G. Maheswarudu

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And several Developmental News items

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Repetitive Spawning of *Penaeus indicus* without Eyestalk Ablation , Hatching Rate and Growth upto Juveniles

G. Maheswarudu, E.V. Radhakrishnan, N.N. Pillai, S. Mohan, M.R. Arputharaj, A. Ramakrishnan and A. Vairamani

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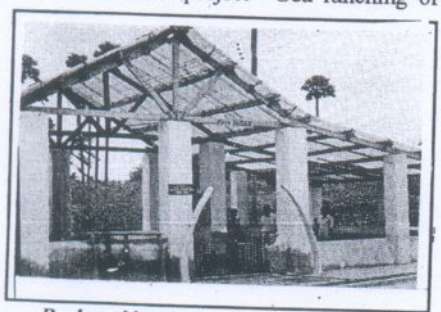
Currently all shrimp hatcheries in India are using mother shrimp for spawning once or twice after eyestalk ablation. It is well known that the spawn size and quality decreases as number of spawnings increase after eyestalk ablation. This leads not only to sacrificing more number of spawners from wild but also to increase in costs. Anticipating this situation, CMFRI has successfully carried out a series of experiments and developed a technology for mass seed production of *Penaeus indicus* through repeated spawnings of the same spawners without eyestalk ablation. This is in line with the technologies developed by CMFRI for seed production of *Penaeus semisulcatus* (Rao *et al*, 1992 and Radhakrishnan *et al.* 1993), induced maturation of *P. indicus* by eyestalk ablation (Muthu and Laximinarayana, 1979) and spawning of *P. indicus* without eyestalk ablation (Muthu *et al*. 1984 and 1986). The present attempt and technique developed for making *P. indicus* to spawn repeatedly by manipulating environment and feed are presented here.

Experiments were conducted during August 1993 and July 1994 in the following facilities.

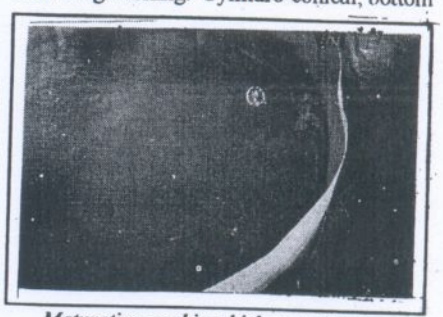
Backyard hatchery: For seed production of penaeid shrimps a backyard hatchery of one million capacity was established in 1987 at Regional Centre of CMFRI, Mandapam for the research project ''Sea ranching of marine prawns''. It is a small four-side-open hatchery shed (12.4 x 7 m) with translucent roofing and is 50 m away from the shores of Gulf of Mannar, housing spawning, larval rearing and post-larval rearing facilities. The infrastructure available in the hatchery consists of two collapsible polyethylene lined aluminium pools (8' dia and 6' dia) for maturation experiments, 6 cylindro-conical and bottom drainable fibre glass tanks of 0.15 t capacity each, 6 oval shape fibre glass tanks of one ton capacity each for larval rearing, 6 rectangular small fibre glass tanks of 150 l capacity each to maintain microalgal culture for feeding larvae, one air compressor run by 0.5 HP electrical motor providing aeration and one hp monoblock pumpset to draw settled seawater from collapsible polyethylene lined aluminium tanks for day-to-day requirements. The seawater from Gulf of Mannar is drawn and allowed to settle in the above said pool (12' dia) of 12 t capacity for a minimum of 6 hours before using (fig.1).



Backyard hatchery of Penaeid prawns at Regional Centre, CMFRI, Mandapam

Maturation system: The experiment was conducted in a 6' dia collapsible polyethylene lined pool in which sand bed (5 cm ht) filter was laid on a perforated aluminium sheet false bottom that stood at about 15 cm ht over the entire bottom of the pool. Water recirculation was maintained by lifting the filtered water from below the sand bed and false bottom with aeration through 6 PVC tubes (4cm dia) that are fixed inwardly in the peripheral region of tank (fig.2) at equal inter distance. About 95% of the top of the pool was covered with a lid made of aluminium sheet and wooden frame to cut light intensity. Once in a week 90% of water was exchanged with fresh seawater. Water bed above the sand bed was 0.5 m. Water pH was regulated between 8.0 and 8.3 by addition of Sodium carbonate whenever necessary. The rate of water recirculation was 15 times per day. The temperature varied between 26-31 degree centegrade; water salinity range was 28.0-36.5%; dissolved Oxygen varied between 3.5 - 4.4 ml/l; light intensity below the lid varied between 40-2000 lux (Table.1) during the entire experiment period.

Maintenance of breeders and spawning: Live males and females of *P. indicus* collected from trawl catches in Gulf of Mannar were used in the present study. Four females measuring 144 mm TL/30 g, 170 mm TL/42 g, 175 mm TL/50 g, and 186 mm TL/60g and four males measuring 142 mm TL/26g, 144 mm TL/28g, 146 mm TL/26g and 150 mm TL/30 g were introduced in the maturation pool on 6.8.1993. They were daily fed with clam, intertidal oligochaetes and squid *ad libitum* but shrimp preferred intertidal oligochaetes (marine earth worms), clam and squid in the order of preference. Feed was given in the evening hours and residuals of feed and faecal matter were removed in the morning hours of the following day. All four females were marked by cut-marks in the distal portion of uropods to observe moulting and maturation individually. Cut-marking of females were repeated whenever cut portion of uropod regenerated. Night observations were made with the help of spot-light to find out maturity stages and identified ripe females were shifted to spawning tanks in the following evening. Cylindro-conical, bottom



Maturation pool in which water is being recirculated through sand bed filter by aeration

→

drainable spawning tanks, each of 150 l capacity were filled with seawater filtered through 50 micron mesh and treated with Disodium salt of EDTA at the rate of 0.1 g/ 100 l. Spawners are kept at the rate of one per tank. Mild aeration by a single stone was provided and the tank was covered with a net piece to prevent escape. Lights were cut off in the hatchery during night. Spawning occurred before 2300 hrs on most of the days. Eggs hatched out after 11-14 hrs of spawning. Total number of eggs and nauplii were estimated by subsampling, counting and raising. Whenever they spawned unexpectedly in the maturation pools sampling was done from the pool concerned for estimation of eggs and nauplii. All the females moulted between 20 and 24 hours. Date of moulting of each female was recorded by locating the cut mark in one of the uropods of the moult. Thelycum of the female was examined after moulting to find the spermatophore deposition. Prawns were observed to be burrowed during day time. But immediately after moulting prawns were found to relax "on sand' for one day and avoid burrowing. During night they were very active by walking, swimming and feeding.

All males and females were subjected to dip treatment in 15 ppm Furacin for 30 minutes once in every three months to avoid fungal infection.

Spawning performance: In total 99,34,850 nauplii were produced out of 1,37,26,325 eggs released through 68 spawnings performed by four females during the 337 days experiment period. Table 2 presents the information about the individual spawning performance. First spawning was recorded on day 57 from the start of experiment. Females weighing above 40g has taken 185 days to record first spawning. The total estimation of eggs and subsequent nauplii count from maturation pool were always lower when compared to the spawning tank due to larger volume of water and eggs entangling in the interspace of sand particles. Number of eggs/spawning varied between 0 and 2,70,450. Duration of moult cycle varied from 12 to 29 days. A maximum of 5 spawnings were recorded by a single individual during one moult cycle and the eggs were always viable. Few spawnings before death gave unfertilized eggs due to mating failure. During this period females could not shed the whole exoskeleton in a stroke. The exoskeleton in the abdominal region was found attached to the body while exoskeleton in the cephalothoracic region was already shed. This might be the reason for failure of mating. After spawning continuously for some time females were found to relax for 2-3 moult cycles without spawning and then performed repetitive spawning again. Details of spawning performance of each female are discussed below.

Female 1: Performed 21 spawnings in a span of 296 days while undergoing 16 moult cycles. Out of 40,36,925 eggs spawned, 30,14,600 nauplii were obtained at a hatching rate 74.6%. The average number of eggs and nauplii/spawning were 1,92,234 + or - 68,839 and 1,43,552 + or - 91,889 respectively. For the last three spawnings, unfertilized eggs were obtained due to mating failure. In this shrimp there was no ovary development during five moult cycles in between successful spawnings.

Female 2: Spawned 12 times and moulted 18 times during the entire 337 days of experiment. Totally 21,31,650 nauplii were obtained out of 25,20,250 eggs spawned resulting in 84.4% hatching rate. The average number of eggs and nauplii/spawning were 2,10,020 + or - 49,623 and 1,77,637 + or - 52,223 respectively. This shrimp did not get ovarian maturation during three moult cycles after first spawning in this experiment.

Female 3: This spawner survived 305 days in captivity. Maximum spawnings were obtained from this female that gave 62,65,150 eggs and 40,79,600 nauplii by spawning 28 times during 15 moult cycles in a span of 258 days after first spawning. The averge hatching rate was 65.1% which is low as compared to that of other females due to mating failure after 280 days of initiation of experiment. The averge number of eggs and nauplii per spawning were 2,23,755 + or - 74, 298 and 1,45,700 + or - 1,00, 875 respectively. Prawn relaxed for one moult cycle without ovary development. Unfertilised eggs were released during last 6 spawnings due to lack of spermatophore deposition.

Female 4: It survived and performed 6 spawnings in a span of 223 days in captivity. It underwent 8 moult cycles after first spawning. Prawn relaxed for five moult cycles wihout spawning. The total number of eggs and nauplii obtained were 9,04,000 and 7,09,000 respectively and the resultant hatching rate was 78.4%. The average number of eggs and nauplii/spawning were 1,50,666 + or - 25,060 and 1,18, 166 + or - 30,928 respectively.

Viability of Seed: Out of 99,34,850 nauplii produced by 4 spawners during the full experimental period of 337 days, 3,60,000 nauplii were further reared in the hatchery by following larval rearing method described by Silas *et al.* (1985) and produced 1,10,000 PL 1. The remaining 95,74,850 nauplii were sea-ranched in the Gulf of Mannar. All these post-larvae were transferred to two out-door cemented nursery rearing tanks and reared upto 21 days. Some of these seed (PL 21) were stocked in two earthern tanks (300 m^2 and 800 m^2 waterspread areas) at Fish Farm, Regional Centre of CMFRI, Mandapam at the rate of 50,000/ha. After 111 days of rearing period, all the prawns were collected alive, tagged and released into Palk Bay in March 1994.

The survival rate was 43.6% in Pond I and 75% in pond II. The average size was 111.71 mm TL/10.1 g wt in pond I and 103.06 mm TL/ 8.1 g wt in pond II at the termination of rearing experiment.

The juveniles thus grown in purely marine conditions were tagged and released in the Palk Bay. Few tagged ones were recovered from commercial trawl catches of Palk Bay and Gulf of Mannar off Mandapam. All these prove that the seed produced through repetitive spawning without eyestalk ablation are sturdy and when ranched, survive well in wild and suitable for commercial culture.

Concluding remarks: Among four females introduced into induced maturation system in which water pH and light intensity were regulated, three females measuring above 40 g wt. responded and spawned within 60 days; whereas female 2 weighing 30 g responded only after 185 days. This indicates that females belonging to large size group above 40 g wt. are preferable for induced maturation without eyestalk ablation.

Despite dip treatment of Furacin at 15 ppm concentration for 30 minutes once in every 3 months, female I and 2 were infected with fungus resulted either in non-development of ovary or developing ripe-ovary and reabsorbing after 280 days of initiation. This →

indicates that the females have lost resistance to diseases after 280 days due to prolonged spawning and aging. Female 1 and 3 gave unfertilised eggs for the last 3 and 4 spawnings respectively due to mating failure. Though the prawns were fed with nutritious diet, females became weak after 280 days due to prolonged spawning and partial moulting resulted in non-mating. The result of this experiment discloses the potential of an individual female of *P.indicus* for prolonged induced maturation without eyestalk ablation for at least six months continuously.

Acknowledgement: We are grateful to Dr. P.S.B.R.James, former Director, CMFRI for providing facilities. We re very much indebted to Dr. P. Vedvyasa Rao, Director, CMFRI and founder of sea-ranching programme on marine prawns at Regional Centre, CMFRI, Mandapam for his encouragement during course of study.

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TABLE . 1. Particulars of Experiment Conducted on Induced Maturation of *P. indicus.*

S.No	Parameter	Particulars
1.	Date of Start	6-8-93
2.	Date of end	9-7-94
3.	Durtion of experiment (days)	337
4.	Number of prawns used	8
5.	Sex ratio	1 : 1
6.	Total length range of males (mm)	142 - 150
7.	Total length range of females (mm)	144 - 186
8.	Weight range of males (g)	26 - 30
9.	Weight range of females (g)	30 -60
10.	Polycraft pool size	6' dia
11.	Water depth in the pool (m)	0.70
12.	Water recirculation rate / day	15 times
13.	Water salinity range (ppt)	28.0 - 36.5
14.	Temperature range (°C)	26.0 - 31.0
15.	pH range	8.00 - 8.3
16.	Light intensity range (Lux)	40 - 2,000
17.	Dissolved oxygen range (ml/1)	3.5 - 4.4
18.	Feed given to prawnsClams, Intertidal oligochaetes and Squid	
19.	Antibiotic treatmentDrip treatment of Furacin at 15 ppm for 30 minutes once in every 3 months.	

TABLE. 2. Details of Spawning Performance of *P.indicus* During Experiment Period.

S. No.	Parameter	Spawner Number		
	1	2	3	4
1.Total length in (mm)	170	144	1860	170
2.Weight in g	42	30	60	50
3.No. of spawnings performed	21	12	28	7
4.No. of moult cycles undergone	18	18	18	11
5.Range of spawnings performed/moult cycle	0-5	0-3	0-3	0-3
6.Range of duration of moult cycle in days	12 - 29	12 - 27	12 - 20	12 - 23
7.Lapse of period in days for attaining initial maturityfrom date of start	57	185	63	69
8.Total no. of eggs spawned	40,36,925	25,20,250	62,65,150	9.04,000
9.Total no. of nauplii obtained	30,14,600	21,31,650	40,79,600	7,09,000
10.Average no. of eggs/spawning	1,92,234	2,10,020	2,23,755	1,80,666
11.Average no. of nauplii/spawning	1,43,552	1,77,637	1,45,700	1,18,166
12.Average hatching rate in %	74.67	84.58	65.11	78.42
13.Range of number of eggs/spawning	70,550-3,00,000	87,000-2.55.000	85,000-3.36.000	1,08,400-1.75.000
14. Range of number of nauplii/spawning	0-2,60,550	72,000-2,38,400	0-2,70,450	70,000-1,52,000
15. Range of hatching rate in %	0-92.89	50.00-94.93	0-97.5	40.00-89.62
16. Remarks	Infected with fungus.	Infected with fungus.	Died in the maturation pool.	Died in the spawning tank.