed for zoea$_1$ (Ong, 1964; Brick, 1974; and Heasman and Fielder, 1983). Subsequently experiments were conducted by giving rotifer as feed for zoea$_1$ (Marichamy and Rajapakiam, 1984, 1992; Anil and Suseelan, 1999). Though zoea$_1$ survives on both the sizes of zooplankton, namely *Artemia* and rotifer, survival being lesser with the former, information on actual feeding mechanism and the suitable size of the diet items is lacking. One of the decisive factors for successful larval rearing and seed production of mud crab is to scale up the survival rate from zoea$_1$ to zoea$_2$. Zoea$_1$ is tiny, fragile and is the earliest hatched feeding larva with incomplete development of visual perception organs (sessile eyes) and digestive system. These impose restrictions on the investigator to select suitable sized diet for viable rearing in the hatchery. In view of developing protocol for larval rearing and in particular to find out the suitable diet for the zoea$_1$, feeding experiments with zooplanktons were conducted. Microscopic observations made on feeding pattern of these larvae of *S. tranquebarica* under the presence of two sizes of zooplanktons, and the results of larval rearing experiments with rotifer as feed are dealt with in this paper.

Materials and methods

The study was conducted at the backyard shrimp hatchery of the Regional Centre of Central Marine Fisheries Research Institute, Mandapam, Tamil Nadu, India. Wild-berried females of *S. tranquebarica* were collected from trawl net operations in the Palk Bay (9°20’-25”N 79°5’-10”E), transferred into 40 litres polythene cans onboard the fishing vessels, and transported to the hatchery by providing aeration through battery operated aerator. Upon arriving at the hatchery, the berried female was maintained in 5 t capacity circular flat bottom fiberglass tank under diffused aeration and by feeding with clam meat to satiation. Daily about 50% of water exchange was provided. After the berry turned into dark green colour, the crab was transferred to 1 t capacity flat bottom oval shape tank/rectangular tank for hatching. Total number of hatched zoeae was estimated by taking three sub samples (2 litres each) after thoroughly mixing the water to ensure their uniform distribution. The number in the three sub samples were counted and then raised to the total volume of water in the tank. Active zoeae were used for larval rearing experiments. Zoeae$_1$ that hatched from berried females, which attained maturity and spawned within 30 days in the Rematuration System of penaeid shrimps (Maheswarudu *et al.*, 1996; Radhakrishanan *et al.*, 2000) without resorting to eyestalk ablation, under reduced light (200-500 lux), and by feeding PUFA rich diet (Littoral oligocheate, *Pontodrilus bermudensis*) at the rate of 2% of biomass along with clam meat and squid at satiation. Zoeae$_1$ were stocked in 1 t capacity fiberglass tanks at two stocking densities (75 and 100 no./litre). Triplicates were ensured for each density trial. In the feeding experiments with rotifer (Table 2), *Brachionus rotundiformis* (<100 μm size) at the concentration of 20 no./ml for zoea$_{1;}$ (<220 μm) for zoea$_2$ at the concentration of 25 no./ml; and (<350 μm) for zoea$_3$ to zoea$_5$ at the concentration of 25 no./ml were maintained in the larval rearing tanks. Megalopa was fed with two-day-old *Artemia* nauplii (>500 μm). About 25% of water exchange was provided daily by using appropriate sieve to avoid exit of zoeae$_1$. Zoeae$_2$ count was performed as estimated for zoeae$_1$. Entire hatchery operations were performed with settled and gravity sand filtered seawater. The ranges for salinity and temperature were 32-36 ppt and 27-32 ºC respectively. Rotifer (*B. rotundiformis* and *B. plicatilis*) were developed (Muthu, 1982) and maintained separately under similar conditions to feed the larvae. Size of each live feed was measured under microscope.

***Microscopic study**:* About five zoeae$_1$ were kept in a 90 x15 mm size petri dish with 25-30 ml of seawater and the feeding behaviour and movement of feed in the gut were observed under microscope by introducing a different kind of diet at each time. Fresh and unfed zoeae$_1$ were selected for every observation of each diet. Two kinds of diet such as pelagic copepods and veliger larvae of pearl oyster (*Pinctada fucata*) were used for microscopic study. Pelagic copepods (*Pseudodiaptomus* spp. and *Labidocera* spp.) were collected from 100 t capacity rectangular cement tanks of onshore pearl oyster culture. Concentration of pelagic copepods was at 10 no./ml and veliger larvae at 30 no./ml. Three observations, each one of 40 minutes, for each diet were performed. Seawater used in the petri dish was just enough to submerge the zoeae and they were always lying on the bottom of the petri dish laterally. Illumination was provided from the bottom of the petri dish to make the zoeae transparent. Observations on feeding behaviour and on feed movement in the gut were carried out under magnifications of 5 x 15 and 10 x 15 respectively.

Results

Out of the 7-berried females that produced zoeae$_1$ for the present study, 3 were from wild and 4 rematured in the Rematuration System without resorting to eyestalk ablation (Table 1). Mean zoeae rate was higher in wild-berried females (9307 ± 1200 zoeae/g. wt.) than that of captive developed females (7767 ± 4169 zoeae/g. wt.).

***Feeding behaviour*:** Observations on zoea$_1$ feeding behaviour under microscope (5 x 15 magnification) have revealed the following five activities: 1. First zoea$_1$ gives a flap with its well-developed telson forcing the water towards the mouth along with the prey / food particles in the medium underneath its abdomen. 2. After a short break of less than one minute the non-food particles that stick on to the setae of maxillules are disentangled by inserting the two dorso-lateral long spines and the serrated furcal setae into the inter spaces of the setae of maxillules. 3. Occasionally abdomen along with the telson/fifth abdominal somite exerts gentle pressure on the mouth appendages (maxillules and maxillae) bending furcal spines and setae backwardly (45 º angle) away from the mouth in opposite direction, and comes to normal position. 4. Flap by abdomen resumes for directing the food towards the mouth 5. Abdomen is in flexure bending telson, furcal spines and furcal setae ventrally perpendicular to abdomen in normal position. It was observed that after flap, giving gentle pressure on mouth appendages by telson/fifth abdominal somite (activity 3) coincided with the movement of food in the fore gut, revealing the fact that abdomen assists in passing on the prey/food particles either to the mandibles or into the mouth.

Observations on the gut: The results of the microscopic study conducted with two zooplanktons are furnished in Table 2. Feeding behaviour of all larvae was similar as described above: flapping of the telson for diverting the current of water under the abdomen towards the mouth, and removing nonfood particles that stick to the mouth appendages with the telson irrespective of the availability of suitable diet in rearing medium. Out of 2 kinds of diet

Table 1. *Details of experiments on larval rearing of* Scylla tranquebarica *with rotifer,* Brachionus rotundiformis

S.No.	Brooder size (CW in mm and wt.in g.) and source	No. zoeae hatched	No. zoeae stocked	Rearing tank capacity (t)	Larval density (no./l)	Feed used for zoea$_1$	Survival from zoea$_1$ to zoea$_2$ (%)	Remarks
1	152/750	6724140	100000	1	100	Juveniles (<100 µm) of *B. rotundiformis*	50.5	Zoeae reached up to megalopa
	RMS		100000	1	100		52.0	Zoeae reached up to z$_4$
2	143/580	1140000	100000	1	100	Juveniles (<100 µm) of *B. rotundiformis*	53.7	Zoeae reached up to z$_3$
	RMS		100000	1	100		54.3	Zoeae reached up to z$_4$
3	153/590	7000000	100000	1	100	Juveniles (<100 µm) of *B. rotundiformis*	51.4	
	RMS		100000	1	100		52.0	
			100000	1	100		50.0	Zoeae reached up to z$_3$
			100000	1	100		50.5	
			100000	1	100		51.0	
4	145/725 RMS	6000000	100000	1	100	Juveniles (<100 µm) of *B. rotundiformis*	51.0	Zoeae reached up to z$_4$
5	170/750 Wild	7000000	75000	1	75	Juveniles (<100 µm) of *B. rotundiformis*	52.4	Zoeae reached up to z$_5$
6	190/1050 Wild	8500000	75000	1	75	Juveniles (<100 µm) of *B. rotundiformis*	51.8	First crab instars produced
7	183/1200 Wild	12595000	75000	1	75	Juveniles (<100 µm) of *B.rotundiformis*	55.0	Zoeae reached up to z$_5$
			75000	1	75		52.5	Zoeae reached up to z$_5$
	RMS=Rematuration System							

tried for the zoea$_1$, only veliger larvae of pearl oyster were observed in the gut, and the movement of the food particle was moderate, taking about three minutes to move due to peristaltic movement of the gut, from the second abdominal somite to anal somite, and make an exit through the anal pore as a faecal pellet, but not like a continuous thread as in protozoea of penaeid shrimp. Non-acceptance of an entire pelagic copepod (500-1000 μm) was indicative of its larger size, as confirmed by presence of shredded appendages of copepods in the petri dish.

Larval rearing: As the microscopic study has revealed that zoea$_1$ accepts the veliger larvae (50-55μm) of pearl oyster, feeding experiments were conducted with the juveniles of *B. rotundiformis* (super small) that passed through the 100 µm sieve and it was found that zoea$_1$ moults into zoea$_2$. Details of larval rearing experiments conducted at two larval stocking densities (75 larvae/litre & 100 larvae/litre) are given in Table 2. Survival from zoea$_1$ to zoea$_2$ was marginally high (53%) at lower density than that (52%) of higher density, but not significant statistically. In all the 7 experiments zoea$_1$ advanced either up to zoea$_3$ or first crab stage. Out of the 7 experiments conducted, one had advanced up to first crab stage, one up to megalopa, two up to zoea$_5$ and three up to zoea$_3$ / zoea$_4$. In all the experiments the survival from zoea$_1$ to zoea$_2$ was above 50% and thereafter it gradually declined, and by zoea$_5$ it was about less than 5%. The larval duration from zoea$_1$ to zoea$_5$ has taken 17 days and

Table 2. *Results of the microscopic study on zoea$_1$ of* S. tranquebarica *with two sizes of live feeds*

S.No.	Live feed	Size of live feed (µm)	Concentration of live feed (No./ ml)	No. of zoeae observed each time	Result of the observations on gut (No. of zoeae observed in %)
1.	Pelagic copepods (*Pseudodiaptomus* spp. and *Labidocera* spp.)	500-1000	10	5	Not passed through the gut (100%)
2.	Veliger larvae (20hrs. after fertilisation) of pearl oyster, *Pinctada fucata*	50-55	30	5	Passed through the gut (80%)

megalopa 8 days to metamorphose into first crab stage. Larval duration for zoea$_1$ to zoea$_5$ was 4, 3, 3, 3 and 4 days respectively, at 27.5-31.0 ºC and 35-36 ppt. During first 3-4 days megalopa was in pelagic phase with dark brown body colour and thereafter turned to benthic habitat with pale body colour. The benthic megalopa rests at the bottom, but rises to swim periodically in the water column probably to catch the prey.

Discussion

The study revealed that zoea$_1$ of *S.tranquebarica* preferred the more minute veliger larvae (50-55*µ*m) of pearl oyster than the pelagic copepods (500-1000 *µ*m). Experiments conducted to determine particle size preference for the various larval stages of *Scylla serrata* by feeding with micro bound diets revealed that diet particle size preference increased with the advancement of larval stage, highest ingestion rates by zoea$_1$, zoea$_3$, zoea$_5$ and megalopa were for particle of size range <150, 150-250, 250-400 and 400-600*µ*m, respectively (Genodepa *et al.*, 2004). The feeding experiments on larval rearing of *S. serrata* also revealed that rotifers are needed as first food item from zoea$_1$ to zoea$_2$ and that diet should be supplemented with *Artemia* from zoea$_3$ onwards (Marichamy and Rajapackiam, 1992; Ruscoe *et al.*, 2004; Davis *et al.*, 2005). Other studies from different regions also revealed that survival was low when fed with *Artemia* nauplii alone from zoea$_1$ to zoea$_5$ (Ong, 1964; Baylon and Failaman, 1999; and Zeng and Li, 1999; Ruscoe *et al.*, 2004). According to Baylon *et al.* (2004) zoea$_1$ exhibited prey selection in the presence of both *Brachionus* and *Artemia* at equal concentrations, ingestion of the former was four fold higher than the later. A series of experiments conducted by Anil and Suseelan (1999) with different combination of feeds on *Scylla oceanica* (= *S. tranquebarica*) revealed that the combination feed (*Artemia* nauplii suspension + *B.plicatilis* + antibacterial chemical Prefuran in the medium) has given highest survival from zoea$_1$ to first crab stage. However, in the presence of bigger size zooplankton (*Artemia*) zoea$_1$ moulted into zoea$_2$, indicating that zoea$_1$ may have alternate mechanism to break down the *Artemia* nauplii into required smaller size. As per the description of Ong (1964) the 3 pairs of serrate setae between the long furca, the innermost pair having 8-10 exceptionally long setules on the inner border, and these play an active role in prey abrasion during flap by abdomen. Garm (2004) while describing the mechanical functions of setae of the mouth apparatus of seven species of decapod crustaceans classified them into 7 categories, the task of serrate setae being to collect, hold, and shred larger prey items. Here the three pairs of serrated setae present between caudal forks do the job of breaking *Artemia* nauplii into smaller pieces and direct them towards the mouth by flapping. Baylon *et al.* (2004) also observed missing of body parts such as the head and appendages of *Artemia* nauplii in the rearing medium and attributed that due to early stages of zoeae not being able to consume the entire nauplius, but manage to size them in order to ingest bits of their prey. In the present study also pelagic copepods larger than 500 µm were noticed without appendages, as zoea$_1$ tackle them for food.

The serrate caudal setae also aid in grooming the setae of maxillules and in removal of rejected non food particles that stick on the setae, as also described by Garm (2004). Non-food particles sticking on to the setae of maxillules may be indicating the role played by these setae in selecting the prey size or regulating the passage of prey item toward the mandibles. It 'therefore' shows that maxillules and maxillae function as more manipulative mouth parts in zoeal stages as in the case of *Menippe mercenaria* (Factor, 1982).

Raja Bai Naidu, (1955) while describing the late first zoea of *S. serrata* reported on the well-developed mandibles with cutting processes. The subsequent study by Ong (1964) illustrated that mandible of first zoea is broader, and relatively a hard structure, with 2 large teeth on serrated edge, aiding in smashing up its food. Electron microscopic study by Factor (1982) on zoeal stages and megalopa of stone crab, *Menippe mercenaria*, describing the development of mandibles and cardiac stomach from zoea$_1$ to megalopa, revealed that mastication of food is performed by the well developed mandibles during five zoeal stages and mastication of food shifts from mandibles to the gastric mill in megalopa stage. He states as "The incisor process of mandible of zoeal stages has series of teeth and denticles and the prominent molar process appears to be well adapted for grinding food. In megalopa the expanded incisor process is rounded and toothless and the molar process is less prominent and has lost its grinding denticles appears to be better adapted for cutting and grasping than for grinding. The developmental trend in the structure of the zoeal mandibles is increase in size, increase in number of teeth and denticles on the incisor process, and increase in concavity of medial surface". These observations by Factor (1982) on stone crab, *M. mercenaria*, mastication of prey by mandibles in five zoeal stages and shifting of mastication of food from mandibles to cardiac stomach in megalopa applies to the larvae of *S. tranquebarica* also. Since the size of the mandible, its denticles and teeth, and concavity of medial surface increase with the advancement of zoeal stage, the ability of zoea to masticate larger size of prey also increases with the progression of zoeal stage. Thus the present study confirms that smaller size (<100 µm) prey/ particle forms suitable diet for zoea$_1$ of *S. tranquebarica* and the size of the prey food/particle increases with the advancement of zoeal stage and megalopa, as also reported for *S. serrata* by Genodepa *et al.* (2004). Due to non-availability of small sized zooplankton in large quantity, the investigator/hatchery is forced to opt for microencapsulated diets of required sizes. However, the main handicap in using artificial diets is to keep them suspended in the rearing medium and to ensure uninterrupted accessibility to the larvae. Other wise in the absence of small size prey, zoea$_1$ has no alternate mechanism other than breaking down the prey into smaller size with furcal serrate setae for which it has to spend some energy. As zoea$_1$ is the first feeding larva and tiny compared to other successive zoeal stages, and if this energy spent to break down the prey could be diverted towards growth, this may in turn promote survival from zoea$_1$ to zoea$_2$.

Larval rearing experiments conducted at two stocking densities with rotifer, *B.rotundiformis* as feed have not yielded any significant variation in survival from zoea$_1$ to zoea$_2$ between two stocking trials, though survival was higher marginally at lower density. All the experiments have yielded above 50% survival from zoea$_1$ to zoea$_2$. Zeng and Li (1999) achieved higher survival by enhancing feed (*B. plicatilis*) concentration up to 60 no./ml. Heasman and Fielder (1983) maximized the survival of zoeae by increasing the concentration of *Artemia* nauplii from 5 to 30 no./ml.

The present study by means of microscopic observations on the feeding pattern of zoea$_1$ of *S. tranquebarica* has revealed the following: 1) telson plays a significant role in prey abrasion as well as in directing the food particles towards the mouth, 2) the telson also aids to remove rejected non-food particles that stick on to the setae of maxillules, 3) the sized up pieces of prey by abrasion that get directed towards the mouth are masticated by the mandibles and finally, 4) the masticated food particles by the mandibles are conveyed to the mouth aided by telson by exerting the gentle pressure on mouthparts such as maxillules, maxillae and mandibles.

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