# PENAEID BROODSTOCK DEVELOPMENT AND MANAGEMENT

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## Introduction

In the context of the global interest in the aquaculture of penaeid prawns, production of large quantities of quality prawn seed in hatcheries has become imperative. For efficient planning of hatchery operations, ripe spawners of the desired species should be readily available at the proper time. In Japan where there is an organised industry for the capture and marketing of live-prawns, obtaining spawners for the hatcheries is not a problem. But in all other countries where there is no such trade, getting spawners from the wild by trawling is expensive and uncertain. This has generated keen interest in the induced maturation of penaeid prawns under controlled conditions and in the development and management of prawn broodstock as anintegral part of the hatchery.

The research work conducted in many parts of the world from 1971 to 1979 on problems connected with induced breeding of marine prawns in captivity were reviewed by Muthu and Laxminarayana (1982). In the present discussion the recent advances in this rapidly growing field of research are included and the results of these investigations carried out during the past decade are evaluated with special reference to management of penaeid broodstock for producing spawners under controlled conditions.

## Reproduction of Penaeid Prawns

At the outset, some aspects of penaeid reproduction relevant to broodstock developmet and management are summarised here. In nature penaeid prawns breed only in the sea. They are sexually dimorphic, the male being generally smaller than the female. The males may become mature even in brackishwater ponds but the females never attain full ovarian development in such ponds. The males produce non-motile sperms which are packed inside spermatophores. At the time of mating the male transfers the spematophores with the help of its petasma to the thelycum of the female. In penaeids with a closed thelycum (e. g. *Penaeus indicus, P. monodon* etc.) mating takes place between a newly moulted "soft" females with immature ovaries and a mature male in the intermoult phase; the sepermatophores are tucked safely inside the seminal receptacles, dorsal to the thelycum where they are retained until the prawn moults again; there is a long time lag between mating and spawning. In penaeids with open thelycum (e. g. *P. styliferus, P. vannamei, P. stylirostris* etc.) mating takes place between a "hard" intermoult female with ripe ovaries and a hard mature male in the intermoult phase; the spermatophores are attached superficially on the surface of the thelycum and can easily be dislodged; spawning takes place soon after mating. In both types of penaeids, at the time of spawning the male is not present; the female simultaneously releases the eggs from the oviduct and the sperms from the spermatophores and fertilization takes place in the seawater.

The hormonal mechanism of reproduction in penaeid prawns is not fully understood but is likely to be similar to that of the other decapod crustaceans discussed by Adiyodi and Adiyodi (1970, 1974). Egg production in prawns, as in other crustacea, is a cyclic phenomenon under the hormonal control of the neurosecretory centres. Among these centres, the X-organ sinus gland complex in the eyestalk seems to produce a gonad inhibiting hormone (GIH) which inhibits vitellogenesis, while the centres in the brain and thoracic ganglia appear to produce gonad stimulating hormones (GSH) which promote vitellogenesis (Kulkarni and Nagabhushnam. 1980, Nagabhushanam and Kulkarni, 1982). During the quiescent phase of the ovary the X-organ seems to produce a high titre of GIH which restrains vitellogenesis either directly or through its action on the neurosecretory centres which produce the GSH. In nature, when the physiological and environmental factors are conducive to reproduction, the titre of the GIH secreted by the X-organ complex is probably reduced, thereby allowing vitellogenesis to take place under the influence of GSH. On the basis of this hypothesis the technique of unilateral eyestalk ablation has been evolved for inducing the penaeid prawns to mature in captivity. By removal of one eyestalk, the titre of the GIH is arbitrarily reduced and this leads to ovarian development.

## Induced maturation of penaeid prawns

By providing optimal environmental conditions and suitable feeds in land-based maturation facilities with flow through seawater systems, many species of penaeid prawns such as *P. californiensis* (Moore *et al.*, 1974), *P. japonicus* (Aquacop, 1975; Laubier - Bonichon, 1978); Laubier-Bonichon and Laubier 1979; Caubere *et al.*, 1979; *P. merguiensis* (Aquacop, 1975; Beard *et al.*, 1977), *P. vennamei* (Aquacop, 1977 b), *P. stylirostris* (Aquacop, 1979; Brown *et al.*, 1980), *P. setiferus* (Brown *et al.*, 1979), *P. indicus* (Primavera *et al.*, 1982) and *Metapenaeus ensis* (Aquacop, 1975) have been successfully made to mature and spawn naturally without eyestalk ablation.

But species which do not mature under these conditions have been induced to mature and spawn in captivity by the well-known eyestalk ablation technique. Some of the important species which have responded to this treatment are *P. merguiensis* (Alikunhi *et al.*, 1975; Nurijama and Yang, 1977), *P. monodon* (Aquacop, 1977 a, 1979, 1982; Santiago, 1977; Chen, 1977; Primavera, 1978 a, b, 1982; Primavera and Borlangan, 1977; Primavera *et al.*, 1978; Halder, 1978; Beard and Wickins, 1980; Tiensongrasmi, 1980; Muthu and Laxminarayana, 1981), *P. duorarum* (Caillouet, 1973), *P. aztecus* (Aquacop, 1975), *P. kerathuras* (Lumare, 1979); *P. plebejus* (Kelemec and Smith, 1980,) *P. orientalis* (Arnstein and Beard, 1975), *P. indicus* (Muthu and Laxminarayana, 1979, 1981). Even species that matured and spawned without eyestalk ablation responded to unilateral eyestalk ablation with more rapid gonadial maturation and repeated spawnings (Aquacop, 1979; Brown *et al.*, 1979; Lawrence *et al.*. 1980; Primavera *et al.* 1982).

## Bilateral vs. unilateral eyestalk ablation

Eversince Panouse (1943) discovered that eyestalk removal led to premature ovarian development, eyestalk ablation has been a research tool for exploring the hormonal mechanisms in crustaceans (Adiyodi and Adiyodi 1970). Idyll (1971) and Caillouet (1973) first applied this technique to induce maturation of penaeid prawns in captivity. But bilateral eyestalk ablation, although leading to rapid ovarian growth, did not result in spawning; the ova got reabsorbed without being released from the ovary (Caillouet, 1973; Duronslet *et al.*, 1975; Aquacop, 1975; Wear and Santiago, 1976). Very soon it was realised that unilateral eyestalk ablation successfully lead to ovarian maturation and subsequent spawning (Arnstein and Beard, 1975; Wear and Santiago, 1976). The lack of success with bilateral eyestalk ablation may be due to the fact that the crustacean eyestalk produces, apart from the GIH, a number of neurosecretory hormones which (1) regulate lipid metabolism and protein synthesis in the hepatopancreas, (2) induce hyperglycaemia in the blood to combat stress, (3) regulate calcium metabolism during cuticle formation, (4) affect the water balance during ecdysis, (5) inhibit production of moulting hormone by the *Y* – organ and (6) influence the movement of pigments in the chromatophores (Hignam and Hill, 1978). When so many vital physiological functions are jeopardised by bilateral eyestalk removal, it is bound to have repercussions of the gonads also. In unilateral eyestalk ablation the presence of one eyestalk seems to ensure normal functioning of all the metabolic processes.

## Methods of eyestalk removal

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, 1973), electrocauterisation (Muthu and Laxminarayana, 1979, 1981), pinching of eyestalk (Aquacop, 1977), squeezing the eyeball contents out (Rodriguez, 1979), incision of eyeball, squeezing out the contents and crushing the eyestalk (Primavera, 1978a and b) and incision of eyeball followed by enucleation of contents (Kelemec and Smith, 1980) are some of the methods used for getting rid of the eyestalk ganglia containing the neurosecretory organs which secrete, store and release the GIH. Electrocautery seals the cut instantly, avoiding blood loss and ensures cent percent survival (Muthu and Laxminarayana, 1981). In the other methods, some loss of blood is inevitable but the procedures are simple.

## Source of broodstock

Broodstock of prawns have been constituted from immature adults caught from the sea (Moore *et al.*, 1974; Brown *et al.*, 1979; Rodriguez, 1979), from large sized prawns cultured in brackishwater ponds (Primavera, 1978a; Muthu and Laxminarayana, 1981) or from postlarvae grown to adult size in controlled systems (Laubier – Bonichon, 1979; Aquacop, 1975, 1977b, 1979; Beard *et al.*, 1977). The source of the prawns is immaterial as long as the specimens have attained the size and age at which the species normally matures, The most convenient source of broodstock is, of course, the grow-out ponds where some of the prawns can be allowed to grow for a longer period to attain maturation size.

## Age of broodstock prawns

The Age at which the prawns are capable of maturation varies with the species.

| Species | Age at first maturation in months | Reference |
|---|---|---|
| *P. merguiensis* | 4–5 | Aquacop (1975) |
| *Metapenaeus ensis* | 8 | |
| *P. japonicus* | 12 | |
| *P. aztecus* | 12 | |
| *P. monodon* | 7–12 | Aquacop (1979) |
| *P. vannamei* | 12–15 | |
| *P. stylirostris* (Panama strain) | 7–9 | |
| *P. stylirostris* (Mexican strain) | 5–7 | |
| *P. merguiensis* | 6–7 | Beard *et al.* (1977) |
| *P. monodon* | 12–15 | Primavera *et al.* (1978) Santiago (1977) |
| *P. monodon* | 5 | Primavera (1978 a) |
| *P. indicus* | 4 | Primavera, *et al.* (1982) |
| *P. indicus* | 4–6 | Personal observations. |

Primavera (1982) has shown that in *P. monodon* the quality of the eggs (in terms of viability of the larvae) produced by the 5 month old pond reared females are inferior to the eggs produced by 1–2 year old wild females. She feels that the older females from the sea are more responsive to induced maturation than the younger females from the ponds, although the latter may be equal in size to the former due to the higher growth rate in the pond environment. She recommended that the pond reared *P. monodon* females should be allowed to remain in the grow-out ponds for about one year before they are utilised for induced spawning by eyestalk ablation.

### Sex-ratio

In the broodstock pools usually males and females are kept in the ratio of 1:1. But Aquacop (1975) observed that for *P. merguiensis* 25% males were sufficient for regular fertilization. Caubere *et al.*, (1979) also successfully used only 30% males in their experiments with *P. japonicus*. Primavera (1982) reported that a male : female ratio of 1:2 produced the highest percentage of spawnings, highest average fecundity and the greatest number of eggs in *P. monodon* and recommended the ratio as economical because it maximises the number of females per tank. The present author found that if spermatophores are extracted from the terminal ampoules of male *P. indicus* another set of spermatophores become ready for extrusion within two hours thereby indicating that the same male is capable of fertilising more than one female in a day.

### Latency period

Literature on eyestalk ablation reveals that maturation after eyestalk removal can be very rapid. Some females are able to develop full ovaries and spawn in 3–5 days but if moulting occurs soon after eyestalk removal the maturation period is extended to 2–3 weeks (Aquacop, 1979). Generally, the white prawns such as *P. merguiensis, P. indicus, P. vannamei* and *P. stylirostris* mature more rapidly (3–4 days) than *P. monodon* and *P. aztecus* (3 weeks). Lumare (1979) found that ablated *P. kerathurus* kept at a constant temperature of 25° C took 43–69 days to spawn in November – December, 30 days in March and only 10 days in May – June, i. e. they appear to mature faster as their natural breeding season is approached.

### Rematuration

Unilaterally ablated females repeatedly mature and spawn viable eggs (Primavera, 1978b, 1982; Primavera and Borlangan, 1977; Aquacop, 1979, 1982; Lumare, 1979; Brown *et al.*, 1980; Lawrence *et al.*, 1980; Beard and Wickins, 1980). In one intermoult period of 20 – 30 days, *P. monodon* has been observed by Beard and Wickins (1980) to spawn upto 6 times, at intervals of 3–5 days without appreciable decline in number of the eggs spawned; but the hatching rate declined in later spawnings. This shows that one impregnation was sufficient to fertilize several batches of eggs spawned within the intermoult period. The decline in hatching rate of the later batches of eggs perhaps indicates reduction in number and viability of the sperms towards the end of the intermoult period. In two successive intermoult periods a single *P. monodon* spawned eleven times in 2 months, 6 times after the first mating and 5 times after the second mating (Beard and Wickins, 1980).

Even unablated females kept in flow through systems have been observed to remature and spawn repeatedly (Moore *et al.*, 1974; Aquacop, 1975; Laubier-Bonichon and Laubier, 1979 Caubere *et al.*, 1979, Beard *et al.*, 1977 and Brown *et al.*, 1979).

### Egg quality

The quality of the eggs spawned by eye ablated *P. monodon* has been studied by Aquacop (1977 a) who described 4 types of eggs. Type 1 eggs are unfertilized, characterised by several unequal big "cells"; Type 2 eggs have fragmented internal membrane and do not develop into nauplius. Type 3 eggs have asymmetrical embryo that dies in the egg or hatches out as weak nauplius and Type 4 eggs are the normal eggs with symmetrical embryo. They found that if the mature females are isolated and kept in a separate 2 $m^3$ tank and fed with *Troca* flesh for 2 days before spawning, the quality of the eggs produced by such females was better than that of the eggs produced by females which were directly transferred from the broodstock pools into the spawning tanks (Aquacop, 1979). Primavera and Posadas (1981) recognised 5 egg types in *P. monodon,* A1, A2, B, C and D. The A1 eggs are the normal eggs giving rise to healthy nauplii, A2 eggs are slow in development and hatch out into abnormal nauplii with deformities, B eggs are unfertilised with irregular cytoplasmic formations; C eggs are unfertilised with no segmentation at all and D eggs are also unfertilised with the cytoplasm invaded by bacteria. The hatching rate was directly proportional to the number of A1 eggs in the spawning. Primavera and Posadas (1981) recommended that if the hatching rate fell below 30% it is better to discard that batch of eggs, as such spawnings are likely to give rise to weak larvae. They also found that unablated wild spawners from the sea produced the largest number of eggs with the highest proportion of A1 eggs (49.3%), followed by ablated wild stock (38.9%), while ablated pond stock produced the least number of eggs with the lowest percentage of A1 eggs (23.5%). Efforts to improve egg quality in ablated pond stock by better nutrition and broodstock management are urgently needed.

## Physical facilities used for broodstock maintenance

Maintaining marine animals in captivity is difficult, but making them breed in captivity is still more difficult. They should be provided with an environment which simulates their natural habitat. Three types of systems have been used to maintain penaeid brood stock viz. (i) marine pens (ii) flow-through systems and (iii) recirculating systems.

## Marine pens

Bamboo pens 250 $m^2$ in area, constructed in sheltered tidal bays and coves where the depth of water is 4–6 m, have been used in the Philippines by the SEAFDEC (Wear and Santiago, 1976; Santiago, 1977; Rodriguez, 1979) to keep the broodstock of *P. monodon.* The pens are constructed of bamboo slats tied to a framework of bamboo poles and lines on the inside with nylon netting of 1.5 cm mesh. A large net made of similar nylon webbing is hung inside the enclosure touching the mud bottom. The prawns are held in this net which can be hauled up periodically to catch the prawns and examine them for signs of gonadial development. Free exchange of tidal water through the nylon netting brings in fresh oxygenated water and removes the metabolites from the pen. In fact, the prawns are living in their own natural environment which is only circumscribed by the pen.

**Flowthrough systems**

On the west coast of Mexico, Moore *et al.,* (1974) have used 23 x 3 x 0.6 m raceways under inflated polyethelene bubble canopy to hold the broodstock of *P. californiensis.* Seawater from a well on the seashore was pumped continuously through the system, the flowthrough rate being 700% per day.

In France, Laubier-Bonichon and Laubier (1979) and Caubere *et al.,* (1979) employed circular tanks 2.9 m and 5 m in diameter with flowthrough rates of 450 litres and 200 litres per hour respectively. Since they were working with *P. japonicus,* a burrowing species, their tanks had a substratum of sand over a false bottom through which water was recirculated by air lifts to prevent anaerobic conditions from developing in the sand.

In Tahiti, Aquacop (1975, 1977 a & b, 1979) held their prawn broodstock *(P. monodon, P. merguiensis, P. japonicus, P. vannamei, P. stylirostris, P. semisulcatus, P. aztecus* and *M. ensis)* in 4 m diameter circular fibreglass tanks. The tanks had a substratum of coral sand. Oceanic seawater pumped directly from the lagoon outside was supplied to the tanks through perforated concentric PVC pipes embedded in a layer of gravel below the coral sand. The water welling up through the substratum dislodged the detritus from the bottom and kept them in suspension. These suspended particles left the tank along with the out-flowing water which drained through two concentric drain pipes in the centre of the tank. The water exchange rate was 2 - 3 times a day. At the SEAFDEC, Philippines the Aquacop model was adopted (Tolosa, 1978); instead of fibreglass the tanks had ferrocement walls.

Primavera *et al.,* (1978) also tried using a 7.25 x 7.25 x 1 m concrete tank having limited water renewal for *P.monodon* broodstock with less success.

In the U. S. A., Brown *et al.,* (1979) and Brown *et al.,* (1980) used 3 m circular fibreglass tanks with central drain pipe and no substratum on the tank bottom for *P. setiferus* and *P. stylirostris* respectively. The tanks had continuous flowthrough of seawater with a turnover rate of 1.8 to 4 times a day. Lawrence *et al.,* (1980) utilised a 4.9 m circular tank with the bottom, which had no substrate, sloping towards the middle, for holding the broodstock of *P. setiferus;* 80–90% of the water was exchanged every 2–3 days.

**Recirculation systems**

In U. K. Beard *et al.,* (1977) have successfully bred *P. merguiensis* in 2.9 x 1.65 x 0.3 m concrete tanks fitted with sub-gravel filters through which water was recirculated by air-lift pumps. 50% of the water was renewed every week. Beard and Wickins (1980) used similar tanks for *P. monodon* but the water was recirculated by pumping through a biological filter which was kept outside the holding tanks.

Lumare (1979) in Italy made use of 2 x 2 x 1 m cement tanks fitted with sub-sand filter through which seawater was recirculated at 6 times the water volume per day, for keeping a broodstock of *P. kerathurus;* 1/3 of the water was replaced every day.

Kelemec and Smith (1980) in Australia employed a 2.4 x 1.5 x 0.6 m tank fitted with sub-gravel filter and air-lift recirculation for making *P. plebejus* mature in captivity, the recirculation rate was 6 times the water volume every hour and water replacement varied from 2.5% to 5% per day.

At the Narakkal Prawn Culture Laboratory of the CMFRI, 3.6 m circular plastic lined pools fitted with sub-gravel filters, through which seawater is recirculated by air-lifts, are used for the broodstock (Muthu and Laxminarayana, 1980).

## Merits and demerits of the different systems

Among the three types of systems mentioned above, the marine pens offer the most natural environment for the broodstock but they also have a number of drawbacks; (i) Calm protected bays suitable for construction of the pens may not be available in the neighbourhood of the hatchery. (ii) The bamboo enclosures get worn out easily and have to be repaired or replaced frequently. (iii) For examining the prawns the whole net has to be hauled up subjecting the prawns to considerable stress. (iv) There is no control over the environmental parameters.

The other two systems which are land based and relatively more permanent could form part of the hatchery and make use of the seawater pumping and aeration facilities, already available at the hatchery, for the broodstock tanks. They are also amenable to greater environmental control and facilitate closer observation of the maturing prawns. If good quality seawater is freely available, the flowthrough systems are to be preferred; the faster the rate of water flow the better it is for the broodstock, as the metabolites will not be allowed to accumulate in the system. But if the maturation facility is situated far away from the sea, the recirculation systems come in very handy. In this case it is imperative that the seawater in the system be recirculated through a biological filter, which may be inside the tank or outside it, to oxidise the toxic ammonia secreted by the animals into relatively harmless nitrates through the activity of the bacteria that grow on the filter material (Spotte, 1970).

In the land-based maturation facilities a substratum of sand is provided for burrowing species such as *P. japonicus, P. aztecus, P. semisulcatus P. duorarum* and *P. vannamei*. But for non burrowing species such as *P. indicus, P. merguiensis, P. setiferus, P. stylirostris, P. monodon* etc., the tank need not have a sandy substratum. It is advantageous to have a plain bottom from the point of view of tank cleanliness.

In recirculating systems it is advantageous to keep the biological filter outside the holding tanks so that the prawns that dash around the pool at the time of capture do not get injured by abrasion against the filter components. Further a built-in sub-gravel filter or sub-sand filter competes for available oxygen in the tank water and will lead to rapid depletion of oxygen when aeration is stopped due to current failure or other mechanical reasons.

## Factors that affect maturation and Spawning of Penaeid Broodstock

A number of factors have been observed to affect the process of maturation and spawning of the captive broodstock. They are classified under three major heads viz. physical factors, water quality and biological factors and discussed in this section.

**Physical factors**

Some information on the effect of light, temperature and pressure on the reproduction of penaeid prawns in captivity are available.

**Light**

It is well known that light is an important factor influencing the gonadial maturation in a number of animals. Some work has been done on the effect of photoperiod on the maturation of the sub-tropical species such as *P. japonicus* (Laubier-Bonichon and Laubier, 1979; Caubere *et al.*, 1979) and *P. kerathurus* (Lumare, 1979). Although these prawns matured when the photoperiod was gradually increased it cannot be concluded that increase in photoperiod was solely responsible for making them mature because in the experiments on *P. japonicus* the temperature was also simultaneously increased in a gradual manner. Further, Lumare (1979) found that *P. kerathurus* matured faster in November - December when the light period was 9 hrs per day than when it was 12 hrs per day. Therefore, when the evidence is not conclusive even in species living in sub-tropical regions where the seasonal change in photoperiod is relatively marked, it is unlikely that the photoperiod has any effect on tropical penaeids accustomed to a more or less equal day / night regime throughout the year.

However, the intensity of light seems to affect the maturation process. In almost all the investigations referred to in the foregoing sections, the maturation pools were either kept inside a room with weak artificial lighting or the tank had covers that reduced the light intensity to 10-40% of natural day light. In nature the prawns breed on the bottom of the sea where the intensity is bound to be very low. Strong light in the maturation pools may be a source of stress to the prawns, especially the non-burrowing species. In this connection it may be interesting to mention that Brown *et al.*, (1979) found that *P. setiferus* when kept in a circular tank painted white on the inside sustained injuries by dashing against the wall, but quietened down when the walls were painted black. Emmerson (1980) working with eye ablated *P. indicus* found that by painting the broodstock pools black on the inside, the maturation process could be accelerated and the number of eggs produced by the females increased. More work is needed on the relationship between light intensity and maturation in penaeid prawns. The optimum light intensity is likely to vary for different species depending on their burrowing habits, swimming behaviour etc.

The colour of the light may have some influence on the maturation process as the blue component of light is predominant at the bottom of the sea where the prawns live and spawn. The experiments of Caillouet (1973) and Alava (1979) on the effect of colour of light on maturation of *P. duorarum* and *P. monodon* respectively were, however, inconclusive.

**Temperature**

Temperature of the water in the maturation pools is bound to influence the rate of all physiological processes including the maturation of the gonads. Laubier-Bonichon and Laubier, (1979) and Caubere *et al.*, (1979) found that *P. japonicus* matured and spawned when the temperature was gradually increased from 15° C to 24° C over a period of 3 months. Aquacop (1979) found that *P. monodon* matured and spawned throughout the year if the temperature was above 24° C. The tropical species of penaeids have been found to mature rapidly when the temperature of the water in the maturation facilities was between 28 – 30° C.

### Pressure

Since prawns are demersal in habit it was thought that pressure might influence the reproductive process. Caubere *et al.*, (1979) found that mature females, subjected to a pressure of 2.5 kg per $m^2$ (the pressure at 20 m depth in the sea) for 12 hrs, spawned. But mature females also spawned even without pressure treatment. Pressure does not seem to have any effect on the maturation process either, since Beard *et al.*, (1977) have obtained full maturation of gonads in *P. merguiensis* kept in a tank with only 0.3 m depth of seawater.

### Water quality

The quality of water in the broodstock pools has a profound influence on the maturation of the prawns. It stands to reason that the water quality should be as close to that of natural seawater the native medium in which penaeid prawns attain full maturity and spawn.

### Salinity

The fact that even those penaeid species which spend their juvenile life in estuaries and brackishwaters migrate to the sea for spawning, suggests that salinity is an important factor that affects the maturation process. This gains credence from the observations of Morris and Bennett (1952), Johnson and Fielding (1956), Liao (1973), Chen (1976), Muthu and Sampson Manickam (1978), Licatowich *et al.*, (1978) and Rodriguez (1981) who have reported female penaeids with mature gonads from coastal ponds and lagoons where the salinity was equal to that of seawater. In all the maturation facilities the salinity of the water is maintained between 27 – 34 ppt. However, comparative experiments have not been conducted to study the effect of different salinities on the maturation of the penaeids in captivity.

### pH and inorganic carbon

In recirculation systems pH declines rapidly due to the physiological activity of the prawns stocked in the pool and may become a limiting factor when it reaches 7.3 (Wickins, 1976a). The process of bacterial oxidation of ammonia to nitrates by the biological filter also leads to reduction in pH and loss of inorganic carbon from the water, both of which affect the calcification of the cuticle and the normal moulting process in prawns (Wickins, 1976b). So a completely closed system of recirculation is not desirable; at least a part of the water has to be replaced by fresh seawater every day or required amounts of sodium carbonate or bicarbonate added regularly to maintain the pH and inorganic carbon content of the water in the pools. Maturation of the broodstock has been most rapid when oceanic water, having a steady pH of 8.2 was continuously flowing through the system (Aquacop 1975, 1977b, 1979).

## Ammonia and nitrate

The toxicity of ammonia and nitrate to penaeid prawns has been studied by Wickins (1976). He found that the maximum acceptable level of ammonia (the concentration at which growth is reduced only by 1-2% compared to the controls) is 0.1 mg $NH_3 - N$ / litre for penaeid juveniles. Wickins (1976a) also observed that at a nitrite level of 6.5 mg $NO_2 - N$ / litre the growth of juvenile *P. indicus* was reduced to 50% of that of the controls. *P. japonicus* was found to be more sensitive to nitrite, 5% mortality occurring at 0.1 mg $NO_2 - N$ / litre (Mevel and Chamroux, 1981). While these values may be appropriate for normal growth of prawns in culture systems, the broodstock prawns may be more sensitive requiring much lower concentrations of ammonia and nitrite for gonadial maturation. At the NPCL the total ammonia concentration in the broodstock pools fitted with biological filters is as low as 0.02 - 0.07 mg ammonia N / litre and the nitrite level only 0.0003 to 0.0012 mg $NO_2 - N$/litre. It is obvious that as long as the biological filters are functioning properly, ammonia and nitrite will not pose problems for the broodstock prawns.

## Biological factors

Biological factors such as food, physiological stress, diseases and injuries and mating success also affect the reproduction of the broodstock prawns.

## Food

Broodstock prawns have generally been fed *ad libitum* on fresh or frozen mussel, clam, oyster or squid meat. A high protein diet rich in essential amino-acids and long chain polyunsaturated fatty acids appear to be necessary for maturation of ovaries. Deshimaru and Shigueno (1972) found that the aminoacid profile of clam and squid meat is very similar to that of prawn flesh and hence these two organisms are excellent sources of protein for feeding cultured prawns. Brown *et al.*, (1980) and Lawrence *et al.*, (1980) have used polychaete worms to feed the broodstock of *P. stylirostris* and *P. setiferus* respectively with encouraging results. The efficacy of the polychaete diet is attributed to the fact that these worms are very rich in long chain polyunsaturated fatty acids (C 20:4; C 20:5; C 22:6) which are found to be essential components of ovarian lipids of penaeid prawns (Middleditch *et al.*, 1979; 1980a). The prawns are not capable of biosynthesizing these long-chain unsaturated fatty acids (Kanazawa and Teshima, 1977; Kanazawa *et al.*, 1979a 1979b, 1979c) and hence these fatty acids should be supplied in the diet of the broodstock prawns in adequate amounts for proper ovarian growth. On the basis of the fact that in several animals the C 20 fatty acids are precursors of prostaglandians which have been found to play a vital role in the reproduction of higher animals. Middleditch *et al.*, (1979) suggested that the reproduction of prawns is also mediated by prostaglandins derived from these fatty acids. The clams and mussels fed to the broodstock prawns usually have mature gonads which may be supplying the essential fatty acids and carotenoids needed for the ovarian development of prawns. The fact Middleditch *et al*., (1980b) have found that bivalves are rich in C 20:4, C 20:5 and C 22:6 fatty acids.

## Stress

Stress due to handling, overcrowding and poor water quality is a major factor inhibiting the maturation of gonads in captivity. *P. monodon* is particularly sensitive in this respect (Aquacop, 1979); regression of developing ovaries is frequently observed in this species. To avoid handling stress, Aquacop (1979) and Primavera (1982) used an underwater torch at night for determining the stage of maturation of the gonads. The light tied to a long pole is held close to the prawn in such a way that the beam of light strikes perpendicular to the upper part of the body making the dark green mature ovary prominently visible.

Aquacop (1979) found that in broodstock tanks stocked with a biomass of over 300 gm/m$^2$, the prawns did not attain maturity. The usual stocking density in land-based maturation tanks is 3-7 animals per m$^2$, the lower density being preferred for larger species such as *P. monodon* (Muthu and Laxminarayana, 1982)

Prawns stop feeding when the water quality deteriorates due to inefficient functioning of the biological filter, disruption in aeration or recirculation of water, accumulation of unused food etc. and this leads to resorption of the developing ovaries in the broodstock prawns (personal observation).

## Mating success

Although males easily attain sexual maturity in captivity even under brackishwater conditions, instances of inability to mate with females in the maturation facilities have been observed by a number of workers (Arnstein and Beard, 1975; Brown *et al.,* 1979; Beard and Wickins, 1980; Kelemec and Smith, 1980; Aquacop, 1979 and personal observat ions), the reason for this is not quite clear. Primavera (1979) felt that the mating behaviour of *P. monodon* calls for a large pool with sufficient area for swimming about freely if impregnation is to take place normally. But Aquacop (1979); Brown *et al.,* (1679), Beard and Wickins (1980) and Kelemec and Smith (1980) have reported lack of impregnation in broodstock kept in large tanks also. Aquacop (1979) found that the proportion of impregnated females was less in penaeids with open thelycum (eg. *P. vannamei* and *P. stylirostris*) because the spermatophores could be easily dislodged. However, they found that by keeping the males and females in separate tanks and introducing only the ripe females into the male tank a few hours before spawning, the impregnation of the female could be assured. Similarly, Bread and Wickins (1980) observed that even in *P. monodon* which has a closed thelycum impregnation is assured if the female is kept separafe from the males and introduced into the male tank when it is about to moult. Separation of males and females seems to increase the attraction between them.

## Diseases and injuries

Brown *et al.*, (1979) have reported that *P. setiferus* males in the broodstock pools were susceptible to a *Vibrio* infection of the terminal ampoule which damaged the structure of the spermatophore, making it impossible for the spermatophores to stick to the open thelycum of the females. At the NPCL the present author has frequently observed black patches on the thelycum of impregnated female *P. indicus* kept in the broodstock pools; such females invariably produced non-viable eggs. The present author has also observed abnormal sperms without spikes or completely disintegrated sperms inside spermatophores extracted from the terminal ampoule of male *P. indicus* kept in the broodstock pools, specially during the hot summer months. Broodstock kept for a longer time in the pools also get cuticular lesions on the abdomen and such females are not able to produce viable eggs, although they may mature and spawn.

## Conclusion

Development and managemet of penaeid broodstock as part of a hatchery system has benefitted by a multidisciplinary approach. Such diverse disciplines like endocrinology, reproductive biology, nutritional physiology, waste water treatment, patho-biology, hydrology, engineering etc. have contributed to progress in this field. of research. It is now possible to make almost all the commercially important species of penaeid prawns to mature and spawn in captivity, either through manipulation of environmental parameters or through eyestalk ablation. However, more scientific knowledge is urgently needed on (1) the normonal mechanisms involved in reproduction of the prawns (2) the role of dietary components in promoting ovarian growth (3) the spawning triggers (4) the factors that affect fertilization (5) the sexual behaviour of males (6) sperm viability and (7) diseases that affect the broodstock. A greater understanding of the ways in which the environmental and biological factors affect the maturation and spawning processes in prawns is necessary for improving the quality and quantity of the eggs produced by the captive broodstock. New engineering designs to improve the efficiency and reduce the construction costs of the physical facilities such broodstock tanks, biological filters, flow-through and recirculating systems etc. are also necessary.

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