J. mar. biol. Ass. India, 2000, 42 (1&2) : 91 - 100

Repetitive maturation and spawning of the green tiger prawn *Penaeus semisulcatus* by environmental regulation in closed seawater recirculation systems

E.V. Radhakrishnan, G. Maheswarudu, M.R. Arputharaj and A Ramakrishnan

Central Marine Fisheries Research Institute, Cochin - 682014.

Abstract

Maturation and repetitive spawning of the green tiger prawn *Penaeus semisulcatus* were achieved by pH regulation and on feeding the shrimps with clams, squid and the oligochaete worm *Pontodrilus bermudensis*. Females and males stocked in a recirculation system at a ratio of 1:1 and exposed to an average light intensity of 500 lux spawned an average 3 times in each moult cycle. In Experiment 2 lasting 60 days, 80% of the females responded, whereas in Experiment 3 lasting 90 days 60% of the shrimps matured and spawned viable eggs. Spawning interval varied from 4 to 7 days. Average number of eggs produced by a single female ranged from 15-20 lakhs from 11-13 spawnings. Though egg production was consistent upto three consecutive spawnings, number of spawnings was decreased beyond moult 3 with no reduction either in spawn size or percent hatch of the eggs. Considerable variability in egg production between spawns within a moult cycle and between spawns of consecutive moult cycles were observed. However, no significant difference in percent egg hatch between the spawns in a moult cycle ($P<0.05$) and between spawns of different moult cycles ($P<0.05$) was noticed. These results suggest that *P. semisulcatus* can be induced to mature and spawn viable eggs in captivity through environmental regulation and by feeding a mixed diet. The advantages of this technique over the conventional eyestalk ablation method and the prospects of its commercial application are discussed.

Introduction

Controlled reproduction is crucial for year round production of seed stock for successful commercial shrimp farming. Unilateral eyestalk ablation has been widely employed for inducing maturation, rematuration and spawning of atleast 21 species of penaeid shrimps (Bray and Lawrence, 1992). But reports on effect of ablation on spawn size, percent fertility and percent hatch of the repeat spawns have been contradictory (Primavera *et al.*, 1979; Emmerson, 1980; Lawrence *et al.*, 1980; Chamberlain and Lawrence, 1981; Browdy and Samocha, 1985b). There is evidence for decrease in viable egg production from ablated animals over a period (Aquacop, 1979; Beard and Wickens, 1980) and also of poor quality of the spawn than those produced from wild females (Bray and Lawrence, 1992). Browdy and Samocha (1985b), on the otherhand, could not find any significant difference in spawn quality between the first three spawns. However, significant reduction in spawn size was noticed within the moult cycle.

Several species of shrimps have been

induced to mature and spawn without eyestalk ablation (Brown *et al.,* 1979 and 1980; Aquacop, 1977, 1979; Emmerson, 1980 and Primavera *et al.,* 1982). Muthu *et al.,* (1986) successfully induced maturation and spawning in pond reared *P. indicus* by maintaining the pH of the maturation pool at 8.1 - 8.2. Browdy and Samocha (1985a) compared the reproductive performance of eyestalk ablated and unablated *Penaeus semisulcatus* and found no significant difference in the spawn size or spawn quality. Maheswarudu *et al.,* (1995) also succeeded in induced maturation and repetitive spawning in *P. indicus* by environmental regulation.

Penaeus semisulcatus is considered as a promising species for aquaculture on the south-east coast of India, where it forms a dominant component of the shrimp fishery. Postlarvae produced in the small scale hatchery at Regional Centre of CMFRI, Mandapam Camp have been regularly released in Palk Bay as part of the searanching programme. While maturation and spawning was normally carried out by unilateral eyestalk ablation, efforts to develop techniques for indured maturation without resorting to eyestalk ablation were made. The main objectives of the study were i) to close the life cycle of the species, ii) to develop a reliable technology for maturation and spawning by controlling environmental factors and not resorting to eyestalk ablation and iii) to test the suitability of recirculation system for maturation of *P. semisulcatus* broodstock and other commercially important penaeid species.

The authors would like to thank the Director, Central Marine Fisheries Research Institute, Cochin for encouragment. We would also like to thank Dr. N. Neelakanta Pillai, Retired Senior Scientist, Crustacean Fisheries Division, CMFRI for his valuable sugestions.

Material and methods

The experimental shrimps were maintained in 1.8 m^3 diameter polythene pools fitted with a sub-sand filter covering the entire floor area of the tank. Seawater was recirculated by an air lift mechanism at an average flow rate of 400 1/hour (600 % exchange per day). The temperature in the system ranged from 25-29°C, salinity, 34-36 ppt, dissolved oxygen, >4.0 ppm and ammonia N-level, 0.01-0.025 ppm. The pH of the recirculated water was checked daily and was maintained between 8.1 - 8.3 by the addition of anhydrous sodium carbonate. The maturation tank was kept in a semi-open hatchery building.

The broodstock tanks were covered partially on the top to reduce the light intensity inside the tank. The light intensity was measured between 8 A.M and 6 P.M using a lux meter. The average ambient light intensity in the maturation tank was 500 lux. The prawns were fed fresh diet in excess twice daily. In the morning the diet included clam alone, where as in the evening the diet was comprised of clams, squid meat and chopped fresh oligochaete worm *Pontodrilus bermudensis.* The feed remains and faecal matter were siphoned out in the morning and the lost water was replaced with fresh seawater. Care was taken to remove minimum water from the tank. 50% of the water was replaced by fresh seawater once a week. The ovarian condition of individual female was monitored daily by external examination of the colour changes and the stage of the ovary. Moults, if any, were recorded in the morning. Uropod clipping of females helped to follow them individually. Any unexpected spawning in the maturation pool was recorded in the morning by sampling the maturation pool for nauplii. Females with ripe ovaries were removed in the evening and kept in individual 150 l capacity FRP cylindro-conical tanks for spawning. The shrimp was returned to the maturation tank on the following morning irrespective of whether they have spawned or not. Unspawned females were kept again for spawning on the following day. The egg counts were made by counting the eggs from three replicate samples and the nauplii counts were also made similarly. The nauplii were then distributed to larval rearing tanks and reared upto postlarvae (PL_{10-20}). Some of the postlarvae were used for farming purpose and others were searanched.

Experiment I

In this experiment, hatchery produced and laboratory reared shrimps were used. Nauplii obtained from spawning of a gravid female brought from Palk Bay were reared and the postlarvae obtained were further reared in an outdoor cement tank for 18 months until used for this study. Five females and four males measuring an average 138 mm in total length and 26 g in weight were stocked in the maturation system. Monitoring of environmental parameters and feed management parameters were carried out as explained above.

Experiment 2

Maturation and spawning of shrimps measuring an average 161.3 ± 5.9mm in to-tal length and 35.8 ± 3.3 g in weight were followed for 60 days. The ripe females five in numbers which were brought from the wild after the spawning, were introduced along with an equal number of males into the recirculation system. Moulting, spermatophore transfer, ovarian maturity and spawning were monitored.

Experiment 3

Another batch of mature shrimps collected from the wild and once spawned were stocked in the recirculation system. Five females measuring 165.3±4.7 mm in total length and 38.5 ± 3.6 g in weight were used. Equal numbers of males were also introduced. Moulting, mating and spawning were monitored for 90 days.

Data were analysed to test the differences between the first, second and third spawn within a moult cycle. Similarly, test of significance was also carried out on spawns obtained from all the spawnings over the moult cycle (Campbell, 1967). Spawn size (total number of eggs) and percent hatch (number of nauplii/by number of eggs x 100) were computed and evaluated. Percent hatch of each batch of eggs has been taken into consideration to evaluate the spawn quality.

Results

In Experiment 1, one of the females spawned viable eggs 19 days after introducing into the recirculation system in which pH and light intensity are controlled. A total of 1,15, 500 eggs were released with a percent hatch of 88.5%. The larvae were reared to postlarvae, giving rise to the first generation population and thereby successful closure of the life cycle of the species.

In Experiment 2, four out of the five females showed response favourably, three spawning consistently to the treatment and the fourth occasionally. Out of a total of 27 spawnings, 18 were collected from the spawning tanks and 9 were detected in the maturation pool. 11 spawnings were used for larval rearing.

In Experiment 3, 60% of the females matured and spawned repetitively and the rest have spawned only erratically. A total of 28 spawnings were obtained of which 20 were in spawning tanks and 8 were noticed in the maturation pool. 11 spawns were used for larval rearing.

In these experiments, complete spawning was noticed in all the shrimps. Within a moult cycle, a single female spawned a maximum of four times in Experiment 2 and three times in Experiment 3. On an average, three spawns per female were obtained from each shrimp within a moult cycle. In experiment 2, the shrimp responded for the first time 11-13 days after exposure to the treatment whereas in Experiment 3, the shrimp took 15-20 days for the first spawning. High variability in the number of eggs per spawn per female was observed in both the experiments. In both Experiment 2 and 3, the initial spawn was smaller. No apparent reduction in

Table 1. *Average number of eggs and nauplii spawned per female over the moult cycle in Experiment 2*

Spawn number	No. of females	Moult 1	Moult 2	Moult 3	Moult 4
Eggs					
Spawn 1 (Mean ± SD)	5	87750± 20250	167000± 52000	172500± 22500	108000± 42000
Spawn 2 (Mean ± SD)	5	93000± 3000	123750± 11250	137000± 22000	142500± 7900
Spawn 3 (Mean ± SD)	5	140000± 10100	128600± 22450	91000± 4000	
Nauplii					
Spawn 1 (Mean ± SD)	5	73500± 9000	14800± 58000	158750± 14250	80000± 35000
Spawn 2 (Mean ± SD)	5	79000± 2000	112750± 7750	120750± 20250	130500± 3320
Spawn 3 (Mean ± SD)	5	135000± 9760	117350± 6920	74250± 8250	

spawn size due to repetitive spawning was noticed either between the spawns within a moult cycle or between the spawns in different moult cycles. In fact, the third spawn in the first moult cycle was significantly larger than the first and second spawning (Table 1). In experiment 2, largest number of eggs were produced in Moult 2 and 3 (4.2 and 4.0 lakhs each, respectively), when all the three spawns are combined (Fig.1). However, total number of eggs produced in Moult 4 was comparatively lesser(3.7 lakhs). On an average, a single female produced 15 lakhs of eggs during the experimental period of 60 days. The performance of shrimps in Experiment 3 was relatively better. Shrimps survived for five moults and spawns were obtained in all the moult cycles. As in Experiment 2, maximum number of eggs were obtained from moult 2 and 3 (5.9 and 6.3 lakhs each, respectively) (Fig.2) and the quantum of eggs produced in subsequent moults, (moult 4 and 5) were only 50% of that obtained from moult 2 and 3. (Table 2). Furthermore, only two spawnings were obtained in moult 4 and 5. Though total egg pro-

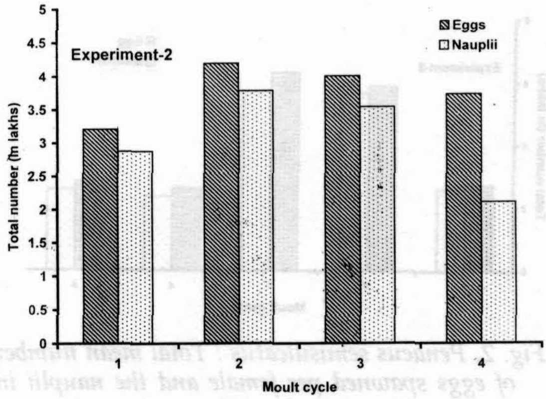

Fig. 1. *Penaeus semisulcatus : Total mean number of eggs spawned per female and the nauplii in each moult cycle in Experiment 2.*

Table 2 : *Average number of eggs and nauplii spawned per female over the moult cycle in Experiment 3*

Spawn number	N	Moult 1	Moult 2	Moult 3	Moult 4	Moult 5
Eggs						
Spawn 1 (Mean ± SD)	5	55500± 4500	130500± 25524	204000± 51846	135000± 39000	81000± 21000
Spawn 2 (Mean ± SD)	5	94500± 28500	139500± 34500	141000± 27000	126000± 0.0	200000± 0.0
Spawn 3 (Mean ± SD)	5	122400± 18200	320000± 6500	286400± 11400		
Nauplii						
Spawn 1 (Mean ± SD)	5	48500± 3500	123467± 29428	191000± 52235	120000± 36000	75000± 18000
Spawn 2 (Mean ± SD)	5	91500± 28500	131000± 36000	102000± 21000	114000± 0.0	180000± 0.0
Spawn 3 (Mean ± SD)	5	118210± 2450	312000± 8360	276800± 5280		

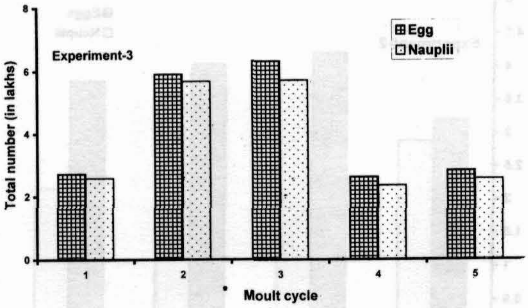

Fig. 2. Penaeus semisulcatus : Total mean number of eggs spawned per female and the nauplii in each moult cycle in Experiment 3.

duction declined over the moult cycle, individual shrimp still produced large number of eggs as can be observed in the 10th spawning of one specimen in moult cycle 5 (Table 2).

In Experiment 3, the first spawn in the first moult cycle was smaller than the subsequent spawns. Spawn size gradually increased with each spawn and the third spawning in second moult cycle yielded 3.2 lakh of eggs (Table 2). An increasing trend in the spawning performance similar to Experiment 2 was observed (Table 2). As in Experiment 2, a single female spawned 2,00,000 eggs in the elevanth spawn with a high percent hatch (90.0%). Average number of eggs produced by the shrimps per spawn in these experiments (1,42,417 ± 52,020) were significantly not different from that had been produced by wild spawners (1,52,243 ± 45,955) of the same size (p<0.01). The cumulative production per spawn during the entire experimental period (Experiments 2 and 3) is shown in Fig.3.

Percent hatch of eggs in captive specimens (89.6±7.8) were higher but not significantly different (P<0.05) from the wild specimens (82.0 ± 11.7). There was no significant difference in the percent hatch of repeat spawns with in a moult cycle nor was there significant difference in the percent hatch over the moult cycle (Table 3 and 4). There was no apparent trend toward reduced hatchability of eggs over the first three spawns in Experiment 2 and hatchability of spawn 3 was in fact higher (96.4%) (Table 3). When percent hatch of all respective spawns in each of

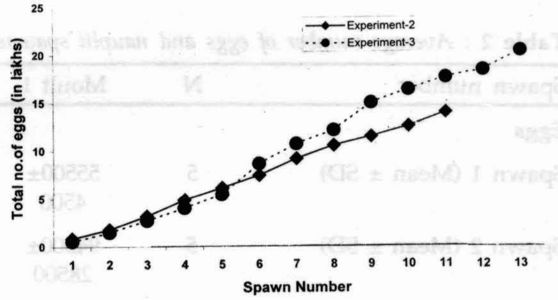

Fig. 3. *Cumulative production of eggs by Penaeus semisulcatus in Experiments 2 and 3.*

Table 3 : *Percent hatch of spawns over the moult cycle in Experiment 2*

Experiments	Spawn No.	Moult 1	Moult 2	Moult 3	Moult 4
Experiment 2	Spawn 1	86.0±9.6	86.2±7.9	92.3±3.8	72.5±4.3
Experiment 2	Spawn 2	85.0±0.6	91.3±2.0	88.1±0.7	91.6±0.0
Experiment 2	Spawn 3	96.4±0.0	91.3±0.0	87.1±0.3	

Table 4., *Percent hatch of spawns over the moult cycle in Experiment 3*

Experiments	Spawn No.	Moult 1	Moult 2	Moult 3	Moult 4	Moult 5
Experiment 3	Spawn 1	87.5 ± 0.8	94.0 ± 3.7	92.8 ± 3.0	88.6 ± 1.1	93.1 ± 1.9
Experiment 3	Spawn 2	96.6 ± 1.1	93.3 ± 2.8	72.2 ± 1.1	90.5 ± 0.0	90.0 ± 0.0
Experiment 3	Spawn 3	96.6 ± 0.0	97.5 ± 0.0	96.6 ± 0.0		

the moult cycle from Experiment 2 and 3 are pooled, no declining trend in percent hatch was noticed over the moult cycle.

Table 5 show the spawning periodicity of *P. semisulcatus* from Experiments 2 and 3. In Experiment 2, the shrimps took an average 5.5 ± 2.5 days after the moult for the first spawning, 4.0 ± 2.0 days for the second spawning and 4.0 ± 1.0 days for the third spawning. In Experiment 3, the spawning periodicity was 7.0 ± 1.0 days, 5.2 ± 0.6 days and 4.0 ± 1.5 days for the first, second and third spawnings, respectively. No trend toward an increase in the spawning interval over the first eight spawns were noticed.

Discussion

Repetitive spawning and consistent egg production of viable eggs have been achieved by environmental control and feed management in *Penaeus semisulcatus* without resorting to eyestalk ablation. Domestication of the species was also achieved by applying the same method. This suggests that with proper broodstock management, reliable egg production could be achieved in *Penaeus semisulcatus* without eyestalk ablation. Browdy and Samocha (1985 a and 1985 b) achieved maturation and spawning of this species for the first time by unilateral eyestalk ablation and then in subsequent experiments without ablation. Though maturation and spawning of several penaeid species have been achieved without eyestalk ablation (Chamberlain and Lawrence, 1981; Emmerson, 1980, 1983; Muthu *et al.,* 1986), using different methods, the observations were mostly restricted to a single moult cycle. Beard and Wickens (1980) reported no reduction in the number of eggs per spawn over the moult cycle in *P. monodon* whereas Primavera *et al.,* (1979) observed an increase in the number of eggs per spawn over time. Browdy and Samocha (1985 b) found a reduction in the size of consecutive spawns

Table 5. Spawning periodicity in *Penaeus semisulcatus*

Experiment	Spawning interval (days) Spawn 1 Mean ± SD	Spawn 2 Mean ± SD	Spawn 3 Mean ± SD
Experiment 2	5.5 ± 2.5	4.0 ± 2.0	4.0 ± 1.0
Experiment 3	7.0 ± 1.0	5.2 ± 0.6	4.0 ± 1,5

within the moult cycle of *P. semisulcatus.* In the present study, an increase in the spawn size over time was noticed upto three moult cycles indicating that repeated spawning neither affect the spawn size nor the hatchaility. Primavera and Borlongan (1977) observed a decrease in hatch rate for successive spawns of ablated *P. monodon.* But no such reduction in percent fertility, percent hatch and percent metamorphosis of repeat spawns were observed in ablated and nonablated *P. semisulcatus* (Browdy and Samocha, 1985 b).

In the present study, high quality spawn could be collected over a period of 60 to 90 days after exposing the shrimps to a steady pH of 8.1-8.3, light intensity of 500 lux and feeding with a mixed diet. The experiment was discontinued after mortality of a few specimens. The shrimps did not survive beyond this period and it is not known whether the mortality was due to weakness consequent upon repeated spawning. Hepatopancreas of these specimens were found to be orange in colour after few days of beginning of the experiment and whether this is indicative of any stress is not understood. Maturation and repetitive spawning of viable eggs from captive specimens of *P. indicus* (144-186 mm in total lenght and 28-60 g in weight) were achieved over a period of *nine months in the same* laboratory by applying same technique (Maheswarudu *et al.,* 1995).

Recirculation system with high circulation rate and with partial exchange once in week (600% exchange in a day) was found to be very effective in broodstock management in this study. The consistent reproductive response of the shrimps in the present study may be due to their acclimation to the stable environment of the recirculation system unlike the flow through system where the environmental parameters are likely to fluctuate. However, completely closed recirculation system may not be desirable for shrimp maturation unless the altered pH and inorganic carbon levels are restored (Wickens, 1976; Bray and Lawrence, 1992). Muthu *et al.* (1986) induced maturation of *P. indicus* by maintaining the shrimps in recirculation system in which pH was maintained between 8.1 and 8.2. Several investigators have used recirculation systems with either inbuilt or external filters (Beard *et l.,* 1977; Lumare, 1979). Reduction in pH and inorganic carbon in the recirculation system can be effectively regulated by regular addition of sodium carbonate or sodium hydroxide to the water. Stocking shrimps in excess of the carrying capacity of the filter and poor water recirculation are factors which may upset the carrying capacity of the filter. Accumulation of ammonia and organic matter are also possible if the recirculation system is not maintained properly and their build up may interfere with the maturation processes. The spawning success obtained in the present study shows that environmental conditions in the recirculation systems are favourable for inducing maturation, mating and spawning. Besides, the light intensity (mean, 500 lux) in the maturation system and the food also plays a major role in maturation of the shrimps. Browdy and Samocha (1985 b) obtained good results with ablated and nonablated *P. semisulcatus* when exposed to light intensity of 0.1 to 0.3 micro Einstein/m^2/sec.

The present study also shows that adequate nutrition is necessary for successful maturation of *P. semisulcatus.* Feeding the shrimps with fresh clams, frozen squid meat and especially chopped oligochaete worm *Pontodrilus bermudensis* probably provided the nutrients necessary for quick ovarian development. Middleditch *et al.,* (1980) provided circumstantial evidence that fatty acid component of the bloodworms are responsible for the ovarian stimulation in *P. setiferus.* The consistent egg production obtained in the present study may be related to feeding the shrimps with a mixed diet containing the worms and further study has confirmed the role of *Pontodrilus bermudensis* in inducing maturation in the crayfish *Procambarus clarkii* (Maheswaudu *et al.,* 1995). Chamberlain and Lawrence (1981) are of the view that a combination diet incorporating squid, shrimp, bloodworms, and clams enhance maturation of *P. vannamei* and *P. stylirostris,* rather than feeding with a single diet.

Induced maturation of penaeid shrimps by environmental regulation has several advantages over the conventional eyestalk ablation technique, as it avoids the removal of the eyestalk which harbours several hormones that control various physiological activity in the shrimp. Bray and Lawrence (1992) have summarised the results obtained by several investigatiors on effect of eyestalk ablation on reproduction of penaeid prawns. Ablation has been reported to result in high mortality, reduced fecundity, lower hatch rate over time, and decline in spermatophore transfer. However, Browdy and Samocha (1985 a) observed no significant reduction in broodstock survival and percent hatch of repeat spawns in ablated *P. semisulcatus,* compared to the unablated shrimps. Since the shrimps in the present study were not subjected to any hormonal stress, they might have responded favourably for a longer period.

The data from this study indicates that the reproductive potential of the captive broodstock could be maintained for a prolonged period with proper broodstock management practices. A small number of breeding stock is sufficient to produce massive quantity of eggs needed for stocking commercial hatcheries, which could substantially reduce the investment for buying the expensive broodstock. Reliable maturation and spawning of broodstock is required for a commercial hatchery to run for a prolonged period without depending upon the wild spawners. Commercial application of this maturation technique may need further standardization of the broodstock holding facility, environmental conditions and the diet by which captive reproduction of the penaeid shrimp could be controlled and reliable production of quality spawns could be achieved for a prolonged period.

References

Aquacop. 1979. Penaeid reared broodstock : closing the cycle of *P. monodon, P. stylirostris* and *P. vannamei*. *Proceedings World Mariculture Society* 10 : 445 - 452.

Beard, T.W., J.F. Wickens and D.R. Arnstein. 1977. The breeding and growth of *Penaeus merguiensis* de Man in laboratory recirculation systems. *Aquaculture* 10 : 275-289.

—— and —— 1980. Breeding of *Penaeus monodon* Fabricius in laboratory recirculation systems. *Aquaculture,* 20 : 79-89.

Bray, A.W. and A.L. Lawrence. 1992. Reproduction of *Penaeus* species in captivity. 5 In : *Marine Shrimp Culture : Principles and Practices* (Edited by A.W. Fast and L.J. Lester) Elsevier Science Publishers B.V., USA, 93-169.

—— and —— 1985 a. The effect of eyestalk ablation on spawning, molting and mating of *Penaeus semisulcatus* De Haan. *Aquaculture,* 49 : 19-29.

Browdy, C.L. and T.M. Samocha. 1985 b. Maturation and spawning of ablated and nonablated *Penaeus semisulcatus. Journal World Mariculture Society,* 16 : 236-249.

Brown, A., McVey, J. P., Middleditch, B. S and Lawrence, A. L. 1979. Maturation of White Shrimp (*Penaeus setiferus*) in captivity. *Proc. World Maricul. Soc.,* 10 : 435-444.

Campbell, R.C. 1967. Statistics for Biologists. Cambridge University Press, London.

Chamberlain, G.W. and A.L. Lawrence. 1981. Effect of light intensity and male eyestalk ablation on reproduction of *Penaeus stylirostris and Penaeus vannamei. Journal World Mariculture Soceity,* 12 (2) : 357-372.

Emmerson, W.D. 1980. Induced maturation of prawn *Penaeus indicus. Marine Ecology Progress Series,* 2(2) : 121-131.

—— 1983. Maturation and growth of ablated and nonablated *Penaeus monodon* Fabricius. *Aquaculture,* 32: 235-241.

Lawrence, A.L., Y. Akamine, B.S. Middleditch, G. Chamberlain and D. Hutchins. 1980. Maturation and reproduction of *Penaeus setiferus* in captivity. *Proceedings of the World Mariculture Society,* 11 : 481-487.

Lumare, F. 1979. Reproduction of *Penaeus kerathurus* using eyestalk ablation. *Aquaculture,* 19 (3) : 203-214.

Maheswarudu, G., H. A. Sharma, P. S. Reddy and M. Fingerman. 1995. Isolation of organic compounds from the littoral oligochaete, *Pontodrilus bermudensis* that induce ovarian maturation in the red swarmp crayfish, *Procamberus clarkii. American Zoologist,* 35(5) : 68A.

——, E.V. Radhakrishnan, N. Neelakanta Pillai, M.R. Arputharaj and A. Ramakrishnan. 1995. Repetitive spawning in *Penaeus indicus* without eyestalk ablation, hatching rate and growth up to juveniles. *Fishing Chimes,* 16 (3) : 21-23.

Middleditch, B.S., Missier, S.R., Hines, H.B., Chang, E.S., McVey, J.P., Brown, A. and Lawrence, a. L. 1980. Maturation of penaeid shrimp : lipids in the marine food web. *Proceedings World Mariculture Society,* 11 : 463-470.

Muthu, M.S., Laxminarayana and K.H. Mohamed. 1986. Induced maturation and spawning of *Penaeus indicus* without eyestalk ablation. *Indian J. Fish.,* 33 (2) : 246-250.

Primavera, J.H. Young, T. and De Los Reyes, C. 1979. Survival, maturation, fecundity and hatching rates of unablated and ablated *Penaeus indicus* H.M. Edwards from brackish water ponds. *D. Res. Rep. Aquacult. Dep. Southeast Asian Fish. Dev. Cent.*3(3): 14-17.

——, J.H. and E. Bolongan. 1977. Notes on ovarian rematuration of ablated sugpo (prawn) *Penaeus monodon* Fabricius. *Quarterly Research Report of the Southeast Asian Fisheries Development Centre,* 3 : 5-8.

Wickens, J. F. 1976. The tolerance of warm-water prawns to recirculated water. *Aquaculture,* 9 : 19-37.