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#### BREEDING AND HATCHERY TECHNOLOGY DEVELOPMENT OF SPINY LOBSTERS AND CRABS - A REVIEW

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#### ABSTRACT

Though maturation and breeding of spiny lobsters have been successful under captivity, prolonged larval phase of spiny lobsters has been the major constraint for seed production. Protracted larval phase of the spiny lobster poses several problems. In India, larvae of *Panulirus homarus* were reared upto the 7<sup>th</sup> stage in 60 days. Partial success has been achieved in the larval culture of *P. polyphagus* and *P. ornatus* also. However, more information on the appropriate food for different larval stages, the feeding behaviour and optimum water quality requirements for faster moulting and growth of the larvae is required for successful development of lobster hatchery technology.

As in lobster farming, lack of adequate supply of seed has been the major limiting factor in mud crab farming. Several countries in Asia including India have been successful in hatchery production of mud crab seeds but with low survival due to cannibalism during megalopa and early postlarval stage. Among the marine crabs, the blue crab, *Portunus pelagicus* is the most preferred in the export market and is suitable for farming. Hatchery technology for *P. pelagicus* has been developed and with improved feed and hatchery management higher production is possible. Successful hatchery technology is expected to pave the way for commercial scale seed production and crab farming in India.

#### **INTRODUCTION**

Lobsters and crabs are valuable crustaceans of high export value. Increasing demand and attractive price for live products in the export market have been luring fishermen to catch more from the sea, which has resulted in excessive fishing pressure and consequently depletion of the natural stocks. Laboratory studies and pilot scale culture experiments show that spiny lobsters are capable of adapting well to artificial conditions and their growth rates can be increased considerably by feed and water management. Mud crabs and marine crabs, especially the most wanted blue crab, Portunus pelagicus can also be farmed or fattened in ponds, cages or pens. Though the potential is high, both lobster and crab culture did not grow to commercial scale obviously due to lack of a proven hatchery technology. Lobsters and crabs are highly fecund crustaceans but only a small percent of postlarvae return to the fishery after completion of their larval phase due to various biological reasons. Sustainable farming operation cannot depend upon wild source of seeds, as their availability is either restricted to certain seasons or continued exploitation leads to depletion. Therefore, commercial farming of lobsters and crabs is possible only through hatchery production of seeds. While success has been achieved in breeding and complete larval development of a few species of lobsters, hatchery technology for commercial seed production is yet to be made available. Recently, there are reports of successful development of hatchery technology for large scale seed production of mud crabs.

The current status of lobster and crab breeding technologies in India and other countries, the constraints and the future prospects have been reviewed in this paper.

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#### **BREEDING OF LOBSTERS**

Tropical lobsters breed throughout the year. However, in certain months breeding activity is higher, which is related to the availability of more food. While *Panulirus homarus* breeds in the shallow waters, *P. ornatus* and *P. polyphagus* move to deeper areas for breeding. *P. homarus* attains sexual maturity at a carapace length (CL) of 55 mm and *P. ornatus* at about 90 mm CL. Repetitive breeding has been reported in all the three species. In the lobsters, mating, egg extrusion and hatching take place during intermoult period when the shell is hard. 3-4 cycles of breeding within a moult cycle has been reported. The pelagic phyllosoma larvae are carried away by the currents even to oceanic regions and moults several times before metamorphosing to the postlarva (puerulus). The puerulus swims towards the shore and settles in the near shore region. Juveniles and sub-adults are seen more in the shallower areas.

#### **Captive breeding**

Egg bearing females for the hatchery operation can be obtained from the wild or from captive broodstock. If depending upon the wild ovigerous females, the hatchery should be nearer to the source of the broodstock, as holding and transportation to long distances may lead to stress and microbial infestation of the eggs leading to either premature shedding of eggs or hatching of weak larvae. Therefore controlled reproduction is inevitable for successful hatchery production of lobsters. The broodstock can be built up either by growing juveniles to sexual maturity or bringing sexually mature adult lobsters and induce them to breed under controlled conditions. Juveniles of the spiny lobster *P. homarus* were reared to sexual maturity and successfully bred in captivity. In the case of *P. homarus* broodstock maintained in indoor flow through or recirculation system under low light intensity (<500 lux) and on daily feeding with mussel meat breed regularly almost through out the year. Food plays a major role in inducing maturation and for spiny lobsters, highest breeding activity was observed on feeding with mussel meat. For successful mating a female to male ratio 2:1 was found to be sufficient.

The fecundity of *P. homarus* ranges from 50,000 to 5,00,000 depending upon the size of the lobster. Though the fecundity of captive lobsters was estimated to be 20% lower than those of the wild, egg quality was comparable to that of wild lobsters and hatching gave viable larvae. The fecundity, yolk, carotenoid content, hatch percent of eggs and larval viability will depend to a great extent on broodstock nutrition. Mating normally takes place during night and the spermatophore is deposited externally on to the sternal plates. The time lag between spermatophore deposition and oviposition or egg extrusion varies from 1to17 days and this may vary from species to species and also will depend upon favourable environmental conditions. Under unfavourable conditions or if no mating takes place ovary gets resorbed and the haemolymph colour changes from light blue to deep red. Colour of haemolymph is also indicative of secondary vitellogenesis when carotenoproteins will be transported from various extra ovarian sites to the ovary through the haemolymph. Infact, spiny lobsters are easy to be bred in captivity. Fresh mating is required after each moult as the spermatophore is lost along with the moult. Broodstocks were maintained under optimum environmental condition (salinity, 31 - ppt, pH 8.0-8.2, light intensity, < 500 lux and feeding with mussels twice daily).

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#### Larval rearing

The incubation period in tropical lobsters range from 20 to 25 days depending upon the water temperature. Newly deposited eggs on the abdominal pleopods change colour from deep orange to deep brown before hatching. Microbial infestation is relatively lesser in captive breeders. The breeder has to be transferred to the hatching tanks containing sterilized seawater as soon as the egg bearing lobster is sited in the broodstock tank. Moderate aeration is to be provided. Hatching takes place during night and the larvae are highly phototactic. Healthy larvae actively swim on the surface of water and can be collected and transferred to larval rearing tank after estimating total larval numbers.

Phyllosoma larval culture experiments have been carried out in Japan for more than 50 years. Larval rearing studies were also conducted in many other countries including India. However, complete larval development of a spiny lobster was achieved in 1988 when Kittaka achieved success in rearing the phyllosoma larvae of the spiny lobster *Jasus lalandii*. Later his team of scientists succeeded in culturing the larvae of five other species from egg to puerulus. Survival percent for different species ranged from 0.03 to 10%. The larval phase of *P. cygnus* was completed in Australia and New Zealand also succeeded in producing puerulus of *J. edwardsii*. The larval period of different species ranged from 132-319 days (Table 1&2) The reasons for success by Japanese workers is summarized below:

Improving the upwelling larval culture system originally designed for American lobster larvae by Massachusetts Institute of Technology.

- Introduction of micro algae *Nannochloropsis* sp. for the first time in culture water, which maintained larval culture water quality. Later larvae were successfully cultured in clear water also.
- Mussel meat as food for the phyllosoma larvae. Newly hatched *Artemia* nauplii, *Sagitta* sp. and fish fry also were used. Though mussel meat is a good food it entangles with the appendages causing mortality in still water conditions. Water quality also deteriorates causing build up of pathogenic bacteria in the system. This could be managed with the introduction of probiotic bacteria.
- Maintenance of water quality throughout the culture period. Safety level of COD was set at 1.2 ppm.

The larval culture experiments provided lot of information on the feeding behaviour and food requirements of phyllosoma larvae during different stages. The food should meet the requirements of the feeding apparatus and nutritional requirements of the larvae, as food intake directly affects the duration of the phyllosoma stage. The total larval period can be shortened by feeding nutritionally balanced food and by providing optimum environmental conditions. Improving culture methods including the scale of the culture tank and the nature of food will be necessary to achieve a natural level of health.

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Species	Larval phase (days)	Number of instar	Number of puerulus	Survival (%)	
Jasus lalandii	306	15	1	-	
Jasus (hybrid)	319	15	2	0.11 10.0	
J. edwardsii	303	17	16		
J. verreayxi	205	17	168		
Palinurus elephas	132	9	4	0.08	
Panulirus japonicus	306	12	4	0.03	

Table 1. Larval development of spiny lobsters in the laboratory (Kittaka, 1994 b)

Table 2.	Water quality parameters in larval culture system of spiny lobsters
	(Kittaka, 1994 a)

Parameters	Values			
Temperature ° C	20.0-21.5 (25.0)*			
Salinity (ppt)	33.5-35.5			
pH	8.0 8.6			
Ammonia (mg/l) (upper limit)	8.0			
COD (ppm) (upper limit)	1.2			
Zinc (mg/l) (LD 50)	6.0			
Copper (mg/l) (LD <sub>50</sub> )	0.3			

\* Panulirus japonicus(sub-tropical)

#### Larval culture experiments conducted in India

The Central Marine fisheries Research Institute (CMFRI) at the Kovalam Field Laboratory of Madras Research Centre initiated breeding and larval rearing of spiny lobsters in 1976. Larvae of *P. homarus* were reared to stage 6 in 60 days and then to stage 7 at Calicut Research Centre. Several other laboratories also attempted larval culture but with little success. An improved larval culture system was set up at Cochin. The upwelling system used by the Japanese were modified in such a way that a major portion of unfed *Artemia* and waste could be flushed out by circulating water through the system. This improved the heath status of the system to a great extent. Mussel meat was not much acceptable to the early *P. homarus* larvae. Phyllosoma larvae is known to feed on the chaetognath *Sagitta* sp. and probably live feed culture of several planktonic organisms will have to be developed to meet the food requirements of the phyllosoma larvae. Enriched and embedded *Artemia* may be good for late stage larvae.

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If the water quality deteriorates due to organic build up in the system, larvae are prone to be infested with ciliates and filamentous bacteria. Once infested with *Zoothamnium* sp. and other vorticellids, larvae are immobilized which interferes with the swimming and feeding activity. Though formalin at 25 ppm is effective in containing ciliates to a certain extent, prolonged treatment will affect the larvae.

#### **Research directions**

The research conducted so far on larval culture of spiny lobsters has shown that larvae could be grown under captivity and maintained for a prolonged period. Appropriate food for the late stage larvae and optimum environmental requirement for moulting and growth are the key areas on which the future research should focus. Larvae of lobsters require special culture systems so that larvae are suspended in water. Major draw back of the Japanese system is difficulty in removal of unfed food and wastes. The system developed at CMFRI and described elsewhere is an improvement over the Japanese system. The specialized feeding behaviour of the larvae necessitates identification of suitable food for different stages of phyllosoma larvae. The priority of research on phyllosoma culture in India has to be on feed development, culture system design and water quality requirements of the larvae. Once complete culture of larvae is accomplished, the focus shall be on scaling upto mass seed production with higher survival. Larvae use their walking legs to target the food, which needs to be soft and slow moving.

Culture of phyllosoma larvae is technically feasible. The larval phase is prolonged and may take 100-300 days to complete the larval phase. Those species with a shorter larval phase is promising. However, their suitability for aquaculture needs to be evaluated. If hatchery technology could be developed for species with good aquaculture potential but with a relatively longer larval period, the attempts will be worthwhile as lobsters are high value seafood with high consumer demand. For the species in which success has already been achieved, the focus should be on improving survival and reducing the larval period by feed, environmental or hormonal manipulations. The small laboratory scale larval culture systems will have to be scaled up for mass production of seeds to meet the demands of aquaculture industry. A longer-term goal will be selective breeding of individuals with the fastest growing or most disease resistant larvae and juveniles.

#### **MUD CRAB BREEDING**

The mud crabs, *Scylla serrata* with greenish colour and prominent polygonal marking on all appendages and *S. tranquebarica*, brownish in colour with polygonal marking on last two pairs of legs are the two species with high aquaculture potential. The former has more demand because of the larger size. There are attempts to develop hatchery technology for mud crabs in the southeast Asian countries and in Australia where they contribute substantially to aquaculture production.

Most of the hatchery research has focused on gonadial maturation, spawning and hatching and larval rearing with the intention of large scale production of seeds. Ovigerous females obtained

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from the wild are suitable for hatching provided they are brought to the hatchery without stress and the eggs are not exposed to a long period outside water. Eggs of females purchased from the traders are normally highly infected due to poor holding conditions. As in spiny lobsters excessive stress will lead to premature egg shedding or bacterial infection of the eggs. The breeders may be treated with 50 ppm formalin overnight. Another source of the breeder will be adult females, which can be induced to mature and spawn by eyestalk ablation under controlled conditions. The quality of eggs from ablated females is comparable to that obtained from the wild. Ripe females can be obtained from captive females that undergo moulting and mating.

Moulting in adult females takes place normally after several rounds of spawning by the repetitive use of the sperm available in the spermatheca. Maintenance of broodstock crabs in captivity is expensive. The best source of breeders will be those mated females with ripe ovary brought from the wild, which will oviposite eggs within 1-3 weeks. Feed is important in inducing maturation. Molluscan meat is the best for inducing maturation.

The incubation period ranges from 8 to 12 days depending upon the water temperature and egg colour changes from yellow to orange and finally to black just before hatching. Higher percent of hatching is obtained in seawater (32-35ppt). No feeding is required during the entire period of egg incubation to prevent water quality deterioration due to dissolved organic material in the system. The newly spawned crabs are placed in sterilized seawater to avoid contamination of eggs with microbes. Hatching normally takes place during night. The fecundity of *S. serrata* weighing 500g is about 3 million. The larvae are highly phototactic and healthy larvae swims at the water surface. Continuous flushing with sterilized water will prevent multiplication of bacteria in the hatching tank.

#### Larval rearing

Several research workers in India have made attempts in the last 15 years to rear mud crab larvae in the laboratory. Though small scale production has been achieved, wide variation in percent survival between larval culture experiment was reported. The factors, leading to low survival have to be identified and the technology perfected before commercial production is attempted. Many Asian countries such as Philippines, Indonesia, Malaysia, Thailand and Bangladesh have also attempted mass production but with little success. The major constraints have been mass mortality during early zoea stage or high cannibalism during megalopa and early crablet stage.

Newly hatched larvae after flushing several times in fresh seawater are stocked in larval rearing tanks. Larval rearing tanks of different sizes (25 1 to 10 tonnes) and shapes (circular, oval, rectangular, cylindrical) are used. Survival during early stages depends upon the stocking density. In Philippines, stocking of 50 larvae/l has been recommended, which gave a mean survival of 77% at stage 2. In India, a stocking density ranging from 10 to 75 numbers/l was used. Various combination feeds constituting live, fresh and artificial feed were used by different workers (Table 3). In Birbie Island Aquaculture Research Centre (BARC) mostly rotifers are used as the sole diet up to the Z<sub>3</sub> stage at a concentration of 10-15/ml Higher densities of rotifer are used in India at Tuticorin Research Centre of CMFRI (30-60/ml). There are contradictory reports on using *Nannochloropsis* sp. or *Chlorella* sp. mixed green water for culture of zoea

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larvae. No significant difference in larval survival with and without microalgal was noticed.' However, majority of workers preferred green water system. In BARC *Nannochloropsis oculata* was maintained at  $5 \ge 10^5$  cells/ml and from Z<sub>3</sub> onwards *Isochrysis* sp. was used to enrich the *Artemia* fed to the larvae. On the other hand in CIBA, mixed phytoplankton cultures were used. Studies conducted in CMFRI show that *Nannochloropsis* sp. is superior to *Chlorella* sp. The micro algae not only improve the water quality but also acts as food for the rotifers. Addition of *Artemia* nauplii from Z<sub>3</sub> stage onwards at 5 to 50/ml has been reported to give higher survival. At Tuticorin minced clam meat was used at Z<sub>5</sub> stage in addition to *Artemia* and decapsulated *Artemia* embryo. Megalopa larvae were fed on prawn-egg-custard in addition to *Artemia* with a higher survival (Radhakrishnan personal observation). In BARC, Australia, seven-day-old *Artemia* workers vary from 0.05 to 21%. In recent experiments conducted in CMFRI a consistent survival of 20% were obtained.

Larval stage	Intermoult Duration(days)	Feed used by different workers			
Zoea Z <sub>1</sub> - Z <sub>3</sub>	Zoea <sub>1</sub> : 2-5 Zoea <sub>2</sub> : 3-5 Zoea <sub>3</sub> : 2-5	Microalgae (Chlorella, Nannochloropsis, Chaetoceros etc.) rotifers (5-60 nos/l), artificial feed (0.5 g/tonne), frozen Artemia nauplii			
$Z_4 Z_5$	Zoea₄: 4-7 Zoea₅: 5-8	*Microalgae, Artemia nauplii (5-50 nos/ml) custard, artificial feed, decapsulated Arten embryo (20 nos/ml), minced clam meat.			
Megalopa	6-11	2-day old live Artemia (50 nos/ml), shrimp/mollusc/squid macerated meat (150-200 g/t of water), copepods, egg custard, artificial feed.			
Crablets	Z <sub>1</sub> first crab instar	Shrimp /clam/squid meat @ 3-5% biomass, artificial feed @ 0.5 g/t of water.			

Table 3.	<b>Different larval</b>	satages of Mud	Crab and	food	supplied by	different w	orkers
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\* Microalgae added to maintain water quality and not as feed.

#### **Research directions**

Research on mud crab hatchery production should focus on improving the survival at different stages of the larval development. Maintenance of optimum water quality will be necessary to avoid organic matter build-up and consequently multiplication of opportunistic pathogens. Studies on larval behaviour during megalopa and improved nursery rearing methods to prevent cannibalism are other areas, which need immediate attention.

#### BREEDING OF THE MARINE CRAB PORTUNUS PELAGICUS

*P. pelagicus* the marine blue crab is in high demand in the International market. Farming experiments with hatchery produced seed has shown encouraging results. The seeds of *P. pelagicus* were produced at the marine hatchery, Regional Centre of CMFRI, Mandapam Camp. 20 seed production experiments were conducted and an average 15% survival was obtained. Larval period ranged from 15 to 17 days. Combination feeds constituting micro algae, rotifer, *Moina* sp., Artemia sp. and prawn-egg custard were fed to the larvae during different stages. Feeding schedule followed was almost similar to that of mud crab larval rearing.

#### **Future prospects**

The crab farmers in the country are awaiting for a reliable hatchery production technology for crabs. The seed production trials conducted in various laboratories have been able to standardize the feed combinations and percentage of survival could be enhanced. However, production is still inconsistent. Fresh feeds such as minced fish or bivalve meat are to be avoided in larval culture systems as they may attract pathogenic bacteria. Availability of hatchery produced crab seeds can be expected in the near future, for which more intensive studies are needed.

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