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LABORATORY REARING AND SEED PRODUCTION OF THE MUD CRAB *SCYLLA OCEANICA* (DANA)

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ABSTRACT

The green mud crab *Scylla oceanica* (Dana) was successfully reared from egg to crab instar under laboratory conditions. Maximum period of egg incubation was noted to be 13 days. The larvae were reared through five zoeal and one megalopa stage to crab instar. It took a minimum of 17 days to complete all the zoeal stages and a minimum of 6 days to moult from megalopa to crab stage. Effect of individual food items such as *Chlorella salina, Artemia, Brachionus,* microencapsulated feed and egg custard as well as their three combinations *viz.,* combination of frozen *Artemia* nauplii, *Brachionus* and microencapsulated feed (Treatment A) combination of frozen *Artemia* nauplii, *Brachionus* and *Chlorella* (Treatment B) and combination of *Artemia* nauplii suspension, *Brachionus* with an antibacterial chemical prefuran (Treatment C) on the development and survival of the larvae was also studied. Treatment C gave the best survival of 23%.

INTRODUCTION

UNLIKE prawn farming for which hatchery technology is well developed crab farming depends entirely on seed obtained from wild. The diminishing seed abundance in natural nursery areas will be an impediment for the development of crab farming in India (Suseelan, 1996). Realising the imperative need, development of hatchery technology for mud crabs has been given top priority in most of the crab culturing countries in the World (Chong, 1992, Cowan, 1984). Experiments on rearing larval stages to juveniles under controlled conditions have been conducted in Malaysia, Hawaii, Australia, Taiwan and Japan (Ong, 1964; Brick, 1974; Haesman and Fielder, 1983; Cowan, 1984; Jamari, 1992) with varying degrees of success. Hatchery techniques for production of juveniles have been developed in Taiwan and Japan (Cowan, 1984). In India, experimental work on larval rearing and seed production of mud crabs was initiated as early as 1984 at the Tuticorin Research Centre of the Central Marine Fisheries Research Institute (Marichamy and Rajapackiam, 1984). Mass rearing of larvae of *Scylla serrata* could be successfully carried out during the above experiments. Since no information is available on these aspects for *S. oceanic*, a series of experiments were carried out on incubation and larval development on this species and the results are described in this paper. The existence of *S. oceanica* as a valid species in Indian waters has been recently established by Anil and Suseelan (MS).

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MATERIAL AND METHODS

Berried specimens of *S. oceanica* were obtained from trawlers operating off Kochi. The live specimens were immediately transferred to water of same salinity in 50 litre aerated plastic jars and transported to the field laboratory of CMFRI, Narakkal. Then they

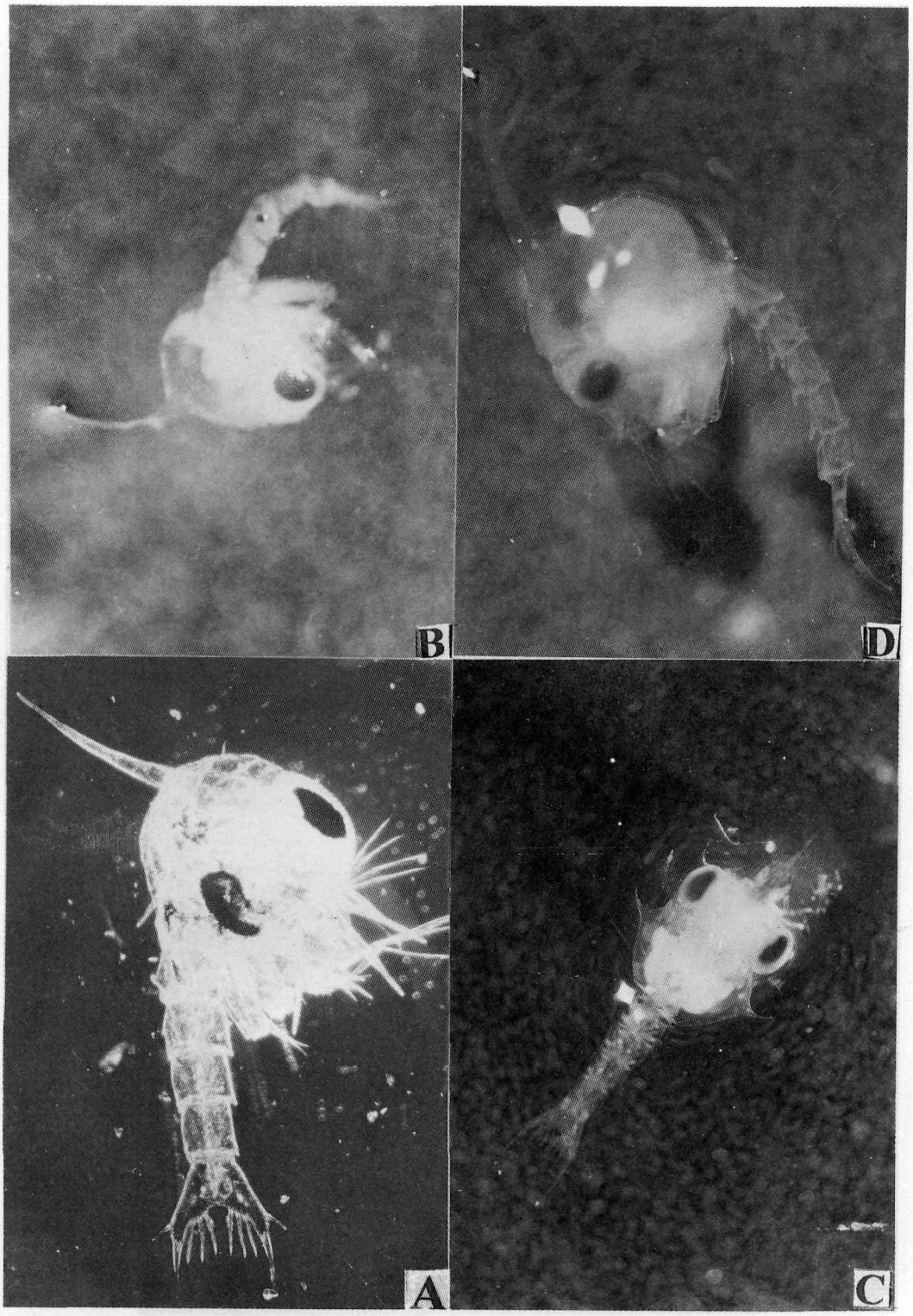

PLATE I. *Larval stages of* Scylla oceanica *A. Zoea I, B. Zoea II, C. Zoea III and D. Zoea IV.*

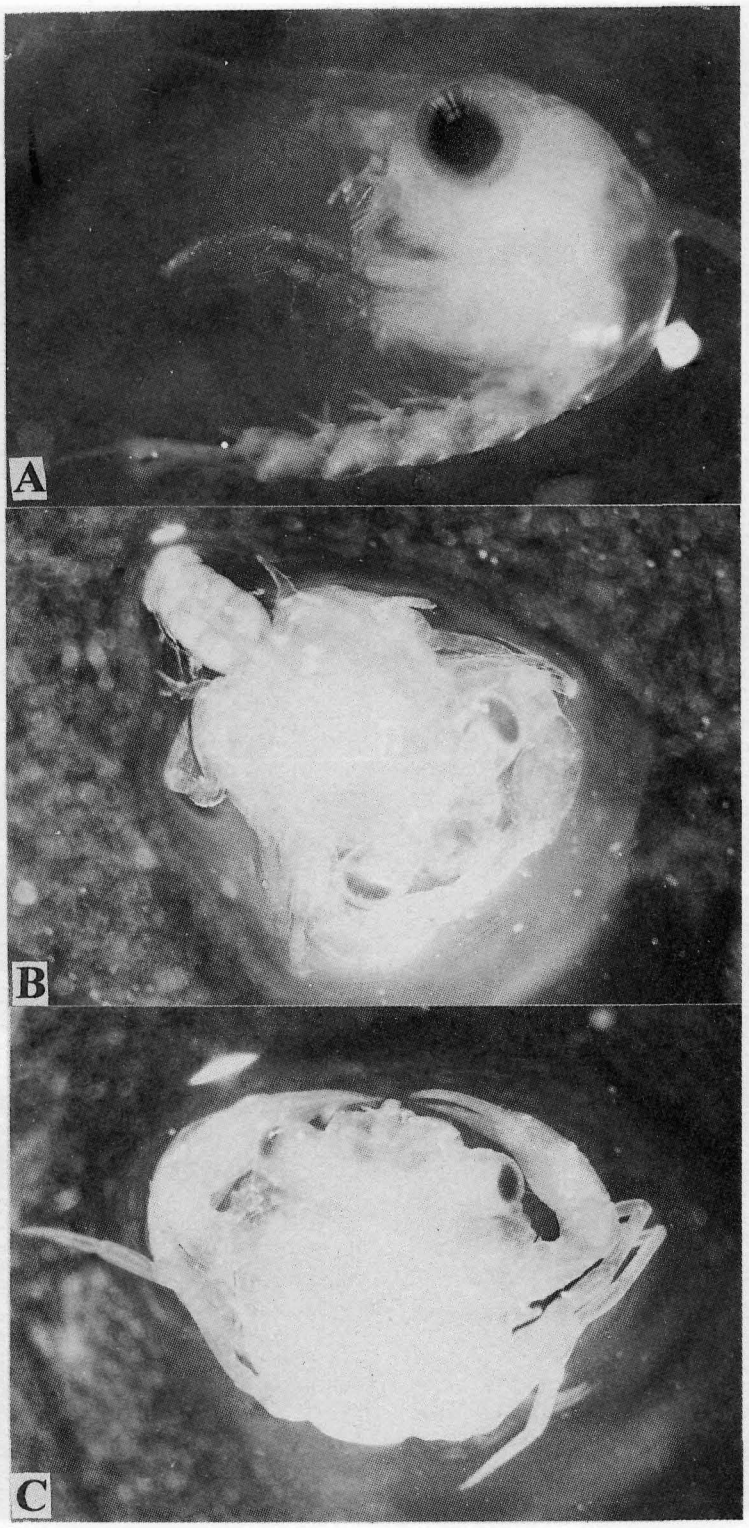

PLATE II. *Larval stages of* Scylla oceanica *A. Zoea V, B. Megalopa and C. Crab Instar I.*

were transferred to 2 ton fibreglass tanks filled with clean filtered and aerated sea water. Live clams were opened and given to the animals as feed. Every day half of the water was exchanged with fresh seawater after siphoning out the excess food, excreta and shed out eggs. Continuous aeration was given throughout the incubation period and the development of the egg was closely observed.

On egg hatching, active zoeae were collected from the spawning tanks for larval rearing studies. In order to facilitate replication of the experiments, zoeae were reared in 2200 ml glass troughs of hemispherical shape filled with 2 litres of filtered and aerated seawater. Each trough was stocked with larvae at the rate of 50 numbers/litre. For every larval feed three replicates were tried. Salinity was maintained at 34-35 ppt., pH 7.8-8.2 and temperature 27-30°C. Every day larvae were counted and transferred to fresh seawater using wide bore pipette. Troughs were arranged in such a way to ensure uniform light conditions to all the containers.

Five larval feeds, namely *Chlorella marina, Brachionus plicatilis, Artemia salina* nauplii, egg custard and microencapsulated feed were given to the larvae individually and in combination. The algal culture was maintained using 'Conwy' medium and the rotifers were fed with fresh *Chlorella* culture. The *Artemia* cysts were procured from Prime Artemia Incorporated, USA and Ballarpur Industries, Gujarat. *Artemia* suspension was made by grinding the nauplii in a mixer for few seconds. Fresh chicken eggs were used for making egg custard. Egg white and yolk were taken in a bowl and blended with little fresh water. This mixture was then steamed till it hardened well. This egg was passed through 200 micron sieve to produce desired particle size. Microencapsulated feed used was obtained from Sanders Brine Shrimp Comp. INC., USA, with a composition of protein 46%, lipid 18%, HUFA 2.5%, ash 0.12% and moisture 8.5%.

RESULTS

Fecundity examined from 17 berried specimens of *S. oceanica* in the size range 13.4 cm to 18.2 cm carapace width gave a total eggcount varying from 25,39,683 to 70,58,823 with an average of 41,23,494.

In freshly acquired condition, the berry appeared yellowish and was very compact. As the development proceeded the colour of egg mass changed slowly to greyish yellow and finally to brownish black or black. The ovigerous eggs were spherical in shape, with yolk content visible through microscope as yellow granules and dividing the egg surface into large polygonal areas. As development advanced there was a decrease in yolk volume. The organogenesis and pigmentation of the embryo were clearly discernible during incubation. Towards the end of incubation period the egg mass became loosened and the abdomen tilted dorsally. The twitching of heart was clearly visible one day before hatching.

The berried animals were sluggish and did not feed regularly during incubation. The actual hatching or release of zoea from the eggs took place in the morning between 6 and 10 a.m. The maximum period of incubation noted was 13 days.

The larvae were reared through 5 zoeal stages and one megalopa stage to crab instar (Plates I and II). Frozen *Artemia* nauplii and *Brachionus* were given as feed for the first two zoeal stages whereas newly hatched *Artemia* nauplii were given to the other zoeal stages. Pieces of clam meat and shrimp were given to the megalopa stage. Though the first zoea was found feeding on *Artemia* nauplii it was

difficult for them to capture actively moving nauplii. Maximum mortality was observed during the first stage which ranged from 40 to 100%. Thereafter the mortality was gradual. The minimum time taken for moulting between stages, size range and salient distinguishing characters of different developmental stages from zoea I to crab I are given in Table 1.

TABLE 1: *Minimum time taken for moulting, sizes and distinguishing characters of developmental stages of* S. oceanica.

Stages	Moulting period (days)	Size range (Mean size) (mm)	Distinguishing characters
Zoea I	4	1-1.3 (1.25)	Sessile eyes, abdomen 5 segmented, 3 pairs of setae between telson furca.
Zoea II	3	1.5-1.7 (1.56)	Eyes stalked, a pair of small setae between inner pair of setae of caudal furca.
Zoea III	3	1.8-2.1 (1.94)	Abdomen 6 segmented, lateral spines on segments 3-5 longer.
Zoea IV	3	2.4-2.6 (2.53)	Pleopod buds on segments 2-6, lateral spines on segments 3-5 more elongated.
Zoea V	4	3.6-5.1 (4.32)	Pleopods on abdominal segments well developed, its exopodite with setae.
Megalopa	6	4.8-5.3 (5.12)	Typical portunid megalopa, Ist pereiopod modified into cheliped.
Crab I	4	3.8-4.0 (3.82)	Margin of carapace serrated, 9 anterolateral spines.

As larval feed is an important factor for better survival, two series of experiments were conducted to study the efficacy of different food items individually and in combination to support development and survival of the larvae of *S. oceanica*. In the first series of experiments, food items such as *Chlorella, Artemia, Brachionus*, microencapsulated feed and egg custard were given individually. In the second series, feeding with different combinations of the above food items was attempted. In all the experiments, larval density was maintained at 50 numbers/litre, Megalopa was reared at a reduced salinity of 23 ppt.

Results of the first series of experiments are graphically represented in Fig. 1 a-f. The first food item tried was *Chlorella salina*. A concentration of 50,000 cell/ml was maintained in the culture medium throughout the rearing period with 100% water exchange daily. Larvae very active during the first day but on the second day morning only 34.3% of them were alive. Even among the live ones some of the larvae were not active and seen lying at the bottom with limited movements. On the third day morning, only 11.7% of the larvae were alive but a greenish tinge was seen in the alimentary canal of the live larvae indicating intake of algae. On the fourth day, 3.7% of the larvae were alive and most of them remained at the bottom of the container with limited movements. Total mortality was observed on the fifth day of rearing.

In the second treatment egg custard was given as feed. The feeding rate was 70 mg/larvae/day in four split doses at a 6 hourly interval. Larvae were active during the first day of rearing but only 12% of them survived on the second day morning. The live ones were also not active. Total mortality occurred on the second day night.

Microencapsulated feed was used as the third treatment and the feeding rate was 50 mg/larvae/day in four split doses at six hourly interval. The survival rates recorded were 22.7, 13.3, 5 and 1.7 per cent on the second, third, fourth and fifth day of rearing and larvae did not survive beyond that. From the second day onwards a greenish tinge was noticed in the alimentary canal of the larvae indicating the presence of feed in the stomach. Even among the surviving larvae only 1/3 of them were active and the others were totally inactive.

In the fourth treatment, the rotifer *Brachionus* was used as food at a feeding rate of 20 numbers/ml. The second zoeal stage was noticed on the sixth day of rearing but the survival rates were low with 26.3, 18.7, 10.3 and 5.75 per cent of larvae surviving on the 2nd, 3rd, 4th and 5th day respectively. Only 2.3% of the larvae metamorphosed into zoea-II. Total mortality was observed on the 10th day of rearing.

In the fifth treatment newly hatched and frozen *Artemia* nauplii were given as feed at the rate of 10/ml for the first and second zoeal stages and from the third zoeal stage onwards newly hatched *Artemia* nauplii were given at the rate of 15/ml. The zoeae were seen nibbling the nauplii on the first day itself. This treatment supported zoea through all the five zoeal stages, the megalopa stage and the crab stage. After the first stage, successive zoeal stages were observed on the 5th, 8th, 11th and 14th day of rearing. Maximum mortality (67%) was recorded on the second day of rearing and after that mortality was gradual. The percentage survival was 18.3, 9.7, 5 and 2% on the 5th, 10th, 15th and 20th day of rearing. The percentage of larvae reaching Zoea-II, Zoea-III, Zoea-IV, Zoea-V, megalopa and crab stages were 13, 8, 6, 4, 2 and 2 respectively. Megalopa stage was observed on 17th day and crab stage on 23rd day of rearing.

In the control set in which no feeding was done, 85% mortality was recorded on the second day of rearing and 3% of larvae survived on the 3rd day. Total mortality was observed on the 4th day.

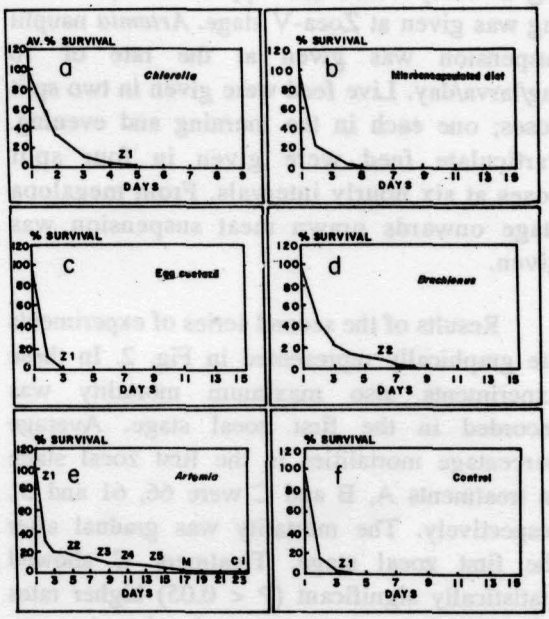

FIG. 1 a-f *Survival rates of developmental stages in different feed trials.*

In the second series of experiments consisting of three treatments, namely A, B and C combinations of feed used in the first series were tried. In the treatment A, a combination of frozen *Artemia* nauplii, *Brachionus* and microencapsulated feed was used, while in treatment B, combination of frozen *Artemia* nauplii, *Brachionus* and *Chlorella* and in treatment C, combination of *Artemia* nauplii suspension and *Brachionus* were used. An antibacterial chemical prefuran was also used in the treatment C to avoid bacterial contamination. In this treatment from Zoea III onwards, *Artemia* nauplii suspension was supplemented with freshly hatched nauplii at

the rate of 15/ml which was increased to 20/ml at Zoea-V stage. Rotifer *Brachionus* was given at the rate of 20/ml till Zoea IV. Microencapsulated feed was given at the rate of 50 mg/larva/day which was increased by 10 mg at every moult and approximately 90-100 mg was given at Zoea-V stage. *Artemia* nauplii suspension was given at the rate of 70 mg/larva/day. Live feed were given in two split doses; one each in the morning and evening. Particulate feed were given in four split doses at six hourly intervals. From megalopa stage onwards prawn meat suspension was given.

Results of the second series of experiments are graphically represented in Fig. 2. In these experiments also maximum mortality was recorded in the first zoeal stage. Average percentage mortalities in the first zoeal stage in treatments A, B and C were 66, 61 and 31 respectively. The mortality was gradual after the first zoeal stage. Treatment C showed statistically significant ($P < 0.05$) higher rates of survival at all the successive larval stages compared to treatment A and B, with average percentage survival of 46, 37, 32, 27 and 23 at Z-3, Z-4, Z-5, megalopa and crab stages respectively. Among the combinations A and B, the latter showed better survival at Z-2 and Z-3 with average percentage survival 39 and 27 as compared to the survival of 34 and 25 percent in the case of treatment A. The differences noticed between the two treatments, however were not statistically significant. From Z-3 onwards the percentage survival was almost similar in the case of treatments A and B. The average production of megalopa with feed combination A, B and C were 11%, 12% and 27% and that of crabs 8%, 9% and 23% for the three treatments respectively. Cannibalistic tendency was observed from megalopa stage onwards and it was the main reason for mortality from that stage. Shelters such as oyster shell and untwisted nylon rope were placed in the container and they were found effective in reducing cannibalism.

The result indicated that the period of transformation between different developmental stages did not vary significantly with treatments. The minimum time taken to attain megalopa stage was 17 days in treatment A and B and 18 days in treatment C. The crab stage was reached in 23 days in treatments A and B and 24 days in treatment C.

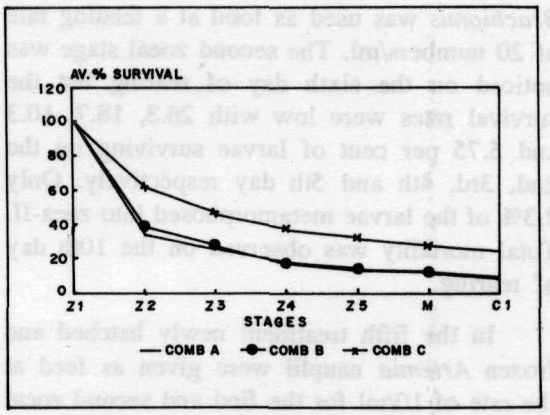

FIG. 2 *Survival rates of developmental stages in different feed combinations.*

DISCUSSION

Studying the early development of *S. serrata*, Ong (1964) observed about 18 days for the completion of the first 5 zoeal stages at 31+2 ppt salinity and 7-8 days for metamorphosis of megalopa to crab stage at 24 ± 2 ppt salinity. During the present investigation *S. oceanica* took a minimum period of 17 days to complete all the five zoeal stages and 6 days by megalopa to moult to crab stage in more or less the same duration. The larval morphology also closely followed the descriptions given by Ong (1964). Brick

(1974) noted a minimum of 17 days and a maximum of 26 days for transformation of zoea 1 to megalopa and 9-11 days more to reach the crab stage. In the experiments conducted by Haesman and Fielder (1983) it took 18-20 days to reach megalopa at 30±2 ppt salinity and another 7-8 days to reach crab stage at 26-28 ppt salinity at a temperature range of 24.8-27.4°C. Marichamy and Rajapackiam (1984, 1992) reported 18-20 days for zoea to reach megalopa and 8-11 days for megalopa to crab instar I at 32±2 ppt salinity and 26-30°C temperature whereas Jamari (1992) reported 25-28 days from zoea through megalopa to crab stage at 28-30°C temperature and 25-30 ppt salinity. In the present observation the minimum period taken at successive zoeal stages were 4, 3, 3 and 3 days for Ist, 2nd, 3rd and 4th zoeal stages and the total duration for the transformation of zoea I to megalopa stage varied from 17 days to 21 days and megalopa stage to crab stage took 6-9 days. In most of the experimental trials the period ranged from 18-20 days for the phase upto megalopa and 7-11 days for megalopa to crab stage in *S. oceanica*. This observation is in conformity with the findings of most of the earlier workers in mud crabs (Ong, 1964: Haesman and Fielder, 1983: Marichamy and Rajapackiam, 1984, 1992).

Among the feed trials, the first series of experiments in which five larval feeds viz, *Chlorella*, egg custard, microencapsulated feed, *Brachionus* and *Artemia* nauplii were given individually, only *Brachionus* and *Artemia* nauplii supported moulting of zoea. Eventhough *Chlorella* did not support moulting of zoeae it enhanced their survival period as compared to control (Fig. 1 a-f). Simon (1974) observed that mixed diatom culture given to early zoeal stages favoured good survival, but did not moult. According to Haesman and Fielder (1983) the better survival with *Chlorella* and antibiotics is due to reduced risk of bacterial infection and/or build up of potentially harmful metabolites or breakdown products thereof. In the case of microencapsulated feed also survival was better than in control, and this may also be attributed to the antibacterial properties of algal fractions which was indicated in the manufacturer's label. But both *Chlorella* and microencapsulated feed failed to support moulting of zoea. Jamari (1992) successfully used 5-10 rotifers /ml in the morning and evening supplemented with artificial shrimp larval feed. Studying the development and survival of the larvae of *S. serrata*, in Hawaii, Brick (1974) observed that rotifers and wild zooplankton as food source failed to support zoeal survival to the onset of metamorphosis. Marichamy and Rajapackiam (1992) however, was able to raise the first two zoeal stages by feeding rotifer (*Brachionus plicatilis*). During the present study *Brachionus* supported one moulting, but very few larvae could reach the 2nd zoeal stage. *Artemia* nauplii supported the larvae through all the zoeal stages, but the production of crab was as low as 2%. Ong (1964) and Du plessis (1971) reported survival rates of 1% and 4% respectively for crab and megalopa stages with *Artemia* nauplii as feed. Haesman and Fielder (1983) achieved the best survival rate of 26% of first zoea using a specially designed recirculatory system in which the larvae were fed exclusively with newly hatched San Francisco brine shrimp. The residual brine shrimp nauplii were selectively flushed from the rearing vessel each day by exchanging the 142 micron filter sleeve with 939 micron substitute for 30 minutes. These authors could maximise the survival rate by increasing the *Artemia* nauplii concentration from 5 to 30/ml.

In the second series of experiment the Combination-C with *Artemia* nauplii suspension

and *Brachionus* along with antibacterial chemical Prefuran (0.5 ppm) gave the best production rate of 23% at crab stage as compared to Combination-A (Frozen *Artemia* nauplii, *Brachionus* and microencapsulated feed) with a production rate of 8% and Combination-B (Frozen *Artemia* nauplii, *Brachionus* and *Chlorella*) with a production rate of 9%.

In all the three feed combinations maximum mortality was noticed during the 1st zoeal stage with mortality rates of 66%, 61% and 39% for Combinations, A, B and C respectively. Thereafter the mortality rate remained steady in all the three cases. Several authors have reported maximum mortality in the first zoeal stage of mud crab (Ong, 1964; Brick, 1974; Haesman and Fielder, 1983; Jamari, 1992; Marichamy and Rajapackiam, 1984, 1992). Several reasons were assigned to the low survival of larvae. According to Ong (1964) the most important reason for mortality was the failure to supply natural food for feeding the larvae. He opined that *Artemia* nauplii were too fast and big for majority of the early zoeae. During the present study also *Artemia* nauplii were found to be too big and moving fast that the zoeae could not feed them easily. Because of this only frozen nauplii were given in the initial stages but the dead nauplii were found to pollute the rearing medium quickly. According to Jamari (1992) sudden death of larvae occurred due to the inability to moult. The zoeae were killed occasionally by the chitin destroying bacteria attacking near the carapace spine (Ting *et. al.*, 1981). In the case of *P. pelagicus*, Raman *et. al.*, (1987) recorded a survival rate of 35.7% at megalopa stage which was attained in seventeen days from first zoea, by feeding different items like *Tetraselmis* sp., egg custard suspension, *Brachionus plicatilis*, egg, and mussel tissue and minute particles of trash fish flesh at different stages of development.

The highest production rate obtained with Combination-C feed can be attributed to the *Artemia* nauplii given in suspension form and the presence of antibacterial chemical in the medium. *Artemia* suspension would have given better accessibility of feed to the zoea and the antibacterial chemical helped in reducing the bacterial contamination.

Brick (1974) observed that the antibiotics enhanced premetamorphic survival of zoea while leaving the rate of zoeal development and the success of metamorphosis to megalopa unaltered. He used the antibiotics such as carbonate buffered potassium penicillin-G at 40 ppm and Polymyxin-B sulphate at 10 ppm, and observed that water filtration and ultraviolet sterilization did not significantly affect larval survival.

During the present study cannibalism was encountered from megalopa stage onwards and the mortality recorded from this stage was mainly due to cannibalism. Similar observations were also made by Ong (1964), Marichamy and Rajapackiam (1984) and Haesman and Fielder (1983) in *S. serrata*. According to Jamari (1992) mortality upto 60% occurred at crab stage due to cannibalism within the initial few days at a stocking density of 10 pcs/litre. He further pointed out that cannibalism continued even when adequate food was given. During the present study the mortality due to cannibalism was controlled by giving shelters such as oyster shells, untwisted nylon rope etc. Marichamy and Rajapackiam (1992) also found that shelters would reduce cannibalism to some extent.

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