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Larval rearing and seed production of mud crab *Scylla tranquebarica* (Fabricius, 1798)

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Abstract
The larval rearing and seed production experiment, the berried *S. tranquebarica* transferred to 1 ton tank filled with clean filtered and aerated seawater and 0.1ppm Treflan (44% trifuralin) every three days in the hatching tanks. Salinity was maintained at 35 ppt, pH 7.8-8.2 and temperature 30-32.5 ^{0}C. Everyday larvae were counted for check the survival. The rearing water replaced at 20% daily after 5 days of stocking of larvae. Feeding starts after 3 hours of hatching of the larvae. Feeding schedule zoeae I was fed with *Brachionus rotundiformis* twice per day morning and evening (*ad libitum*). The zoeae II to IV were fed with *B. rotundiformis* and the enriched *Artemia* nauplii (2 times). Zoeae V and megalopa were fed with the enriched *Artemia* nauplii and the formulated feed respectively (2 times). The period of incubation noted and it was 12 days. The complete larval development of *S. tranquebarica* consists of five zoeal and one megalopal stages before moulting into crab instar stage. The zoeal stages (I to V) required the minimum time of 4,3,3,3 and 3 days respectively, megalopa required 6 days to metamorphose into crab instar stage. The complete larval development was took place within a span of 22 days. The survival of the first zoeae to crab instar stage was 6.9%.

Keywords: mud crab, *Scylla tranquebarica*, seed production, larval rearing.

1. Introduction
The mud crabs are being fished and traded for a high price by small scale fishermen and thus they are acting as an important source of income for coastal communities. As declining catches have been reported from most of the major mud crab producing countries, a renewed interest in controlled reproduction and larval rearing (seed production) has aroused.
The natural resources of mud crabs have been seriously depleted in many countries and there is an urgent need for the promotion of crab culture. The sustainability of aquaculture however depends on the availability and continuous supply of seed and feed for which a strong technical backup is required. Before any commercial hatchery technology for mud crabs is developed, a thorough study of its larval biology is a must. Further several studies related to the survival of the larvae of mud crab had used brine shrimp, rotifers and algae as food, since the nutrition turns to be a vital to the larval survival. However, the techniques of mass production of these larvae is not yet standardized and well developed. Besides the above, the increasing demand for the mud crabs in the market and decrease in the wild stock due to the environmental hazards and unlawful indiscrimate fishing is pushing the mud crab fishery into its unrecoverable state. So it becomes important to maximize the larval production in the hatcheries, not only for ranching to improve the natural stock but also to cater the needs of the aquafarmers to increase the production of mud crabs to supply and fulfill the requirements of the consumer. Many of the problems faced in different hatcheries appeared to be site specific. But the water quality and bacterial infestation are still major stumbling blocks. All the hatcheries still depend on antibiotics for reasonable success. The need to use live feed makes the process expensive and subject to total collapse due to crashes of feed cultures. Further, at times, the live feed can also act as disease vector. The culture of mud crab larvae is currently based on a diet of the brine shrimp, *Artemia* sp. particularly in the late larval instars. The declining survival near the end of the larval cycle and a high incidence of failure to complete the first metamorphosis indicate a possible nutritional deficiency. One further option is to explore the use of other live food sources, such as copepods, that are potentially a nutritionally complete prey item for crab larvae.

Thus determining the critical nutritional requirements of the larvae is an important step towards developing reliable hatchery techniques.

The seed production of aquatic species is almost entirely depending on the successful production of live food organisms, principally rotifers, followed by *Artemia* and that marine fish larvae fed diets enriched with these live marine organisms are of improved quality. The N-3 highly unsaturated fatty acids (n-3 HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are particularly important for fish larvae.

The following are some of the remarkable attempts on larval rearing and seed production made in *S. serrata*, in *S. tranquebarica*, in *S. oceanica* and in *S. paramamosain* by Nghia *et al.* 2001.

So far, in a successful attempt to culture the larvae of *S. serrata*, studies on water quality, phytoplankton and food organisms, use of recirculatory system, amount and different diet combinations have been studied. But no such comprehensive study is available for *S. tranquebarica* and hence the present study has been carried.

2. Materials and methods

Berried specimens of *S. tranquebarica* were obtained from trawlers operating off Parangipettai coast (Lat.11^{O}29'N; Long.79^{O}49'E). The live specimen (Fig. 1A) were immediately transferred to seawater and transported to the laboratory. Then they were introduced into 1 ton capacity cement tank filled with clean filtered and aerated seawater. To counteract fungal and ciliate infections, the berried females were treated with 0.1ppm Treflan (44% trifuralin) every three days once in the hatching tanks until the berry hatched out (Fig. 1B). The cooked clam meat was given to the animals as feed in the morning and evening hours. Every day the excess food, excreta and shed out eggs were siphoned out. Continuous aeration was given throughout the incubation period and the development of the egg was closely observed through MEIJI binocular microscope. The different stages of the embryonic development were observed daily.

On hatching, the active zoeae were collected from the hatching tank for larval rearing studies using fine mesh net. In order to facilitate replication of the experiments, the zoeae were reared in the filtered and aerated seawater. Each tank was stocked with larvae at the rate of 50 nos/l. The antibiotics such as Ciprofloxacin and Oxytetracyclin were applied in the tanks for first five days (morning and the evening) in 2:1 ratio. The salinity was maintained at 35ppt, pH 7.8 - 8.2 and temperature 30-32.5 ^{0}C. Every day the larvae were counted to assess the survival rate and checked for the healthiness. Twenty percent of rearing water was replaced daily after 5 days of stocking of larvae.

The larvae were examined each day before changing water for the presence of live, dead larvae and exuviae. Sample of each larval stage and exuviae were preserved in 10% formalin for further observations. Dead larvae and exuviae were siphoned out during this time to prevent contamination. Larval numbers were estimated daily by counting 8 replicates taken in 500 ml beakers from the rearing tanks. Assessment of each zoeal stage was done at completion of different levels of metamorphosis to determine feeding and survival rates.

After the metamorphosis of the V zoea into megalopa the larvae were stocked at the rate of 2 nos./l. Blacknets and PVC pipes were provided as shelters when the megalopa metamorphosed into crab instars.

2.1 Live feed culture

2.1.1 *Chlorella marina*

The inoculum of *C. marina* was inoculated into the seawater enriched with ammonium sulphate, super phosphate and urea in a ratio of 10:1:1. The green colour developed within 3 to 4 days was the indication of *C. marina* development (Fig. 1C & D).

2.1.2 Rotifer (*B. rotundiformis)*

After inoculating the rotifer (30 individuals per ml) into the *Chlorella* tank, the yeast was added daily as supplementary feed to the rotifers. After the microscopical observation on 3 or 4th day rotifers were harvested and to the tank an equal amount of *Chlorella* with the medium was added for further culture of rotifer. Continuous vigorous aeration was given and the temperature was maintained 30±20 ^{0}C throughout the culture period.

2.1.3 *Artemia* nauplii (OSI Brine shrimp eggs, USA)

The *Artemia* nauplii harvested from the *Artemia* hatching tank and placed in a plastic tub with required quantity of water. The enrichment solution (Culture Selco - INVE, Belgium) was added at a concentration of 0.1%. The nauplii were enriched for 12 hours and after washing in seawater the nauplii were fed to the crab larvae (Fig. 1E & H).

2.1.4 Formulated feed

For the formulation of feed the ingredients such as prawn, clam, eggs, milk powder, cod liver oil, lecithin and vitamins were used. After proper mixing, grinding and cooking, the feed mixture (wet dough) was rubbed against a sieve of 1mm mesh size. The particles thus obtained were stored in the refrigerator on the previous day itself. Just before feeding, the feed was washed well.

2.1.5 Feeding Regime

Feeding was started when the larvae hatched after 3 hrs. The zoea I was fed with the rotifer *B. rotundiformis* (Fig. 1G); zoea II to IV were fed with rotifer dominated *Artemia* nauplii feed and zoea V and megalopa were fed with *Artemia* nauplii dominated formulated feed. The feed was given twice a day at 8'O clock in the morning and 5'O clock in the evening *adlibitum*.

3. Results

The berried crabs were sluggish and failed to take feed during the incubation period (12 days). The colour change of the berry was golden yellow, yellowish orange, orange, brown and black. The gradual change in the colour of the berry was noticed once in 2-3 days. After the completion of the embryonic development, the eggs measured about 0.82 mm in diameter. The actual hatching or release of zoeae from the berry took place in the morning between 6 and 10 a.m.

The treatment with Treflan to the berried crab, once in 3 days, gave better result which prevented to berry from infection. The water treated with the antibiotics such as Ciprofloxacin and Oxytetracyclin also recorded better survival and reduced the mortality of the larvae considerably. The salinity was maintained as 35 ppt during all the zoeal stages and when zoea V metamorphosed into megalopa the

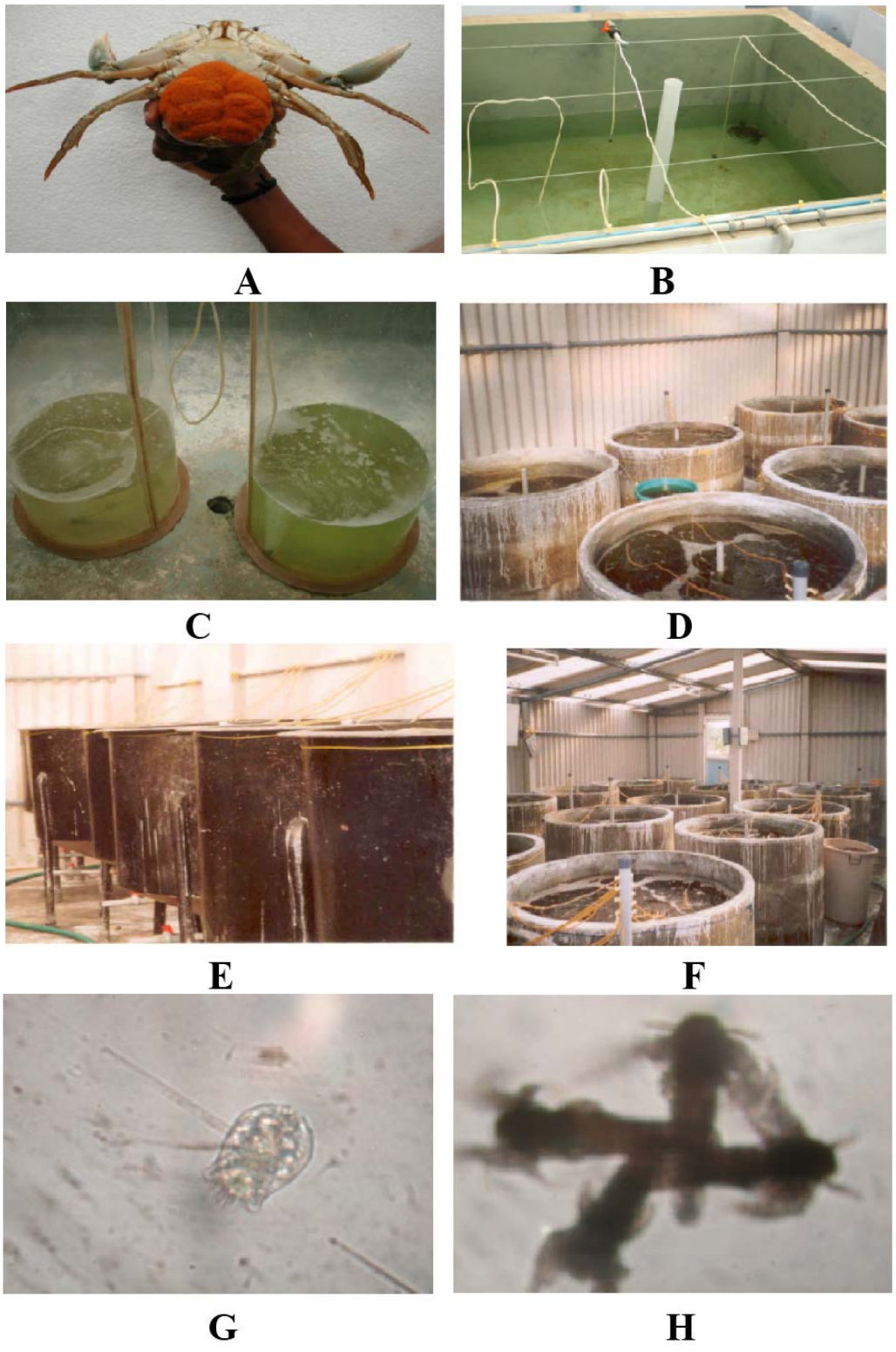

Fig 1: Crab seed production facilities (A - Berried crab, B - Broodertank, C - *Chlorella* inoculum, D - Algal culture tanks, E - *Artemia* culture tanks, F - Larval culture tanks, G – *B. rotundiformis* and H - *Artemia* nauplii)

salinity was reduced to 26 ppt. Any drastic fall in temperature and the cloudy weather resulted sudden mortality. The maintenance of temperature, in the larval rearing tanks, ranging between 32.5 to 33 ^{0}C was found to speed up the moulting and thus the metamorphosis. The complete larval development of *S. tranquebarica* consisted of five zoeal and one megalopal stages before moulting into the crab instar stage (Fig. 2). The zoeal stages (I to V) required the minimum duration of 4,3,3,3 and 3 days respectively, and the megalopa required 6 days to metamorphose into crab instar stage. The complete larval development took place within a span of 22 days. The details of the intermoult duration of the different larval stages are given in Table.1.

Table 1: Intermoult duration of different larval stages

Larval stage	Duration of moulting (Days)
I Zoea to II Zoea	4
II Zoea to III Zoea	3
III Zoea to IV Zoea	3
IV Zoea to V Zoea	3
V Zoea to Megalopa	3
Megalopa to Crab instar	6
Total	22

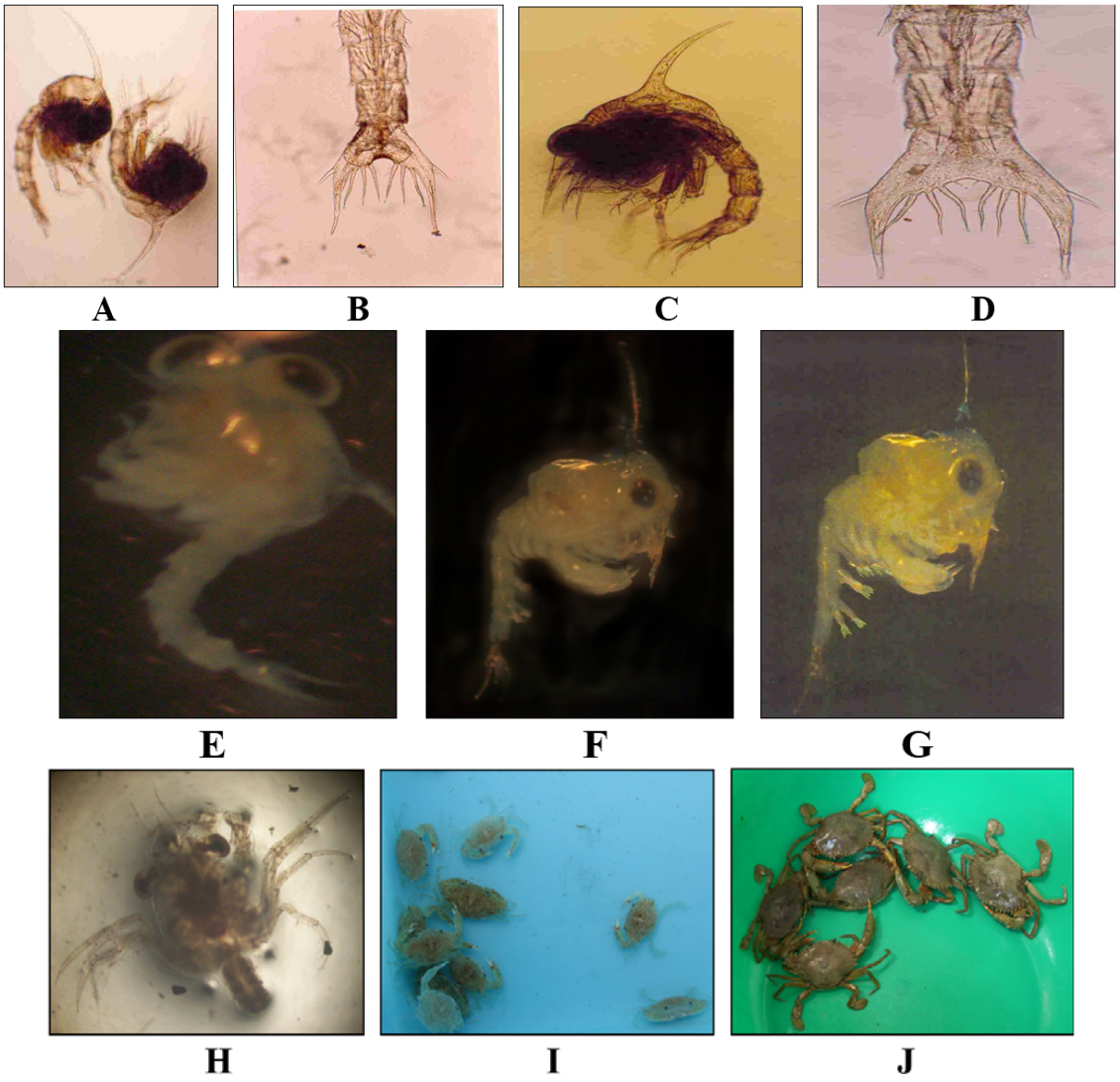

Fig 2: Larval stages of *S. tranquebarica* (A – Zoea I, B – 3 pairs of setae, C – Zoea II, D – Four pairs of setae, E – Zoea III, F – Zoea IV, G – Zoea V, H – Megalopa, I – Crab instar and J – Juvenile crabs)

A maximum of nearly 37% of mortality was observed during the first zoeal stage. Thereafter the mortality was gradually increasing (Table.2). The survival of the first zoeae to crab instar stage was 6.9%. The percentage of survival in each larval stage is presented in Table. 2 and Fig. 3.

Table 2: Percentage of survival of different larval stages

Larval stage	Survival (%)
I Zoea to II Zoea	62.7±13.1
II Zoea to III Zoea	44.8±11.9
III Zoea to IV Zoea	33.1±8.4
IV Zoea to V Zoea	20.6±7.5
V Zoea to Megalopa	12.5±4.9
Megalopa to Crab instar	6.9±2.5

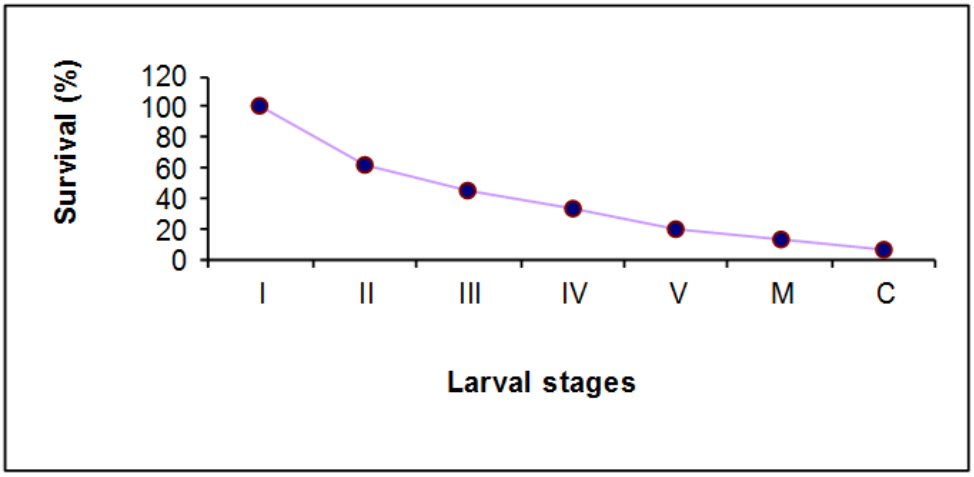

Fig 3: Survival (%) of different larval stages in S. *tranquebarica* (I to V Zoea, M – Megalopa and C – Crab instar)

The rotifer *B. rotundiformis* and enriched *Artemia* nauplii were given as feed for the first zoeal stage and other zoeal stages upto zoea IV respectively. Whereas the formulated feed was given to the zoea V and megalopa stages. The rotifer and the enriched *Artemia* nauplii were supported the larval development very much and hence better survival could be observed. No ciliate and bacterial infection was noted during the study period. The formulated feed washed properly then given to megalopa and crab instars. Feeds were prepared according to the capturing ability of the megalopa and crab instars through their tiny chelae.

Cannibalistic tendency was observed from megalopa stage onwards and it was the main reason for the higher mortality from that stage. The shelter provided to this larval stage was found effective and reduced the cannibalism considerably. The structural morphology of each larval stage (Fig. 2) during the larval development is described in Table. 3.

Table 3: Identification characters of different larval stages

Larval stage	Identification characters
Zoea I	Sessile eyes Abdomen 5 segmented 3 pairs of setae between telson of furca (Figs. 2A & B)
Zoea II	Eyes stalked A pair of small setae between inner pair of setae of caudal furca (Figs 2C & D)
Zoea III	Abdomen 6 segmented (Fig. 2E)
Zoea IV	Pleopods buds on segments 2-6 Lateral spines on segments 3-5 more elongated (Fig. 2F)
Zoea V	Pleopods on abdominal segments well developed, its exopodite with setae (Fig. 2G)
Megalopa	Typical portunid megalopa Ist periopod modified into cheliped (Fig. 2H)
Crab lnstar	Margin of carapace serrated 9 anterolateral spines (Fig. 2I)

4. Discussion

The form of larval rearing seed production will be determined following works on temperature stability, water management regimes, stocking density, examination of survival rates following provision of substrates for settlement, enhancement of *Artemia* using supplements, identification of larval diseases and strategies to manage them. The antibiotics such as Ciprofloxacin and Oxytetracyclin were used in the present investigation. Preliminary antibiotic studies included measuring effects of penicillin-G, streptomycin and polymyxin-B, individually and in combination, on survival and development of the crab larvae of *S. serrata*.

The incubation period was noted as 12 days for *S. tranquebarica* in the present study. It has been reported 7 to 14 days as incubation period at 25 to 30 ºC in *S. serrata*. It was also inferred that the temperature is influencing the incubation period in crabs. It observed two or three times longer incubation period at 18 to 20 ^{0}C than at 26 to 28 ^{0}C in the same species *S. serrata*.

The water salinity maintained at 35 ppt due to spawning and embryogenesis and hatching of eggs generally takes place in coastal regions because the first zoeal stage of the species is unsuited to estuarine conditions. Here the larvae in this present experiment were observed to be very active and they appear to like the high salinity of the culture water (35ppt). The result of this study confirms that survival and development of the zoeal stages require high salinity water in the ocean. So far, there has been no report what so ever on the exact location where metamorphosis to megalopa takes place in the natural environment. However the megalopa moves the near shore, moult to crablet stage and recruit in the mangroves to feed.

The present study indicates that the most suitable range of

temperature for crab larvae is 30-32.5 ^{0}C. The fluctuations in water temperature resulted in a sudden drop in the larval survival during the first 3 days of culture. The study noticed that the temperature shock causing larval stress and mortality has been surmised when unintentional temperature of 5 ^{0}C fluctuations due to equipment failure lead to abnormally high mortality rates.

The collapse of the microalgae also contributed to the fouling of the culture water. The earlier study reported that the presence of *Chlorella* sp. in the culture medium of *S. serrata* zoeae had a beneficial effect on the production of megalopas in terms of survival and suggested that the effectiveness of the algal supplementation was the release of oxygen and the removal of metabolites. The presence of phytoplankton was reported to have a beneficial effect in larval fish cultures in term of survival.

The five zoeal stages were completed 16 days after hatching. Duration of each zoea stage was 3 to 4 days and 6 days for megalopa. Earlier study reported 3 to 4 days duration for each of the 5 zoeal stages and 8 to 11 days for megalopa. The megalopa stage lasted for 6 days before metamorphosis to the first crab instar. Thus, it took totally 23 days from first zoea to first crab instar stage. Here the megalopa attained 16 days from the hatching day and the crab instar came the 22nd days of the culture.

The survival rate from the first zoeae to crab instar was 6.9% only. Several authors reported maximum mortality in the first zoeal stage of mud crab *S. serrata*. Here also the first zoeal mortality is nearly 37%. Pioneer studies reported that the survival rate differed from 2.5% to 26.9% in *S. serrata* and the survival rate from the fifth zoeal stage to the megalopal stage was clearly lower than that from the first to fifth zoeal stages in different trials, whereas other reports noticed that the survival from zoeae I to 3-4 days old megalopa was 3-7%. The most encouraging results were reported in Vietnam where hatchery of *S. paramamosain* has been consistently achieved with survival for zoeae I to crab instar of 10-15%. Survival from zoeae I to megalopa with no antibiotics was poor at 1.3 to 1.5% but much better at 30% with oxytetracycline and the survival from megalopa to crab instar is very good at 70-80%.

The major food items of crab larvae are *Brachionus* sp. and *Artemia* nauplii. Live and moving animal food preferred over plant food by crabs. Larval survival and developmental improve with increasing density of *Brachionus* and *Artemia* nauplii in small scale culture. For mud crabs, low survival rates were obtained crabs fed rotifers only until the zoeae V stage.

Lipids are important as sources of fatty acids for metabolic energy, and to maintain structural integrity of cellular membranes. Fatty acids, specifically n-3 highly unsaturated fatty acids (HUFA) such as 20:5n-3 (eicosapentaenoic acid; EPA) and 22:6n-3 (docoshexanoic acid; DHA) are essential components in the diet of crustaceans. Enrichment live feeds through the enriched diets (bioencapsulates) to increase the nutritional efficiency of the live feeds such as rotifer *Brachionus* sp. and *Artemia* nauplii have significantly contributed to the larval survival and quality, in marine fish and crustacean hatcheries all over the world.

The enrichment of *Artemia* nauplii with n-3 HUFA also affected carapace width and survival of mud crab larvae. The mud crab larvae definitely need EPA for survival, while DHA was required for carapace growth. *Artemia* enriched with n-3 HUFA from the zoeae III stage in order to improve survival rates. Likewise the suitable feeding schedule for different larval stages of crab when fed rotifers and enriched *Artemia* at the zoeae II to megalopal stages. The larval crabs were given live food containing n-3 HUFA, especially *Artemia*, the moulting rate of the megalopa and the intermoult period were much improved. Therefore, the enriched *Artemia* appears to be a better vehicle for fatty acid delivery to the crab larvae. These results suggests that the crab larvae should be fed *Artemia* containing n-3 HUFA from the zoeae II to obtain high survival rate at the first crab stage.

The thinning out of the population of late zoeal stages were made to reduce the cannibalism, the aggregating behaviour of the larvae. Stocking density of megalopa in tank allowed 2 individuals per liter. Blacknets and cut PVC pipes distributed as shelters when megalopa becomes instars. The larvae have metamorphosed to the megalopa stage, additional substrate suspended within the culture tank to provide a surface for settlement of megalopa and crab instars. The megalopa was constructed of flyscreen mesh, which suspended vertically along a length of twine weighted at one end and with a float at the other. As provided the shelter to megalopa the cannibalistic feature somewhat controlled, because they are attached with the shelter.

5. Conclusion

Three predictions relevant to the aquaculture of mud crab *S. tranquebarica* are a judicious use of eyestalk ablation should provide year-round supplies of berried females which would be required to supply even very large aquaculture ventures, high survival rates with rapid growth, from I zoeae to first crabs are possible using the rearing techniques and the mass rearing technique used here could easily be adapted to run on a large scale with relatively small capital expenditure. The technique is not labour intensive and should be relatively inexpensive to maintain.

The survival rate was less from zoea larvae to first crab instar stage in the present trial, it is hoped that the further successful rearing will result in the development of a suitable hatchery techniques for commercial seed production for the mud crab *S. tranquebarica* in the near future. The research should address the main biological limitations, namely low survival of larvae and early crab stages, the problem of cannibalism in all stages, and the need for cost effective feeds. The suitable and farming systems should be developed, while protecting natural stocks from our harvesting to assist culture which helps the market development including value added products.

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