CAPTIVE BROODSTOCK DEVELOPMENT, INDUCED BREEDING AND LARVAL STAGES OF MUD CRABS (SCYLLA spp.)

CAPTIVE BROODSTOCK SYSTEM FOR THE PRODUCTION OF BERRIED FEMALE MUD CRABS TRANQUEBARICA INDUCTION OF MATURITY MATING BEHAVIOUR Procurer adult male and female crabs from wild A. Pre-mating embrace Size of broodstock holding tanks Females subjected to unilateral B. Copulatory moulting in female REFUGE SYSTEM WATER MANAGEMENT centimeter height sand bed 32-35 ppt saline water FEEDING C. Mating process Clam and squid meats twice a day @ 10 % of stocked biomass

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केन्द्रीय खारापानी जलजन्तु पालन संस्थान

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CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE

(Indian Council of Agricultural Research)
101-B, MAHALINGAPURAM MAIN ROAD, CHENNAI - 600 034.



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S. SRINIVASAGAM, M. KATHIRVEL AND S. KULASEKARAPANDIAN

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PREFACE

Among the edible brackishwater crustaceans of India, mud crabs belonging to Scylla spp. command an unique status by virtue of their delicacy and greater demand for consumption in local and export markets. Though the mud crabs are marine dwellers, they immigrate into brackishwater systems during their postlarval stages, grow fast, attain maturity and form a lucrative fishery in estuaries, backwaters and coastal lagoons. They emigrate into inshore seas for breeding. Ever since the export trade to foreign countries has started, mud crabs are in the lime light, resulting in their increased exploitation, especially from brackishwater regions.

As an alternative crop to the disease-ravaged shrimp farming sector, culture of mud crabs has been carried out on a small scale by the shrimp farmers in the coastal states, namely, West Bengal, Orissa, Andhra Pradesh, Tamil Nadu and Kerala. At present, the stocking materials for culture operation is obtained from wild. Two types of farming is carried out, one by growing sub-adult and adult crabs to marketable size and the other by rearing 'water crabs' to gain weight in suitable fenced earthen ponds.

The limited wild resource, greater exploitation and export of adult mud crabs are likely to affect the overall availability of mud crabs in the coming years. Appropriate technologies for mud crab hatchery seed production are the need of the hour to enhance the natural stock, on which, both the capture and culture fisheries are dependent. For the hatchery seed production trials, the availability of egg-bearing or berried females is a pre-requisite. Since the availability berried crabs in the wild is erratic, the other alternative source to obtain such berried crabs is through the captive broodstock development.

In this context, the Central Institute of Brackishwater Aquaculture has taken up R & D programmes in the captive broodstock development of two species of mud crabs, namely, Scylla tranquebarica (larger species) and S. serratta (smaller species). Repeated trials were carried out for holding the

adult crabs in suitable tanks, induction of maturity in brood crabs, water and feed management for broodstock and production of berried crabs. The technology package developed for the production of berried fermale crabs is presented in this Bulletin. It is hoped that the information with appropriate figures presented in this Bulletin would serve as a guide to those scientists, administrators, entrepreneurs and farmers interested in mud crab culture in general and hatchery seed production in particular.

I appreciate my collegues, namely S/Shri M. Kathirvel, S. Srinivasagam and Dr. S. Kulasekarapandian, Senior Scientists for their sincere efforts in developing a suitable technology for the production of berried female mud crabs under controlled conditions and the preparation of this Bulletin.

Chennai-34 2-3-2000 DR. G. R. M. RAO DIRECTOR

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Mud crabs, namely, Scylla tranquebarica and Scylla serrata are highly considered for their delicious meat, nutritional and medicinal values and export trade. The larger species S. tranquebarica grows to a maximum size of 2.4 kg and the smaller species S. serrata to 0.7 kg. Though mud crabs are marine in origin, they are euryhaline and migrate during their postlarval/juvenile stages into brackishwater bodies such as estuaries, backwaters and coastal lagoons, where they grow and attain adulthood. In India, mud crabs are utilised for local consumption and export trade. Since the export of live mud crabs began in 1987-88, they have been considered as a valuable foreign exchange earner. About 870 tonnes of mud crabs valued at Rs 7.5 crores were exported during 1987-98. At present, mud crabs collected from the wild are being either cultured or fattened on a small scale in the maritime states like Andhra Pradesh, Kerala, Orissa, Tamil Nadu and West Bengal, as an additional source for the live crab export trade and also as a means of diversification in the brackishwater aquacultural practices. As the mud crab seed resources from the wild are limited, future expansion of mud crab farming will have to depend upon hatchery produced seed, which in turn depends on the availability of berried or egg-bearing females. As part of the R & D programmes in brackishwater aquaculture, the Central Institute of Brackishwater Aquaculture has undertaken research on controlled reproduction of two species of mud crabs (S. tranquebarica and S. serrata) and evolved a technology package for captive broodstock development and production of berried females which is presented in this Bulletin.



REPRODUCTIVE BIOLOGY OF MUD CRABS

2.1. Candidate species

2.1.1. Larger species - Scylla tranquebarica (Plate 1A)

The presence of polygonal markings on all limbs and two spines on the outer margin of the wrist of chelipeds are the important features of S. tranquebarica.

2.1.2. Smaller species - Scylla serrata (Plate 1B)

The absence of polygonal markings on limbs and presence of one sharp or blunt spine on the outer margin of wrist of chelipeds are the significant external features for identification of *S. serrata*.

2.2. Identification of sexes

Sexes can be morphologically distinguished based on the shape of the abdominal flap. Though the shape of this flap is similar in both immature and mature male (Fig. 1A), it is different in mature and immature female. In mature female the shape of abdominal flap is half-round (Fig. 1B), while in immature female it is broad and triangular (Fig. 1C).

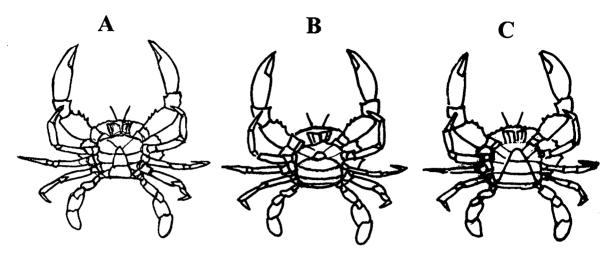


Fig. 1. Shape of abdominal flap. A. Immature/mature male; B. Mature female; C. Immature female.

2.3. Size at maturity

The size range at first maturity in males and femals of S. tranquebarica and S. serrata is presented in Table 1.

Table 1. Size range at first maturity in S. tranquebarica and S. serrata

Species	Sex	Size-range at first maturity (Carapace width in mm)
S. tranquebarica	Male Female	125-133 129-135
S. serrata	Male Female	80-89 85-96

2.4. Male reproductive system

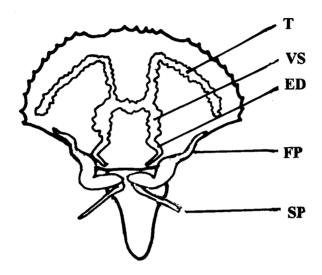


Fig. 2. Male reproductive system. T-Testis; VS-Vas Deferens; ED-Ejaculatory duct; FP-First pair of pleopods; SP-Second pair of pleopods.

The male reproductive system consists of a pair of testes, a pair of vas deferentia and a pair of ejaculatory ducts internally and a pair of pleopods externally as accessory reproductive organs, present on the inner side of the abdominal flap (Fig. 2). The ejaculatory ducts open into a small genital papilla. The ejaculate consists of non-motile sperms and seminal plasma. Sperms are enclosed in numerous small spermatophores and stored in the anterior vas deferens, while the seminal plasma is produced and stored in the posterior vas deferens. The first pleopod is made up of two segments, the basal one is broad, rectangular and flattened and positioned close to the sternal wall and the terminal one is long tube-like and tapering towards the tip, which is actually inserted into the seminal receptacle of the female during copulation (Plate 3A). The second pleopod helps in passing the spermatophores from the ejaculatory ducts into the funnel-like portion of the first pleopod.

2.4.1. Maturity stages of testis

The maturity stages of testis are described below:

Immature (Plate 2A): Transparent/creamy in colour; occupying less

than 1/6th of body cavity; without a prominent

vas deferens.

Maturing (Plate 2B): Creamy white; occupying 1/4th of body cavity.

Mature (Plate 2C): Milky white with thick vas deferens; occupying

full body cavity.

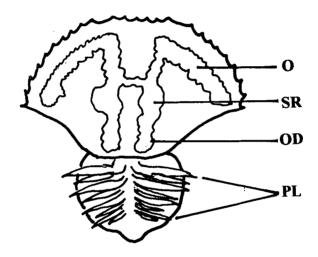


Fig. 3. Female reproductive system. O-Ovaries; SR-Seminal receptacle; OD-Oviduct; PL-Four pairs of pleopods.

2.5. Female reproductive system

The female reproductive system consists of a pair of ovaries, a pair of seminal receptacles and a pair of oviducts internally and four pairs of pleopods externally as accessory reproductive organs, present on the inner side of the second to fifth segments of the abdominal flap (Fig. 3 & Plate 3B). The seminal receptacles or spermathecae are the enlarged portion of the oviducts. The oviducts open to the exterior through the female genital openings situated on the left and right sternites of sixth thoracic segment (Plate 3B). Each

pleopod consists of a basal propodite from which arise the medial endopodite and lateral exopodite. The exopodite bears a large number of pinnate setae. A cluster of long and very smooth setae are present on the endopodite, to which the extruded eggs are attached at the time of spawning.

2.5.1. Maturity stages of ovary

The maturity stages of ovary are described below:

Immature (Plate 2D): Transparent / yellowish in colour; occupying

1/6th of body cavity; without a prominent

seminal receptacle.

Maturing (Plate 2E): Pink in colour; occupying 1/3rd of body cavity.

Mature (Plate 2F): Orange-red in colour; with a prominent seminal

receptacle; occupying full body cavity.



BROODSTOCK DEVELOPMENT AND MAINTENANCE

3.1. Source of broodstock

Live mature male and female S. tranquebarica and S. serrata can be obtained from the inshore sea and brackishwater regions where these mud crabs form a commercial fishery.

3.2. Transport of broodstock

Generally live mud crabs are tied with jute/nylon thread to impede the movement of chelipeds for easy handling. Live adult crabs obtained from the fishermen/merchants are dipped in fresh and clean sea/brackish water in order to moisten the gills and packed in bamboo baskets with wet seagrass/seaweeds to provide a cool and moist condition during transport (Fig. 4). The packed crabs are transported either by road or rail or air. By this method of packing, mud crabs are known to survive for a maximum period of 3-4 days. If the duration of transport is longer, those crabs with all limbs intact are given another dip in sea/brackish water and packed in new bamboo baskets en route. This will ensure their survival for another 3-4 days. In another method, adult crabs weighing 350-700 g are packed at the rate of one crab per packet in polythene bags each containing six litres of oxygenated seawater after placing plastic sticking tape over the chelipeds and a tight plastic tube over the tip (dactylus) of walking legs and transported by road or rail or air for a duration of 24 hours.

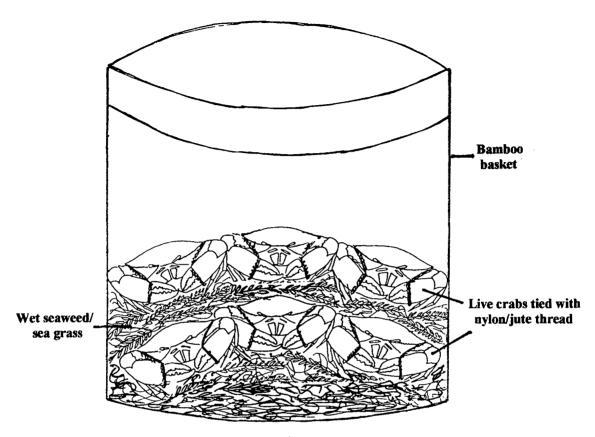


Fig. 4. Transport of live adult mud crabs

3.3. Prophylactic treatment of broodstock

Mud crabs obtained either from the wild or from the culture ponds should be disinfected individually in 100 ppm formalin for 30 minutes before transferring them to the broodstock holding tanks (Fig. 5).

3.4. Acclimatisation of broodstock

After disinfection, the adult mud crabs should be held in tanks containing sea water (30 to 35 ppt), for a week, in order to acclimatise them to the hatchery conditions, before the initiation of trials on induced maturation and breeding.

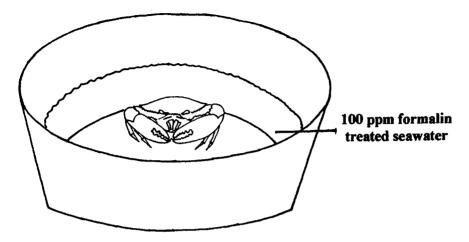


Fig. 5. Prophylactic treatment of adult crab



INDUCED MATURATION

4.1. Selection of mature crabs

Mature male (Fig. 1A) and female (Fig. 1B) crabs with all limbs intact and measuring above the size mentioned in Section 2.3 should be considered for induced maturation. Females with triangular shaped abdominal flap (Fig. 1C) should not be selected as they are immature.

4.2. Eye-stalk ablation

The X-organ-sinus gland complex in mud crabs plays an important role in the regulation of metabolism. The Hyperglycemic Hormone (HGH) present in the eye-stalk is responsible for metabolic regulation. Eye-stalk ablation not only removes the source of HGH, but also the Moult Inhibiting Hormone (MIH), which inhibits the secretion of Y-organ. Eye-stalk ablation in mud crabs results in increased food intake, faster growth of ovary irrespective of developmental stage or season, significant increase in weight and size of oocytes and advancement of extrusion of eggs. Thus, the female mud crabs may be subjected to unilateral eye-stalk ablation for the inducement of growth of ovary at any required time.

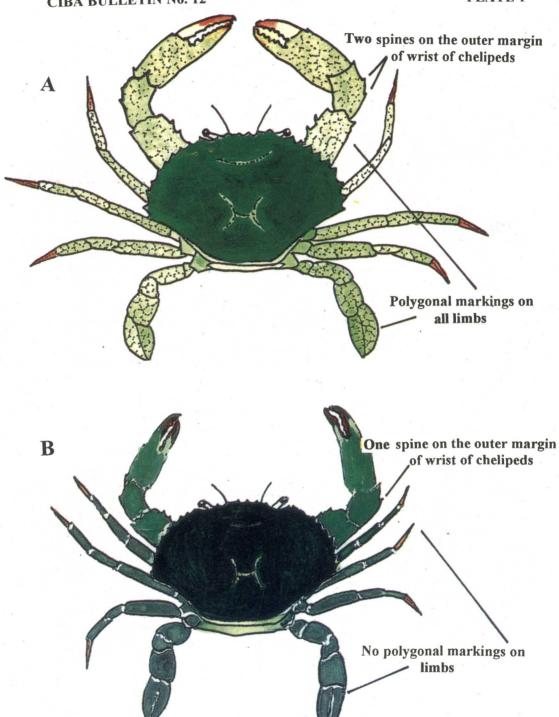


PLATE 1. A. Larger species of mud crab, *Scylla tranquebarica*, B. Smaller species of mud crab, *Scylla serrata*.

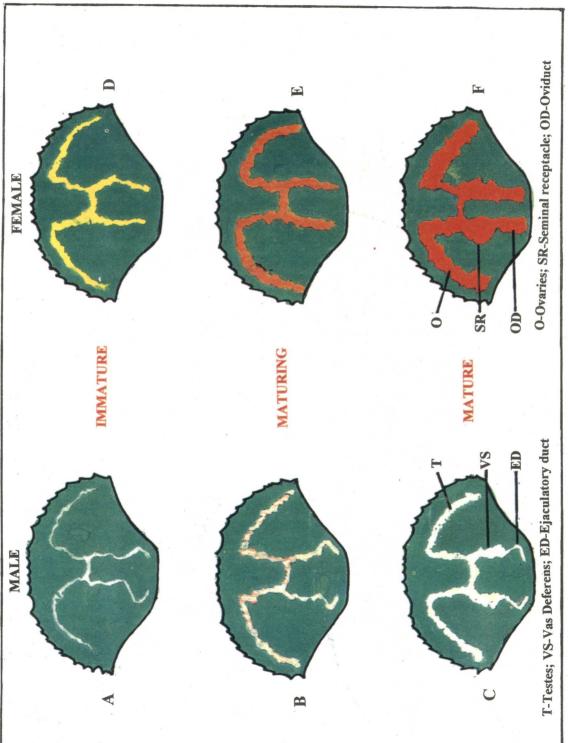
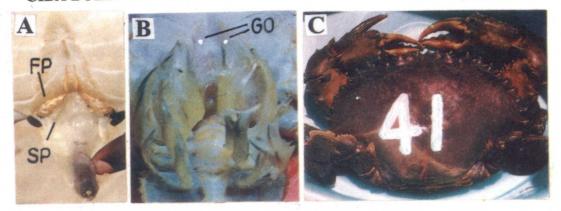
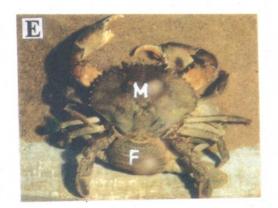


PLATE 2. Developmental stages of testes and ovaries







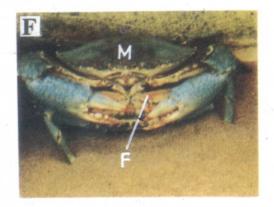


PLATE 3. A. Male pleopods; B. Female pleopods; C. Marked crab; D. Premating embrace; E & F. Mating process. (FP-First pleopod; SP-Second pleopod; GO-Genital opening on sternite of sixth thoracic segment; M-Male; F-Female).

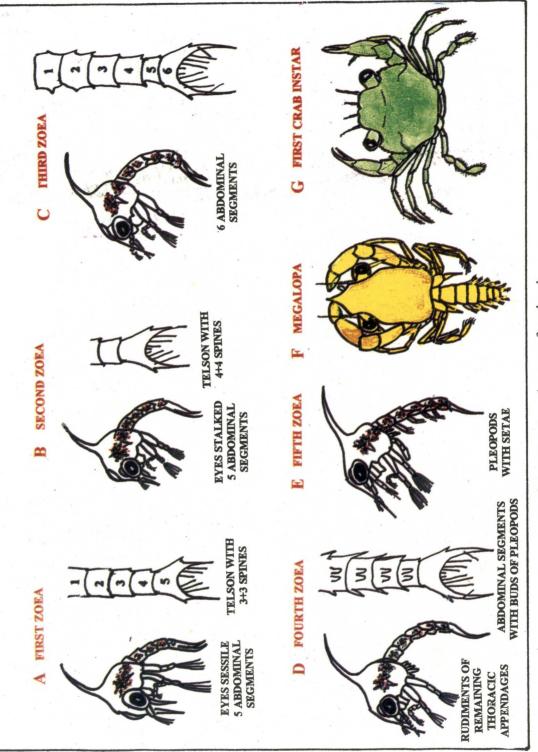


PLATE 4. Larval stages of mud crab.

4.2.1. Methods of eye-stalk ablation

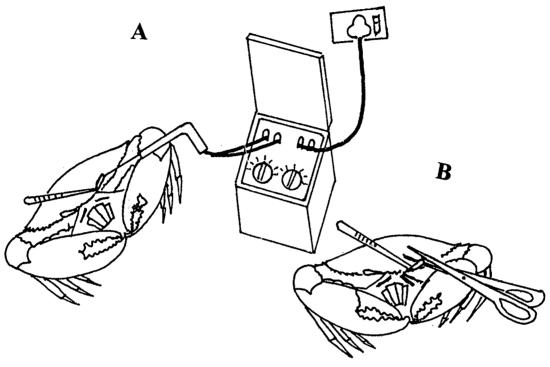


Fig. 6. Method of eye-stalk ablation. A. Electro-cautery apparatus; B. Scissors and forceps.

The right or left eye-stalk of mature female crabs can be removed either by using an electro-cautery apparatus (Fig. 6A) or by cutting the eye-stalk with the help of a pair of scissors and a forceps (Fig. 6B).

4.3. Stocking

4.3.1. Size of the tank

Brick and mortar/RCC circular (Fig. 7A) and rectangular (Fig. 7B) tanks (five or ten tonne capacity) with facility for drawing and draining water are used for holding the brood crabs, both ablated females and unablated males.

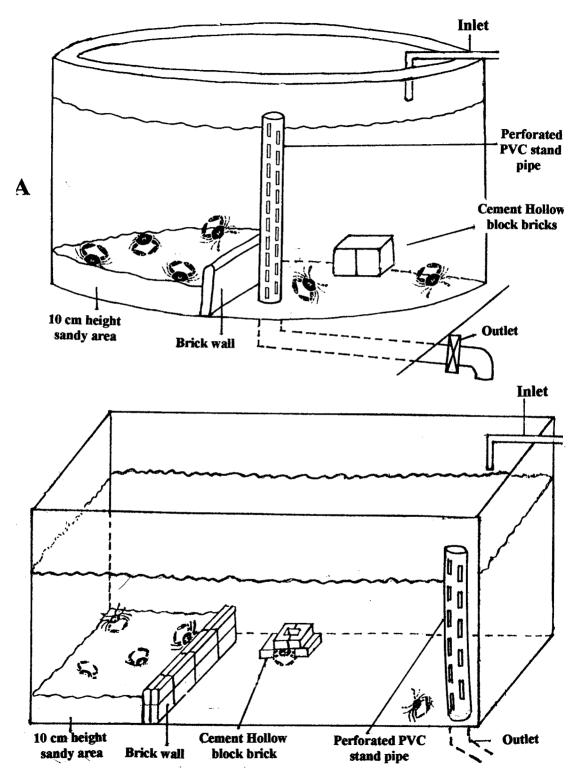


Fig 7. Broodstock tanks. A. Circular; B. Rectangular.

4.3.2. Stocking rate

The stocking rate should be 1-2 crabs per square metre.

4.3.3. Sex-ratio

Adult unablated males and unilaterally eye-stalk ablated females should be stocked either in the ratio of 1:1 or 1:1.5.

4.3.4. Marking of reared crabs

Both the sexes of the stock should be marked on their carapace with white colour epoxy paint, to trace their individual identity (Plate 3C). When a crab moults, its old exoskeleton is shed off. In such cases, the same marking may be painted on the carapace after hardening of the exoskeleton of moulted crab. Thus, the identity of an individual crab in the stock is continuously monitored. Re-marking of crabs may be done, whenever the old marking fades away.

5.3. Water quality management

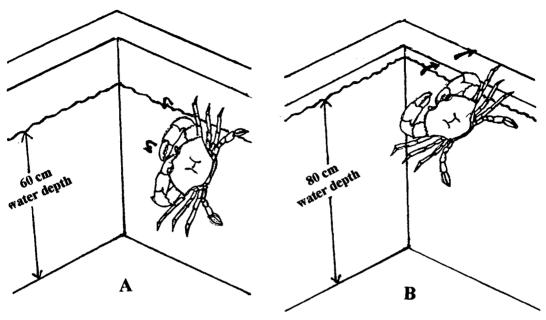


Fig. 8. Water depth to be maintained in broodstock tank.

Sand-filtered seawater drawn from a bore-well or from the open sea, having a salinity of 30-35 ppt should be used for the rearing broodstock. After removing the left-over feed, water should be changed daily @ 60 to 70 % in the morning. The water level should be maintained at 60 cm if the tank height is 100 cm (Fig. 8A). Otherwise, if the water level is maintained at 80 cm, the brood crabs may climb out as shown in Fig. 8B. Water temperature should be in the range of 25 to 29 ° C.

5.4. Feeds and feeding schedules

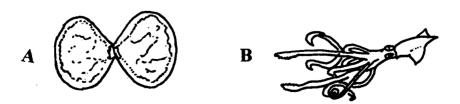


Fig. 9. Feeds for brood crabs. A. Bivalve meat; B. squid meat

Animal flesh such as bivalve meat (Fig. 9A) and squid meat (Fig. 9B) which contain high protein levels may be used as maturation diets. The rate of feeding should be 8-10% of stocked biomass. The daily ration may be divided into two equal quantities and offered at 7 o' clock in the morning and 7 o' clock in the evening. However the crabs should be fed to their satiation.

5.5. Monitoring of broodstock

Daily observations should be made to record moulting, pre-mating embrace, mating, duration of mating and appearance of berry in the brood crabs.

5.6 Moulting

Normally, the crabs undergo moulting during the night. When this occurs, the next morning, both shed cuticle with marking and the freshly moulted crab without marking are seen. These crabs should be re-marked with the same marking as given before moulting.

5.7. Growth

As in the case of penaeid shrimps, the moulted crabs increase in size due to absorbtion of sea water. These crabs should be measured in terms of size (carapace width in millimetre) and weight (total weight in grams) three days after moulting, by which time, their shell will harden. As stated earlier, the moulted crabs should be re-marked to trace their identity.

5.8. Pre-mating embrace

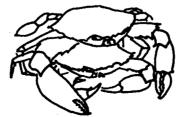


Fig. 10. Pre-mating embrace. M-Male; F-Female.

Pre-mating embrace takes place between a hard male and hard female which lasts for 2-3 days. A typical posture during the pre-mating embrace is shown in Fig. 10 and Plate 3D. Then the pair separates from each other and after an interval of 2-3 days the same female moults. This moulting is called as the pre-copulatory moulting, during which, the male assists the female to cast-off her old shell.

5.9. Mating



Fig. 11. Mating process. M-Male; F-Female.

As soon as the female crab completes moulting, the male embraces the soft female again for actual mating. The male gently turns the female over her back using his chelipeds. The female unfolds her abdominal flap and the male holds the female in position as shown in Fig. 11 and Plate 3 E & F. Though the process of copulation lasts for 5 to 52 hours, the actual time taken for transfer of spermatophores is 5 to 7 hours. Afterwards, the male separates from the female. In some cases, a moulted female allows two different males to copulate one after another with an interval of 1 to 2 days. Thus, the spermatophores deposited by two males co-exist in the seminal receptacle of one female. The spermatophores deposited by the second male only will be utilised for fertilization.



EXTRUSION OF EGGS

6.1. Fertilization

Spermatophores are stored in the seminal receptacles of female till the eggs are extruded. During the process of extrusion, the stored sperms are liberated from the spermatophores to fertilize the eggs and the fertilized eggs are extruded through the genital openings present in the sternites of sixth thoracic segment. These eggs become attached to the smooth setae present in the endopodite of four pairs of abdominal pleopods (Plate 3B).

6.2. Segregation of berried female

The egg mass carried on the abdominal flap is called as berry. Those females carrying a berry are called as berried or ovigerous crabs. When a berried female is noticed in the broodstock tank, it should be collected gently by using a hand net and placed in a 500-litre circular hatching tank for further observations. The tank should be covered with a black cloth to block the entry of light.

6.3. Number of eggs extruded

In the case of *S. tranquebarica*, the number of eggs attached to the abdominal pleopods varies from 2 to 5 million, while it ranges from 1 to 3 million in *S. serrata*. The dead eggs are removed by the mother crab with the help of first and second pairs of walking legs.

6.4. Repetitive extrusion of eggs

Between two copulatory moults, a female can extrude 2 to 3 batches of eggs, indicating that repetitive spawning/extrusion of eggs may occur in the brood crabs.

6.5. Viability of stored sperms

The spermatophores deposited in the seminal receptacles of the female by one or two males during different matings may be utilised in more than two spawnings, indicating the occurrence of multiple fertilization. The viability of stored sperms in mud crabs may last for a period of 9 to 12 months.

6.6. Time interval between eye-stalk ablation and extrusion of eggs

The time interval (range and average) observed between eye-stalk ablation and first extrusion of eggs and subsequently between first and second extrusion and between second and third extrusion is presented in Table 3.

Table 3. Time interval between eye-stalk ablation and serial extrusion of eggs

	Time interval (in days)			
Particulars	S. tranquebarica		S. serrata	
	Range	Average	Range	Average
Between eye-stalk ablation and first extrusion of eggs	26-89	54	6-96	32
Between first and second extrusion of eggs	34-56	47	22-37	30
Between second and third extrusion of eggs	38-42	40		



7.1. Effect of water temperature on embryonic development

The water temperature in the hatching tank is one of the controlling factors for the embryonic development of extruded eggs in the berry. The incubation period ranges from 7 to 11 days at 27 to 30 $^{\circ}$ C and 16 to 17 days at 23 to 25 $^{\circ}$ C.

7.2. Colour change in the berry

The freshly extruded eggs are orange in colour, which gradually changes to brown and finally to black. The black colour of the berry before hatching is due to the black chromatophores present in the eyes of the embryo. The change in colouration of the berry indicates the progress of embryonic development in the eggs.

7.3. Prophylactic treatment for berried crabs

The female crab with black coloured berry before hatching should be given a dip in 100 ppm formalin for 10 minutes.



8.1. Liberation of larvae

Before the liberation of larvae from the berry, the abdominal flap of mother crab makes frequent jerking movements and the egg mass gets loosened. Simultaneously, the jabbing of third and fourth pair of walking legs over the egg mass takes place before hatching. The liberation of larvae generally takes place during the night, when the mother crab is swimming around, presumably for dispersion of larvae. In some cases, the larvae emerge as pre-zoea, which in turn metamorphose into first zoeal stage within 3-4 hours. The hatching rate is more than 90 % in crabs with all limbs intact. The third and fourth pair of walking legs are used for pushing out the freshly hatched larvae from the berry. The hatching rate is low in crabs which have lost the third and fourth pair of walking legs.

8.2. Behaviour of larvae

The hatched out first zoeal larvae are photo-positive and colonize the upper column of water. This facilitates easier separation of larvae from the hatching tank.



LARVAL PRODUCTION

9.1. Species-wise

The production of first zoea larvae obtained through controlled breeding, in both species of crabs S. tranquebarica and S. serrata, is given in Table 4.

Table 4. Species-wise production of first zoea larvae

Species	Size of the crab	No. of larvae liberated per hatching (in million)	
	(CW mm/TW g)	Range	Average
S. tranquebarica	142-144/410-500	0.8-3.5	2.1
	155-160/500-570	0.2-5.0	3.7
	163-168/550-675	0.4-5.5	4.0
S. serrata	101-110/170-200	0.1-1.9	1.1
	112-120/210-2 70	0.1-2.1	1.2
	121-129/230-310	0.2-3.1	1.3
	132-137/340-350	0.5-2.3	1.3

9.2. UTILITY OF FIRST ZOEA LARVAE

The first zoea larvae can be utilised for the following purposes:

- 1. Further rearing of first zoea larvae up to megalopa stage in larval rearing tanks in the hatchery.
- 2. Sea-ranching of first zoea larvae in the inshore sea for replenishment of natural stock.



10.1. Larval and post larval stages

The larval stages of mud crabs include five zoeal, one megalopa and one crab instar (first crab instar). The important external morphological characters of each larval stage are described below in Table 5 and illustrated in Plate 4 for easy identification.

Table 5. Important external morphological characters of different larval stages of mud crabs (Scylla spp.)

Stage	Important external morphological characters
First Zoea	Eyes sessile; 5 abdominal segments; telson with 3+3
(Plate 4A)	spines.
Second Zoea	Eyes stalked; 5 abdominal segments;
(Plate 4B)	telson with 4+4 spines.
Third Zoea	6 abdominal segments.
(Plate 4C)	
Fourth Zoea	Rudiments of remaining thoracic appendages;
(Plate 4D)	abdominal segments with buds of pleopods.
Fifth Zoea	Remaining thoracic appendages developed; pleopods on
(Plate 4E)	abdominal segments with setae; telson with 5+5 spines.
Megalopa	Carapace longer than wider; abdomen with 5 pairs of
(Plate 4F)	pleopods; a pair of chelipeds; 4 pairs of legs.
First Crab	Carapace with 9 anterolateral teeth on either side; 3
Instar	pairs of walking legs; last pair of legs with
(Plate 4G)	paddle-shaped dactylus; resembles parents.

10.2. Utilisation of commercial shrimp hatcheries

At present, some shrimp hatcheries in the corporate sector are lying idle most part of the year. To minimise the high capital investment for establishing a new mud crab hatchery, such shrimp hatcheries can be utilised with suitable modifications, for captive broodstock development, breeding and seed production of mud crabs.



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