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47 Broodstock development and spawning in *Penaeus indicus*

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ABSTRACT

Among cultivable native species, *Penaeus indicus* occupies an important position due to its tolerance to wide range of salinity, fast growth, compatibility in high density culture, wide distribution and demand in export market. *P. indicus* broodstock can be developed using adults collected from the wild or from grow out ponds or by reusing healthy females after spawning in the hatchery. The size of prawn used for broodstock development should preferably be above 145 mm T.L. (20 g) for females and 140 mm T.L. (17 g) for males. Pens, cages, tanks with or without flow through system, recirculation system etc. are in vogue for broodstock development. 100 t tanks with insitu biological filter is used for the broodstock development in CMFRI. Stocking density @ 4 prawns per cubic meter (sex ratio 1 : 1) was found ideal. Three tanks of the above size can meet the broodstock requirements of a hatchery of 18 million capacity. Unilateral eyestalk ablation of females proved to be effective. Electrocautery apparatus was used for this purpose. Experiments also revealed that unablated *P. indicus*, can also be induced to mature and spawn by environmental and feed manipulation. Unablated *P. indicus* maintained in recirculation system and fed with intertidal oligochaetes, clam and squid meat attained maturity and spawned repeatedly over a prolonged period. Maintaining the pH of water between 8-8.2 and controlling the light intensity in broodstock tanks found to hasten maturation.

Introduction

Over fifty countries of the world have taken up shrimp farming during the last one and a half decades consequent upon the high demand for shrimp, coupled with their limited availability in the conventional fishing grounds. India, with its background in traditional shrimp farming, has also started semi intensive and intensive shrimp farming making use of the vast brackishwater and coastal areas. Timely supply of quality seed is the prime requisite in shrimp farming. Collection of seed

from the wild is often beset with problems in conservation of natural resources. Therefore the only option to produce quality seed is by establishing a chain of hatcheries all along the coast. Year round availability of spawners can only ensure successful operation of hatcheries. As spawner availability from the wild is often erratic and undependable, maintenance of captive broodstock has become a necessity. A captive broodstock also reduce the risk of diseases and offer scope for genetic manipulation as well.

Among the cultivable native species *Penaeus indicus* occupies an important position next only to *P. monodon* due to the latter's tolerance of wide range of salinity, fast growth, compatibility in high concentration culture, wide distribution, and demand in export market.

Different aspects on the reproduction and spawning of penaeid prawns have been reviewed by Wickins (1976 b); Muthu and Laxminarayana (1982); Muthu (1983); Rao (1983); Primavera (1983) and Browdy (1992). Some important aspects on the reproductive biology, captive broodstock development and spawning of *P. indicus* are presented in this paper.

Reproductive system and maturity stages

Sexual dimorphism is prominent in *P. indicus*. In males full gonadal maturation is characterised by the presence of fully developed spermatozoa with spikes. Generally, swelling and whitish colouration of the terminal ampoules are considered as an indication of gonadal maturity. Fully mature ovary is dark green in colour, occupies major portion on the dorsal side of the animal along its entire length and clearly discernible through the dorsal exoskeleton. It has lateral expansions in the first abdominal segment. Mature ova measure 240 μ to 270 μ in diameter. Rod like peripheral bodies radiate from the opaque central region of the ovum. Five ovarian maturity stages namely, immature, early maturing, late maturing, mature and spent recovering has been described by Subrahmanyam (1965), Rao (1968) based on histological studies and colour, size and ova diameter respectively. Since the thelycum is 'closed-type' in *P. indicus* mating takes place between hard shelled male (intermoult) and newly moulted (post-moult) female which is having immature gonad, to facilitate insertion of spermatophore in the seminal receptacle in the thelycum. During spawning, female releases eggs through the oviducts and sperm from the spermatophores stored in the thelycum simultaneously and fertilization takes place externally.

Broodstock management

Broodstock can be developed using adults collected from the wild or from grow out ponds or by reusing healthy females after spawning in the hatchery. The age and size of prawns used are critical. Female of *Penaeus indicus* should be larger than 145 mm in total length (20 gram) and the males larger than 140 mm in total length (17 gram) (Silas, *et al.* 1985; Jetani *et al.*, 1996 and Maheswarudu *et al.*, 1996). Primavera *et al.* (1982) used three months old pond reared specimens of *P. indicus* for induced breeding experiments.

The different systems in vogue for broodstock maintenance and development are pens and cages, tanks with or without flow through systems, and recirculation systems. Pens and cages are established in sheltered bays free from pollution having 4 to 6 m water depth, less wind velocity and less wave action. Bamboo pens of 250 sq. m. area were used for *P. monodon* in Philippines by SEAFDEC (Wear and Santiago, 1976; Santiago, 1977; and Rodriguez, 1979). Same types of pens could be used for *P. indicus* also. In flow-through system, rectangular or circular tanks made of cement, ferrocement, fibreglass and collapsible pools are used. Sea water filtered through sand or biological filter is passed continuously through the tanks. Flow through rate is 700% per day (Moore *et al.*, 1974, Aquacop, 1975; 1977 a and b and 1979). Primavera *et al.* (1982) used 12 m³ (4 x 3 x 1) ferrocement tanks for flow-through system in which *P. indicus* attained maturity with and without eye stalk ablation. Water filtered through sand bed was used at the flowthrough rate 200-300% per day.

Experiments carried out by Central Marine Fisheries Research Institute (C.M.F.R.I.) proved that 100 ton capacity out door tanks (10 x 10 x 1 m) can be used for the maintenance of *Penaeus indicus* broodstock. A net of the same size and shape of the tank (1 cm mesh size) is made and kept in position inside the tank before introducing the broodstock, so as to enable periodic observations of shrimps easier. The sand-filtered sea water of 32-34 ppt salinity is pumped into the tank. The tank is then stocked with broodstock at the rate of 4prawns/m³ of seawater. The sex ratio is maintained at 1:1. *P. indicus* hatchery with a production capacity of 18 million seed/annum requires totally 1200 broodstock shrimps, which could be easily maintained in 3 tank of hundred ton each.

In recirculation system water is recirculated through subgraval filter or biological filter fitted *insitu*. Muthu and Laxminarayana (1980) successfully maintained and attained maturity in *P. indicus* (ablated and unablated) by using 3.6 m circular plastic lined pool fitted with subgraval filter through which sea water was recircu-

lated by airlifts. In this system the metabolite ammonia is oxidised into nitrates by the activity of bacteria colonising the biological filter. Muthu (1983) reported very low levels of ammonia (0.02-0.07 mg/l) and nitrite (0.0003-0.0012 mg/l) in recirculation system of *P. indicus* and *P. monodon*. The maintenance of pH at 8.2 in the pool accelerates the ovarian maturation in penaeid prawns (Aquacop, 1975; 1977 b, 1979; Muthu and Laxminarayana, 1977; Muthu *et al.*, 1984 and 1986 and Maheswarudu *et al.*, 1996). The pH can be adjusted to 8.2 by replacing part of the water with fresh sea water every day and by adding sodium carbonate or bicarbonate in required quantities regularly.

Adequate precaution may be taken to avoid transmission of diseases through broodstock as well as to avoid any kind of stress to broodstock animals while maintaining them in captivity. The broodstock prawns are treated with 100 ppm formalin for about half an hour for disinfection before transferring to broodstock tanks. If needed an antibiotic dip treatment with Erythromycin or Prefuran or Oxytetra-cycline can be given for ten minutes at 50 ppm (Joshua *et al.* 1993). *P. indicus* was maintained in maturation system for 357 days by subjecting to dip treatment of 15 ppm Furacin for 30 minutes once in every 3 months (Maheswarudu *et al.*, 1996).

Stress in stocked prawns is mainly caused by over crowding, improper handling and poor water quality. Prawns of a biomass of over 300 g/m² did not attain maturity (Aquacop, 1979). The ideal stocking density advised by Muthu (1983) is 3-7 animals/m² for *P. indicus*. In maturation pool, Muthu (1983) observed that impregnated female of *P. indicus* having black patches on thelycum did not produce viable eggs. He also observed abnormal sperms without spikes or disintegrated sperms in spermatophores extracted from the terminal ampoules of male *P. indicus* during hot summer months. Broodstock get cuticular lesions when kept for a long time in maturation tanks and these prawns cannot produce viable eggs even when they mature and spawn. Females of *P. indicus* after 280 days of maintenance in the rematuration system and infected with fungus showed either non-development of ovary or developing ripe-ovary and reabsorbing (Maheswarudu *et al.*, 1996).

Generally males and females of *P. indicus* are maintained at 1:1 or 1:2 ratio in maturation tanks to ensure successful mating as reported by Emmerson (1980) and Emmerson *et al.* (1983), Primavera *et al.*, (1982), Aquacop, (1983), Muthu *et al.*, (1984 and 1986), Jetani *et al.*, (1996) and Maheswarudu *et al.*, (1996). Muthu (1983) reported that if spermatophores are extracted from male terminal ampoules of *P. indicus* another set of spermatomorphores are ready after two hours and he suggests that one male can fertilise more than one female in a day.

Induced maturation

Induced maturation refers to the ovarian maturation of female prawns only, as males mature readily in captivity without inducement. Endocrinal, environmental and nutritional manipulations are generally used to induce maturity of prawns in captivity.

In penaeid prawns, hormonal mechanism of reproduction is not known fully but it may be similar to that in other decapod crustaceans (Adiyodi and Adiyodi, 1970 and 1974). As in other decapod crustaceans, the process of egg production in the ovary is a cyclic phenomenon which is under the hormonal control of the neurosecretory centres in the X organ sinus gland complex, brain and thoracic ganglia. The studies made by Kulkarni and Nagabhushanam (1980) and Nagabhushanam and Kulkarni (1982) in *Parapenaeopsis hardwickii* revealed that the neurosecretory centres in the X organ sinus gland complex produces hormones (GIH) which inhibit the vitellogenesis. In contrast to this, the neurosecretory centres in the brain and thoracic ganglia produce hormones (GSH) which promote the vitellogenesis. X organ produces high titre value of GIH during the quiescent phase of ovary and this restrains the vitellogenesis either directly or acting through neurosecretory centres which produce GSH. When physiological and environmental factors are amenable or conducive for reproduction the titre of the GIH secreted by X organ sinus glands complex is reduced and then the process of vitellogenesis is accelerated under the influence of GSH. The technique of eyestalk ablation, first demonstrated in crustaceans by Panouse (1943), has been attempted in penaeid prawn for induced maturation on the basis of this process. By removing eyestalk, the titre of GIH is reduced and then GSH accelerates the ovarian maturation. This technique has been used in penaeid prawns for rapid development of ovary in captivity by doing unilateral eyestalk ablation as well as bilateral eyestalk ablation. The various works on this aspect reveal that unilateral eyestalk ablation gives best result of ovarian maturation, spawning, hatching and higher survival rate in larval rearing (Muthu and Laxminarayana, 1977; Emmerson, 1980 and Jetani *et al.*, 1996). Muthu and Pillai (1991) observed that after spawning when the females were subjected to unilateral eyestalk ablation and maintained under controlled conditions and fed with fresh clam meat, they matured and spawned releasing viable eggs on the third day.

The experiments conducted by Primavera *et al.* (1982) has revealed that *P. indicus* mature and spawn either subjecting to eye stalk ablation or without resorting to eyestalk ablation. Ablated females produced ten fold spawnings, eight fold eggs

and six fold nauplii over unablated females. However, high hatching rate of eggs was found in unablated females.

Latency period is the required time for ovarian maturation from the eyestalk ablation. In *P. indicus* the latency period is 9-27 days (Muthu and Laxminarayana, 1977). The interval between two consecutive spawnings is reduced to 3-15 days in ablated females compared to a minimum of 10 days and 2.7 months in unablated wild females (Primavera *et al.*, 1982 and Rao, 1968). A gap of 2 months period is the required time for eggs to mature between the two spawnings in wild females during reproductive season (Rao, 1968). In ablated females, Primavera *et al.*, (1982) observed a decline in hatching rate with successive spawnings in a single intermoult or with successive moult cycles. This may be attributed to the insufficient reserves in hepatopancreas as the interval between two successive spawnings was reduced to as short as 3 days with rapid maturation and over stimulation of spawning.

Emmerson (1980) correlated the events of moult cycle with that of reproductive cycle. Maturation proceeds through intermoult and premoult period followed by spawning during intermoult and premoult period and then mating occur immediately after ecdysis when the female has undeveloped ovaries. Eyestalk ablation during intermoult period is advisable to achieve maturation in less than a week. Ablation during premoult period results in moulting and hence latency period is extended for 2-4 weeks. Ablation during postmoult period leads to mortality due to additional stress on the animal and loss of haemolymph. In maturation tanks unablated females showed higher survival than ablated females and in males survival was higher than females due to non-ablation and handling stress (Primavera *et al.*, 1982).

In penaeid prawns, eyestalk ablation methods used are electrocauterisation (Muthu and Laxminarayana, 1979 and 1980), cutting the eyestalk near the base with a pair of scissors (Arnstein and Beard, 1975 Lumare, 1979), scissor cutting followed by cauterisation with pencil-type soldering iron (Caillouet, 1972), pinching of eyestalk (Aquacop, 1977), squeezing the eye ball contents out (Rodriguez, 1979), incision on eye ball and squeezing out the contents (Kelemec and Smith, 1980). Among these methods electrocauterisation is advisable because haemolymph loss is minimised as cut is sealed immediately.

The important environmental factors that influence ovarian maturation are light, temperature, salinity and pH. Light seems to play a significant role in ovarian maturation of penaeid prawns in captivity. Its effect was studied by Emmerson, (1980); Emmerson *et al.* (1983) Aquacop (1983), Primavera *et al.* (1982). The role of

temperature on ovarian maturation in non-ablated *P. japonicus* and *P. orientalis* was studied by Laubier-Bonichon and Laubier, (1979). *P. indicus* attains maturity rapidly at the water temperature range 28-30°C in the maturation pools (Muthu, 1983). Salinity is a limiting factor for ovarian maturation and *P. indicus* attains maturity at a salinity more or less equal to that of sea water. Ablated females of *P. indicus* matured and spawned when pH was maintained between 8.0 and 8.2 in maturation pool. When pH was dropped to 7.9, ablated females of the same species did not attain maturity (Muthu *et al.*, 1984).

The diet, rich in protein and lipid is essential for ovarian maturation. Mohamed and Diwan (1992) found that in *P. indicus* ovary is autotrophic during initial stages of yolk synthesis and in later stages lipids are mobilised from hepatopancreas to gonad through haemolymph. Among lipids polyunsaturated fatty acids (C 20:4, C 20:5, C 22:6) are essential for ovarian maturation in penaeid prawns (Middleditch *et al.* 1979 and 1980). These long chain unsaturated fatty acids are not synthesised by prawns (Kanazawa and Teshima, 1977; and Kanazawa *et al.*, 1979a, b and c). The incorporation of these fatty acids in the diets for broodstock in adequate quantities for ensuring ovarian maturation is imperative. Clams, mussels and polychaete worms are advisable food for broodstock as these animals are good sources for long chain poly-unsaturated fatty acids (Middleditch *et al.*, 1979 and 1980 a & b). As the aminoacid profile of clam meat and squid meat is similar to that of prawn, these two animals are good source of protein for prawns (Deshimaru and Shigueno, 1972). *P. indicus* performed repetitive spawning for prolonged period in recirculation system when fed intertidal oligochaete, clam meat and squid meat *ad libitum* (Maheswarudu *et al.*, 1996).

The use of frozen mussels as feed for *P. indicus* broodstock alters the reproduction performance. Alvarez del Castillo Cueto and Cahu (1989) obtained double the number of viable eggs from females fed with fresh mussels as compared to those fed with frozen mussels. Dietary vitamin E plays significant role on the reproduction and spawning of *P. indicus*. Broodstock when fed with compound diet containing vitamin E 300 mg/kg produced eggs which gave higher hatching rate and healthy larvae in subsequent larval stages (Cahu and Fakhkakh, 1990). Broodstock are fed *ad libitum* on dry feed and wet feed at the rate 3-5% and 10-30% of the body weight respectively. Total feed is divided into 4-5 doses a day.

Spawning

Impregnated mature females are transferred in the evening to the spawning tanks containing 200 l sea water filtered through a 50 micron mesh. Only one spawner is introduced in each tank and mild aeration is provided. Disodium salt of EDTA at the rate of 0.1 g/100 l is added. Tank is covered with black lid to protect the female from strong light and prevent it from jumping out of the tank. The optimum temperature, salinity and pH are 27-30°C, 30-34 ppt and 8.0-8.2 respectively in the spawning medium (Silas, *et al.*, 1985). Induced maturation experiments carried out at CMFRI revealed that the number of eggs per spawner varied from 7500 to 3,36,000.

Remarks

Summing up, the salient features on the broodstock development, maintenance and spawning of *P. indicus* are: i) *P. indicus* attains maturity in captive conditions with or without eyestalk ablation, ii) females of about 145 mm TL (> 20 g) may be selected for induced maturation: iii) electric cauterisation is ideal for eyestalk ablation as it prevents loss of haemolymph; iv) captive prawns fed with diet having essential aminoacids and long chain PUFA matures faster, v) pH of sea water to be maintained between 8-8.2 for better results, vi) controlling the intensity of light on broodstock tanks can hasten maturation and vii) spawners kept individually can ensure compete and uninterrupted spawning.

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