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Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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BREEDING AND LARVAL RAERING OF MUD CRAB

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Introduction

Aquaculture of mud crabs (*Scylla* spp.) dates back to 1890 in Guangdong, China (Shan and Lain 1994). Since 1970s onwards, a steady interest was noticed in mud crabs culture in many tropical Asian countries due to its importantance in the industry and advantages viz. (1) uncomplicated technology, (2) abandoned shrimp ponds can be converted, (3) international markets, (4) native species to many tropical Asian countries, (5) easy transportation, potential for rural as well as industrialized aquaculture, (6) individual animals are valued in contrast to penaeid shrimps and (7) resilience of resources. However, crab aquaculture is severely constrained by the unperfected hatchery technology. This article summarizes various aspects of mud crab biology and hatchery technology.

Biology of Mud crabs

Taxonomy

Taxonomy of genus *Scylla* has been considerably confused. Estampador (1949) recognized three species and one variety. His classification was mainly based on coloration, morphological characters and behavior. Although many authors accepted Estampodaor's classification, Stephenson and Campel (1960) concluded that there was insufficient evidence for separation of species beyond mono-specific term *Scylla serrata*. Recently, the taxonomy of genus *Scylla* revised and confirmed the existence of four species (Table 1; Figure 1) based on morphometric analysis, allozyme electrophoresis and mitochondrial sequences. However, insufficient evidences from Indian mud crab species (Kathirvel, Personnel communication) to support the classification suggested by Keenan *et al.* (1998), in this article, the classification suggested by Kathirvel and Sreenivasagam (1991) is used. The following Table provides the status of nomenclature of mud crab species.

Life History

Mud crab's natural history can be considered as catadromous: adult spawn in the open ocean but young migrate inshore. The various stages of development are shown in Fig. 2.

Biology of Crab Reproduction

The sex of crabs can easily be determined by external features. Male crabs are characterized by inverted 'T' Shaped abdomen, whereas in females the abdomen is semicircular. In addition the male has relatively larger chelae, and a general trimness for body contour than females. Males have two pleopods that modified as copulatory organs on the first and second abdominal segments. In the case of females first four abdominal segments carry pleopods, which are biramous and possess setae for attachments of eggs for brooding. The female reproductive system comprises a pair of ovaries, a pair of spermatheca (= seminal receptacle) and a pair of vagina. The ovary is 'H' shaped and located dorsally just beneath the carapace. The horns of ovary extends anterolaterally from either side of the gastric mill and dorsal to the hepatopancreas. Two posterior horns, which lie ventral to the heart, extend posteriorly on either side of the intestine on either side of the intestine. The seminal receptacle arises from mid lateral border of the posterior horns. Each antennal receptacle leads into a narrow vaginal tube which further open outside through small circular gonopore situated ventrally. Eggs are produced in the paired ovaries. Sperms produced in the testes opens into coiled tubes (vas deferens) that package mature sperms into gelatinous bundles (spermatophore) for transfer to females. In natural conditions mud crabs attain sexual maturity at between 18 and 24 months.

Table 1: Taxonomic status of mud crabs *Scylla* spp

Kathirvel and Srinivasagam (1992)	Estampador (1949)	Keenan et at (1998)
Scylla tranquebarica	*Scylla oceanica*	*Scylla serrata*
	Scylla tranquebarica	*Scylla tranquebarica*
Scylla serrata	*Scylla serrata*	*Scylla olivacea*
Not reported	*Scylla serrata var. paramamosain*	*Scylla paramamosain*

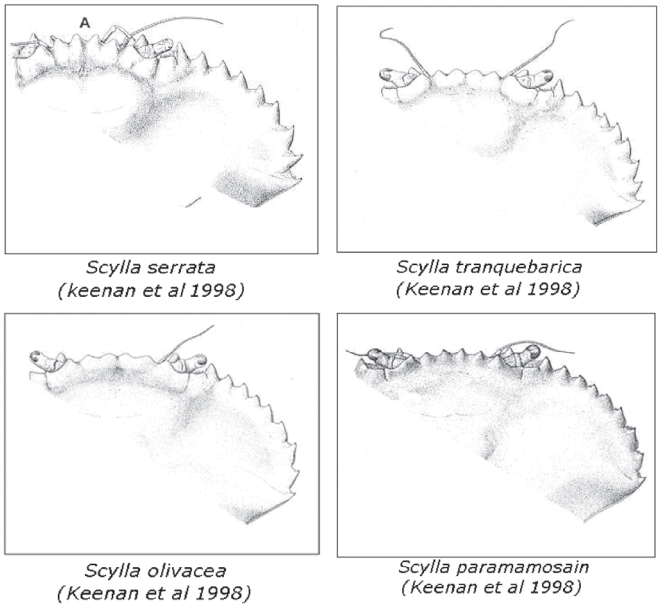

Fig. 1. Taxonomic features of different mud crab species (After Keenan *et al* .1998)

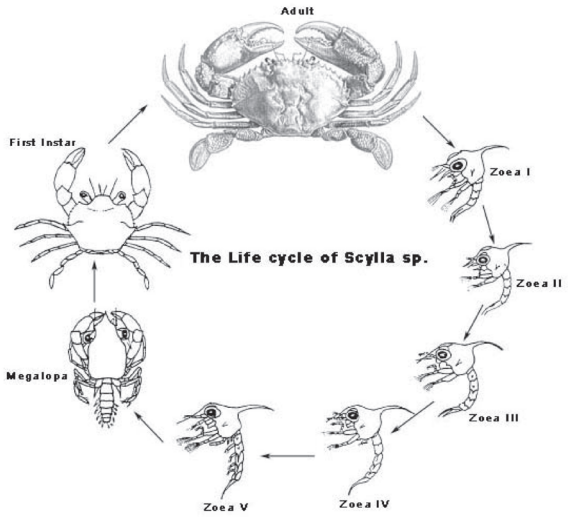

Figure 2 Generalized life cycle of *Scylla* spp.

Mating takes place in the estuarine environment, after which female crabs migrate to the sea where spawning takes place (Arriola 1940, Ong 1966). Berried *S. serrata* females have been caught in trawl nets up to 80 km from the shore in Australia (Poovichiranon 1992, Hill 1994). Spawning appears to occur throughout the year with some seasonal peaks (Heasman et al 1985, Quinn and Kojis 1987). These peaks seem to be related to seasonal rainfall for tropical populations, while in temperate regions reproduction is more strongly related to temperature, with a peak in spawning activity in the summer months (Heasman *et al.* 1985). *S. serrata* is highly fecund with up to 8.36 million eggs per female (Mann *et al.* 1999). The zoeal larvae develop and remain in the open ocean until they reach the megalopa stage, after which they migrate back into the estuarine environment (Keenan 1999). Little is known about the oceanic phases of the life cycle.

Mating

The mature female releases a chemical attractant (pheromone) in to the water, which attract males. The successful male picks up the female and carries her around for several days until she molts. Copulation can occur only when females are in soft shell condition. Male deposits Spermatophore inside the female storage sac (spermatheca) by using male first pleopods. The Spermatophore can remain viable, until fertilization takes place, for weeks or even months. When the eggs complete vitellogenesis they are passed down and fertilized by stored sperms, and extruded onto pleopods. The eggs adhere to the pleopod hairs and female is said to be berry or ovigerous.

Ovarian development

The classification of ovarian maturation provides a guide for broodstock management in hatchery facilities. For hatchery operation, animals with ripe or late maturing ovaries should be selected to minimize the use of resources such as time and money. The colour, size and texture of ovary of mud crabs are closely related to its cellular development. Based on external morphology and light microscopy, Quinitio *et al*., 2007 classified the ovarian development stages of *S. serrata* into five (Table 2).

Incubation

Mud crabs brood their eggs, as all other pleocemata. During the incubation period, females stop feeding and therefore animals generally avoid 'baited lift nets and 'traps'. Egg incubation period generally varies from 7 to 14 days, but the duration of incubation is greatly influenced by the rearing water temperature. Egg incubation period is tested at different temperature (20 to 30 ºC) and found that incubation period decreased exponentially with increasing temperature.

Larval stages

There are five zoeal stages passing through five molts to reach the megalopa stage. At a salinity of 31 ppt development from zoea 1 to megalopa requires 16-18 days; each zoeal stage takes minimum period of 3-4 days before it molts in to the next stage. The megalopa takes 11-12 days before it molts into the first crab stage; at lower salinity in the range of 21-27 ppt this period is reduced to 7-8 days. The faster rate of megalopa in lower salinity indicates that the megalopa in nature move shoreward into brackish water.

Description of Zoea (From Ong 1966)

The zoea are of typical brachyuran type with long rostral and dorsal spines. The abdomen in all stages have has lateral knobs on second and third pleomeres. Identifying characteristics of different zoeal stage of S. tranquebarica is given tin the Table 3.

First zoea: Body length 1.15 mm; eyes sessile. Antenna unsegmented and bears short setae apically. Mandible is broad with two large teeth and serrated edges. Maxillule with two segmented endopodite; maxilla with un-segmented endopodite; the first and second maxillipeds bears four natatory setae. The abdomen is made up of five pleomeres. The telson bears a pair of long dorsolateral spines.

Table 2: Characteristics of ovarian stages of *Scylla tranquebarica* (=*S. serrata*); adopted from Quinitio *et al*., 2007

Ovarianstage	Externaldescription	Histologicaldescription
Immature	Ovary thread-like; sometimes difficult to recognize fromother tissues; transparent to translucent	Oogonia, oocytes and follicle cells apparent in theovarian lobe; follicle cells found around the periphery of the lobes and an area among groups of oogonia and oocytes; oogonial nuclei in different stages of mitosis and meiosis Thin ovary; translucent to off white Lobes clearly separated by connective tissues; follicle cells with variable shapes gradually enclose the oocytes; more advance oocytes found in the periphery
Earlymaturing	Ovary increases in size; yellow	Early maturing Oocyte diameter increases in size; small yolk globules start to appear in bigger oocytes; follicle cells around theoocytes (Fig. 2)
Late maturing	Massive increase in ovarian size; lobules apparent; light orange	Yolk globules occur in the cytoplasm with larger globular inclusions toward periphery; follicle cells hardly recognizable; a few small oocytes visible
Fullymature	Lobules swollen with large ova; ovary occupies availablespace in body cavity; orange to dark orange	Large yolk globules in the entire cytoplasm; nucleus small; follicle cells hardly see
Spent	Ovary similar to Stage 2 or smaller than Stage 3;	Yellow to light orange and sometimes with dark orange on some parts of ovary; flaccid Oocytes of various stages present; yolky oocytes not expelled still recognizable; atretic oocytes evident

Second Zoea: Body length 1.51 mm; eyes stocked; Exopodite of both maxilliped bear six natatory setae. Telson has a pair of small setae at the inner margin of furca.

Third Zoea: Body length 1.9; Larger antennule than second zoea; antenna has developed a small bud. Exopodite of second maxilliped with 9 setae.

Fourth Zoea: Body length 2.4 mm; Antennule bears aesthets in a terminal group and a subterminal group; Flagellum of antenna elongated; first maxilliped bears 10 natatory setae; second maxilliped bears 10 natatory setae and one or two short setae. Rudiments of third maxilliped appears.Abdomen has bud on pleormeres 2-6. The telson grows additional setae between the innermost pair.

Fifth Zoea: Body length 3.43 mm; first maxilliped bears 11 long setae; second maxilliped has 12 setae. All the pereiopods are elongated and shows the signs of segmentation. Pleopod buds are well developed. Five pairs of setae on the telson furca.

Megalopa: Single megalopa stage similar to other portunids; carapace length 2.18 mm; carapace width 1.52 abdominal length 1.87. The abdomen has five pair of pleopods.

Table 3: Summary of different zoeal characteristics of *Scylla tranquebarica* (Ong 1967)

Stages	Size (mm)	Eyes	Setae (2 nd maxilliped)	Appendages (thoracic)	Setae (middle furca)
Zoea 1	1.1	Sessile	4	Nil	3 pairs
Zoea 2	1.5	Stalked	6	Nil	4 pairs
Zoea 3	1.9	Stalked	9	Starts developing	4 pairs
Zoea 4	2.4	Stalked	10	Large	4 pairs+middle one
Zoea 5	3.4	Stalked	12	Large	5 pairs

Hatchery production of mud crabs

This section is dealt with three subsections: Facility, Broodstock management and Larval rearing. For the development of mud crab hatchery, most of the shrimp hatchery can be converted into crab hatchery.

Facilities

Broodstock and larval rearing tanks: Tanks may be made of concrete, fibreglas, or wood lined with rubberized canvas. These can be either circular, oval, or rectangular. However, rounded corners are preferable due to more effective water circulation. Tank capacity may vary from 1-10 mt for broodstock and 1-5 mt for larval rearing tanks.

Algal culture tanks: The green phytoplankton, *Chlorella* is needed for rotifer, *Brachionus* Algal tanks must be shallow to allow enough light penetration. Barchionus are cultured in 5-10 mt tanks.

Spawning tanks: It is advantageous to have smaller round tanks with volumes ranging from 300 to 500 L tanks where berried tanks are held and allowed to hatch their eggs.

Artemia hatching tanks: Nauplii of Artemia or brine shrimp is protein rich organisms give to larvae starting second or third zoea. Tank capacity of Artemia varies from 30 to 50 L.

Reservoir: Storage tanks are necessary for chlorination and holding of filtered and treated water for daily use. An elevated storage tank that can distribute seawater to other tanks by gravity is advantages.

Seawater system: Seawater may be pumped from the sea or sump pit. Water is passed through sand filter, which is usually elevated prior to storage.

Other equipments and accessories: Other equipments and accessory such as refrigerator, weighing balances, Refractometer, pH meter and drainers etc are equally important in hatchery operations.

Broodstock management

Females of *S. tranquebarica* can be obtained form fishers or landing centres. Male crabs are not required for hatchery operations as almost all matured crabs in wild would have mated (Ezilarassy and Subramoniam,). Animals range in size above 500 g (S. tranquebarica) and above 300 g (S. *serrata*) should be selected for larval production. Further, females were identified as being matured by their wide, dark, U- shaped abdomen fringed with setae. Immature females were typically characterized by having an abdomen resembling that of male with slightly convex side and without setae. Maturity can be assessed by observing through gap at the junction of carapace and abdomen (Fig.3).

Transport: Crabs for transport are tied with twine to render the claws immobile. They can be kept out of water in cardboard cartons for two days. The bottom and sides of containers are lined with damp mangrove leaves, wooden shavings, or damp sackings. As dehydration affects survival of crabs, it should not be subjected to drying winds during transport. Likewise exposure of direct sunlight for long period could lower survival.

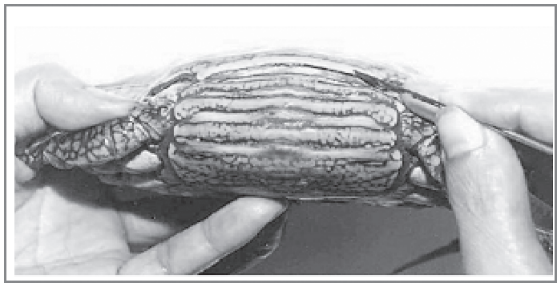

Fig. 3 In vivo evaluation of ovarian stage of *Scylla tanquebarica*

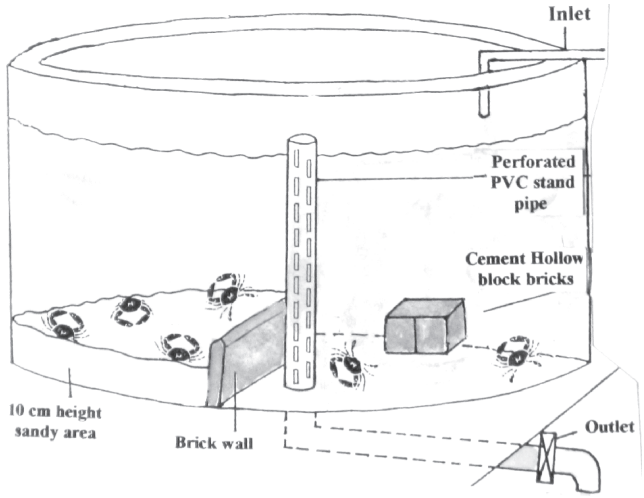

Fig. 3 .Broodstock tank for *Scylla* spp. Half of the portion is provided with a sandy substratum

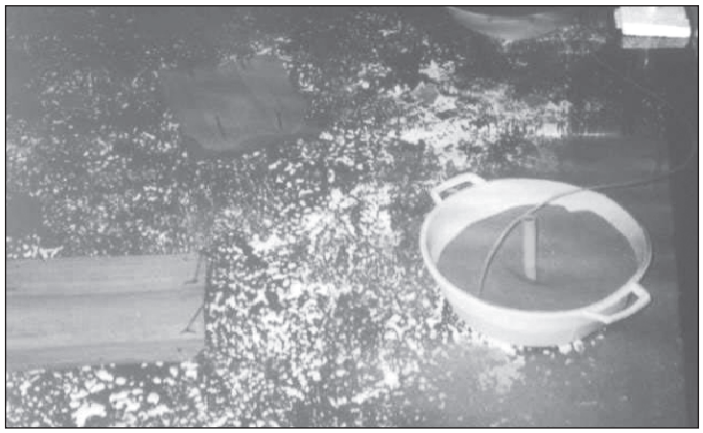

Fig. 4. Broodstock tank of *Scylla* spp sand filled basin are provided for spawning (after Churchil,2003)

Disinfection: Under culture conditions, the ability to control disease is vital because the potential for pathogen proliferation increases with the density of cultured animals. Formalin has extensively been used in crustacean culture for disinfecting and disease prevention. Therefore, newly caught animals should be disinfected with formalin to reduce the number of symbionts and parasites. Formalin doses and exposure time varies widely between studies with doses ranging from 100 ppm for 1 h to 50 ppm for 20 min. (see the box 1 for calculation of formalin)

Eyestalk ablation: As the occurrence of berried crabs in nature is rare, it is essential to develop ovigerous crabs in captivity. Eyestalks are the sites of gonad inhibiting hormones and therefore the removal of eyestalks accelerate the gonad development and spawning. One of the eyestalk is removed. Intact animals can also be used as broodstock, however, the time to get ovigerous crab extended according to the ovarian stages of the animal. Reproductive performance of intact animals are significantly greater in intact animals (Millanema and Quiniito 2000. Table 4).

A sandy substratum should be provided in the spawning tank. Female crabs kept in a tank that has bare floor may often drop their eggs during spawning because eggs fail to remain securely attached to their pleopods. Half of the broodstock tank can be provided with 10 cm sand layer and another half can be bare floor for feeding purpose (Fig 3). Alternatively sand filled trays can be provided (Fig 4). Crabs can be stocked at the rate of one animal per 1 mt or one per sq.m.

Box 1

To calculate how much formalin should be added to provide a concentration of 100 ppm
Formula:
$\mathbf{C_1V_1 = C_2V_2}$
C_1 = concentration of the formalin in the bottle
C_2 = concentration of formalin needed in the tank (100 ppm)
V_1 = Volume of the formalin needed from the bottle
V_2 = Volume of water in tank
C_1 = 40% of formaldehyde [i. e. 40g per100ml=400 g per1000ml=400 000 mg per 1 L =400 000 mg per 1000 g=400 000 mg per 1,000 000 mg OR 400 000 parts per million (ppm)]
C_2 =100 ppm
V_1 =?
V_2 = 500 L or 500 000 ml
Substitute the values
400 000 ppm X V_1 =100 ppm X 500 000 ml
V_1 = 100 ppm X 500 000 ml

$$= \frac{400\ 000\ ppm}{125\ ml}$$

Treating larvae with formalin

The prawn larvae in LRTs should be treated every second day with 30 ppm formalin for up to 1hour, to reduce the incidence of fungus and protozoan infestations.Formalin may be sold almost pure (100%) or as a 40% solution. Read the label on the bottle to determine the concentration. 100% formalin represents 1,000,000 ppm 40% represents 400,000 ppm. To calculate how much formalin to add to an LRT to provide a concentration of 30 ppm, use the formula:

C1 V1 = C2 V2

where: C1 is the concentration of formalin in the bottle

Table 4 .Reproductive performance of ablated and intact Scylla serrata (=S. tranquebarica) (Millamena and Quinitio 2000)

Variables	Ablated	Intact
No viable spawning	8(40%)	12(60%)
Mean eggs per BW	4437	5124
Mean eggs fertilization rate (%)	58	80
Total No of Zoea (million)	15.67	20.49
Broodstock survival (%)	42	83

Feeds and Feeding: Broodstocks are fed with natural feeds such as molluscs, polychaete at the rate of 10% body weight. Un eaten feeds should be removed daily by siphoning the tank prior cleaning

Water management: Seawater with 28-35 ppt is used for crab broodstock, and water in the tank is changed daily. Sand substrate should be cleaned twice week.

Spawning and hatching: Crabs should be checked daily to know whether spawning has occurred or not. Once crabs spawned they are placed in the basin containing 150 ppm formalin for 30 min., and stocked individually in 300-500 L spawning tanks for subsequent spawning.

Larval rearing operations

Preparation of larval tanks for stocking: The tanks should be disinfected with 200 ppm chlorine water for 8-10 h, and scrubbed with a mixture of 200 ppm Chlorine and 5% detergent by using sponge pads. Then the tanks are thoroughly rinsed with fresh water and drained at least for 24 h. Just before filling the tanks it should be rinsed with fresh water. Before stocking zoea, algae (*Chlorella*) should be added at the rate of 50, 000 cells/ml. The tank water should be aerated mildly. Micro algae do not provide any nutritional benefits; it may enhance the water quality.

Acclimation and stocking: The larvae are estimated in the spawning tank directly. Aeration should be taken out before collecting the larvae, and the waste products settled down at the bottom should be siphoned out. Only active larvae are stoked in the larval rearing tank at 10 – 50 individual per litre. Active larvae are photo tactic; hence they swim up to the surface. The zoea received from the hatching tanks should be acclimatized by adding the larval tank water to the acclimatization basin. The acclimatized zoea can be released slowly in to the tank in small quantities.

Feeding: The most critical component of the mass larval rearing of aqua cultured species is the standardization of feeding regime. The feeding regime for mass rearing of *Scylla* has yet to be standardized. Nutrition has been suggested as a possible cause of mass mortalities experienced during the mud crab larval rearing. Absence of an optimal feeding regime may be the fore most reason for the failure of hatchery production of mud crab larvae. Many experiments were conducted in India and elsewhere using a variety of live feed organisms such as veliger of oysters, copepods, rotifers, Artemia nauplii and micro algae. Trials were conducted using these live feed organisms individually or in combination. Heasman and Fielder (1983) reported their highest survival of 26% form zoea to first crab instar when larvae fed solely on *Artemia* nauplii whereas Marichamy and Rajapakyam (1991) reported a maximum survival from first zoea to first crab instar when they used a combination of rotifer and *Artemia* nauplii. In India Anil and Suseelan (1999) conducted experiments on feeding of *S. tranquebarica* (as *S. oceanica*). They used 3 feed combinations: 1) frozen *Artemia* nauplii, rotifer and micro encapsulated feed 2) Frozen *Artemia* nauplii, rotifer and *Chlorella* and 3) *Artemia* nauplii in suspension in addition to the fresh *Artemia* nauplii (15 -20 individual/ ml), rotifer (Brachionus 20 individual/ ml) with antibacterial compound prefuran. The best survival obtained for the third combination (23%). Although *Artemia* alone can be used and successful larval production is achieved by some authors, most of the authors reported with convincing evidence that rotifer is an indispensable component of mud crab larval rearing. Rotifers are significantly smaller (0.5 µg and 45 -200 µm) than Artemia nauplii (2.7 µg and 428 -517 µm) and less

vigorous as well. Measurements of feeding appendages of *S. serrata* larval stages suggest that the optimum food size for Z1 larvae ranges from 100 to 200 µm. Further, early post larvae show a clear preference to slow moving rotifers. There fore Z1 to Z2 should be provided with rotifer. Qunintio et al 2001 suggested that rotifer, should be fed through out the larval rearing cycle. The maintenance of rotifer culture for a long period requires enormous resources, therefore, use of rotifer in larval rearing of mud crab should be limited to the early zoeal stages. Experiments conducted in Australia (Rusoe et al 2004) indicate that although rotifers are essential for the acceptable larval survival, it can be removed from the feeding regime as early as third zoea. Artemia should be provided from second day of second zoeal stage. Production of phytoplankton and rotifers should be synchronized with the hatching operation so that these are available as soon as the eggs hatched to zoea. Suggested feeding regime is given in the Table 5.

Table 5 Suggested Larval-culture sequences and feeding and water management for mud crab hatchery operation

Day	Tank	Volume (L)	Stage	Chlorella Cells/ml	Rotifer	Artemia Ind./ml	Water(%) Ind. /ml
0	ST	300	Z1	-	-	-	-
1	LRT	500	Z1	50000	10-15	-	-
2	LRT	500	Z1	50000	10-15	-	-
3	LRT	500	Z1	50000	10-15	-	30
4	LRT	500	Z1	50000	10-15	-	30
5	LRT	500	Z2	50000	10-15	0.5-5	30
6	LRT	500	Z2	50000	10-15	0.5-5	30
7	LRT	500	Z2	50000	10-15	0.5-5	50-80
8	LRT	500	Z3	50000	-	0.5-5	50-80
9	LRT	500	Z3	50000	-	0.5-5	50-80
10	LRT	500	Z3	50000	-	0.5-5	50-80
11	LRT	500	Z3	50000	-	0.5-5	50-80
12	LRT	500	Z4	50000	-	0.5-5	50-80
13	LRT	500	Z4	50000	-	0.5-5	50-80
14	LRT	500	Z4	50000	-	0.5-5	50-80
15	LRT	500	Z4	50000	-	0.5-5	50-80
16	LRT	500	Z5	50000	-	0.5-5	50-80
17	LRT	500	Z5	50000	-	0.5-5	50-80
18	LRT	500	Z5	50000	-	0.5-5	50-80
19	LRT	500	M	50000	-	0.5-5	50-80
20	LRT	500	M	50000	-	0.5-5	50-80
21	LRT	500	M	50000	-	0.5-5	50-80

ST: Spawning tank; LRT: larval rearing tank; Z: Zoea;M: megalopa

Water management: Water for rearing is treated with 10-20 ppm hypochlorite and neutralized by strong aeration until chlorine residues have evaporated by addition of sodium thiosulfate. Water should be treated with 5-10 ppm EDTA to chelate heavy metals. For better results water is allowed to stand for three days after neutralization before this is used for culture. Water is replaced daily at 50-80% of the total volume starting day 2 or 3. Dead larvae and uneaten feeds are siphoned out prior to water exchange. Salinity of water may be reduced 32 to 26 ppt starting zoea 4 until megalopa. In some cases rearing water is not changed but the volume is gradually increased as larvae grow.

Once the megalopa is reached and water is changed almost daily from 30 to 50% of total volume. A few days prior to crab stage, net substrate and PVC cuttings are placed all over the tank bottom for attachment and refuge.

Nursery: Megalopa may be reared until the crab stage in the same larval rearing tanks or may be transferred to nursery tank and reared until 3-5 g prior to pond stocking. For the crab stage, 30-50% of water exchange is done daily.

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