

## Chapter Two

# MATURATION, REPRODUCTION, AND BROODSTOCK TECHNOLOGY

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Maturation generally refers to physiological maturity or the development of the gonads or primary reproductive organs producing eggs and sperm capable of fertilization. However, maturation may also refer to functional maturity or the ability to mate with the completion of the secondary sexual organs. In penaeids, the secondary genitalia - thelycum and petasma - develop ahead of the ovaries and the testes.

This review describes first maturation in *Penaeus monodon*; endocrine, nutritional, and environmental methods of induced maturation; broodstock constitution; maintenance and other operations; and a comparison of maturation tanks, pens, and cages.

## BROODSTOCK

### Female Maturation

According to Motoh (1981), *P. monodon* females with CL above 47 mm have structurally complete thelyca, consistent with his observation that most wild females of this size were positive for spermatozoa. In ponds, however, minimum size of *P. monodon* with sperm was smaller at 39 mm CL.

Ovarian maturation stages of *P. monodon* have been classified using *in vivo* examination or histological criteria of size and kind of ova. Motoh (1981) described five categories for wild *P. monodon*--undeveloped (Category 1), developing (2), nearly ripe (3), ripe (4), and spent (5)--based mainly on progressively increasing mean ovum diameter (from 35 to 235 microns) and external ovarian appearance. Also using external examination and dissected specimens, Primavera (1982) came up with five similar groupings and gave the equivalent stages used

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in actual sampling of broodstock at SEAFDEC AQD (Stages 0, I, II, III, IV, and V). Based on histology, Tan-Fermin and Pudadera (in press) reduced the number of stages for wild *P. monodon* to only four: (1) previtellogenic stage - oogonia to oocytes in chromatin nucleolus and/or perinucleolus eosinophilic (equivalent to Stage 0); (2) vitellogenic stage - eosinophilic yolky oocytes (Stages I and II); (3) cortical rod stage - oocyte with peripheral rod-like bodies (Stages III and IV); and (4) spent stage - remaining oocytes with yolk and/or cortical rods, thicker follicle layer and few dark irregular perinucleolar oocytes (Stage V). Mean and maximum oocyte diameter were also significantly different among the four groups.

### **Male Maturation**

Male *P. monodon* become capable of mating with the complete fusion of the petasma (by means of numerous hooks) at 34 mm CL (Motoh 1981). Physiological maturity with the presence of ripe sperm was noted at 31 and 37 mm CL for pond and wild males, respectively. However, Motoh (1981) observed that sperm in wild males below 37 mm CL showed only a body (without tail or spike). Primavera (1978) observed that both pond and wild males of at least 40 g body weight produced mature sperm while Ruangpanit et al (1985) noted immature males without sperm from both pond and wild *P. monodon* of unspecified size.

Rather than sacrifice the males in the process of checking under the microscope for spikes, hatchery workers use the more practical method of looking for enlargement and opaque whitening of the terminal ampoules that indicate spermatophores containing mature sperm.

### **Mating**

*P. monodon* is a closed thelycum penaeid in which the prerequisite to mating or spermatophore transfer is the molting of the female.

Precopulatory behavior in *P. monodon* starts with the attraction of one to three hard-shelled males to a newly molted female which they follow as she makes brief upward movements (Primavera 1979). When one male positions itself directly below

the female, the pair engages in parallel swimming movements during which the male tries to align his ventral side to that of the female. If successful, the male quickly shifts from parallel position to perpendicular to the female. He curves his body in a U-shape around the female, flicking head and tail simultaneously, presumably inserting the spermatophores inside the thelycum at this time.

Mating requires a minimum water volume and depth; limited frequency and success have been experienced in small and shallow tanks (Primavera 1979, Poernomo and Hamami 1983). Complete darkness (Aquacop 1980) as well as bright floodlights (Primavera, unpubl.) can also hinder mating in *P. monodon*. Salinity may have no effect on mating because females caught from brackishwater estuaries and ponds possess sperm in their thelyca.

Fertilization of eggs from initial spawnings of captive broodstock is dependent on sperm from mating in the wild or pond environment. Failure of mating in captivity will ultimately lead to unhatched (unfertilized) eggs due to loss of spermatophores once the female molts. The decrease in hatch rates in *P. monodon* spawns, averaging 96% up to 10 days after ablation down to 0% afterwards (Muthu and Laxminarayana 1977), may be traced to non-mating of captive broodstock.

With unsuccessful mating of broodstock, artificial spermatophore transfer is being tested in *P. monodon* and other penaeids. Spermatophores are extracted either manually or electrically and inserted in the thelycum of a newly molted female. While Muthu and Laxminarayana (1984) obtained a low hatch rate of 2.4% from only one out of 10 attempted transfers, higher mean hatch rates of 71.87% and 82.35% were produced by insertion of one and two spermatophores, respectively, by Lin and Ting (1986), all on ablated *P. monodon* females. *In vitro* fertilization gave 49.4-63.1% hatching when the sperm homogenate was added right after spawning (Lin and Ting 1986).

### Source of Broodstock

*P. monodon* broodstock may be obtained from the wild or from ponds. Wild broodstock are caught from coastal waters by tide-dependent stationary gear such as corrals, lift and lever

nets, and by trawlers and other fishing boats. It has been observed that wild broodstock (and wild spawners) from brackishwater areas give lower hatch rates compared to those from offshore waters (Posadas 1986).

In Aquacop (1983), the rearing of *P. monodon* broodstock involves a series of 3-stage pond transfers over 9-12 months during which density from 10-20/m<sup>2</sup> is gradually decreased to 1-2/m<sup>2</sup> with feeding of artificial and fresh feeds. Pond broodstock used at SEAFDEC AQD are grown under extensive conditions of low stocking density and natural food with or without supplementary feeding over 6 months in a single pond without transfer or with transfer to a second compartment. Age at ablation varies from 5 (Primavera 1978) to 15 months (Santiago 1977) from spawning. Satisfactory maturation, spawning, and metamorphosis of larvae to post-larvae have been obtained from females with a minimum age of 8 months (Millamena et al 1986; Primavera unpubl.). In the wild, *P. monodon* attains full maturity and spawning at 10 months (Motoh 1981).

Although pond-reared prawns have produced good quality larvae, the state-of-the-art is to use wild broodstock in places where *P. monodon* is indigenous and easily available. The first reason for this is the non-availability of pond broodstock because holding the prawns beyond the regular 3-4 month cropping period means a longer turnover period and economic loss to the farmer. Second, wild broodstock give a faster turnover and produce in 4-8 weeks the same number of larvae that pond broodstock can yield in 8-12 weeks. Lastly, pond stock require a maturation pellet often not commercially available in addition to natural food such as squid, mussel, and trash fish, whereas wild broodstock can be maintained on natural food alone.

### **Transport, Acclimation, and Prophylaxis**

Ideally, the hatchery should be located close to wild and pond broodstock sources to minimize transport stress and costs. The prawns may be transported in sea water provided with aeration and ice, if necessary to lower temperature. A one-ton tank (canvas, fiberglass, etc.) can accommodate up to 400 prawns if travel time is one hour or less. With periods of 4-5 hours, not more than 200 prawns/ton should be stocked. Early morning or

late afternoon transport is recommended to avoid high midday temperatures; otherwise, ice can be added to the transport water.

Upon arrival, the prawns are disinfected in 25-50 ppm formalin and acclimated over a one-week period. Few or no additional deaths indicate that the animals have recovered and are ready for ablation.

## Stocking

Stocking density of *P. monodon* in tanks is 2-7/m<sup>2</sup> depending on the water quality and exchange rate and prawn size such that biomass should not exceed 300-400 g/m<sup>2</sup> (Primavera 1985).

Sex ratios are generally maintained at 1 ♀: 1 ♂ to ensure mating success. Higher female ratios (1.5-3 ♀:1 ♂) are more economical because egg and larval production per tank are maximized (Pudadera et al 1980a).

## INDUCED MATURATION

Male penaeids generally mature in captivity so that induced maturation mainly concerns females. Three basic approaches have been employed to induce ovarian maturation in penaeids--endocrine (ablation), nutritional, and environmental. So far, maturation in *P. monodon* has been induced only through eyestalk ablation although diet and environmental parameters may enhance reproductive performance.

Table 1 summarizes information on ablation, food, and environmental conditions for *P. monodon* maturation.

### Eyestalk Ablation

In decapod crustaceans, ablation of the eyestalk destroys the X-organ and sinus gland that produces and stores, respectively, the gonad-inhibiting hormone (Adiyodi and Adiyodi 1970).

In *P. monodon*, the eyestalk can be ablated or destroyed by means of incision-pinching, cutting, or cautery with the eye

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Table 1. Physiochemical parameters, stocking, feeding and water management in maturation tanks for *Penaeus monodon* (after Primavera 1985)

References	Ablation	Brood-stock source	Age	Stocking density (no/m <sup>2</sup> )	Sex ratio	Daily water exchange rate	Water management
Alikunhi et al 1975	+	pond			1:1		recirculating
Aquacop 1977a, b; 1979; 1980 1983	+	pond	9-12 mo	5	1:1	200-300%	flow-through
Santiago 1977	±	pond	15 mo	0.8	1:1		
Primavera 1978	+	pond	5 mo	4	1:1	30%/3 days	replacement
Primavera et al 1978	±	pond	1-2 yr	5-6	1-2:1	30%/3 days	replacement
Primavera et al 1979	+	pond		6	2:1	200-400%	flow-through
Pudadera et al 1980a	+	wild		4-5	2:1	200-400%	flow-through
Pudadera et al 1980b	+	wild		5	1-4:1	200-400%	flow-through
Pudadera and Primavera 1981	+	pond		6	1:1	200-400%	flow-through
Vicente et al 1979	+	wild & pond		7.5/ton	3.3:1		
Beard and Wickins 1980	+	captive		1.6	1:12	15%/wk	recirculating
Ruangpanit et al 1981	+	wild		13/ton	1:1		
Ruangpanit et al 1985	+	wild		4	1:1	100-200%	flow-through
Simon 1982	+	pond & wild	5-8 mo	7	1:1	100-250%	flow-through
Poernomo and Hamami 1983	+	pond		2.7	1:1	20-50%/2-3 days	replacement
Emmerson 1983	±	wild		2.2/ton	1:1	30%/2 days	replacement
Hillier 1984	+	wild		2.3	3:1		
Posadas 1986	+	wild		5-6.4	1:1	100%/2 wk	recirculating
Millamena et al 1986	+	pond	8 mo	4	1.3:1		flow-through

Temperature (°C)	Salinity	pH	Light intensity & quality	Photo- period hr light	Food
27.8-30.6	24-31				mysid, shrimp and arti- ficial pellet
25.5-30	34	8.2	10%, 100 lux	natural	squid, mussel, troca and pellet
22.5-33.2	33±1.6		outdoor pens		
23.8-26.2	30-34	7.8-8.1		natural natural	salted mussel salted mussel
24-34	27.6-30.2		60%	natural	pellet, mussel, squid
28.6-33.6		7.0-8.2	40-60%	natural	brown mussel and pellet
25-30.5	15.4-34.8	7.2-8.1	60%	natural	brown mussel and pellet
21.3-33.6	28-36	7.9-8.1	1,210- 3,500 lux: blue, red natural	natural	
26-28.5	32.3-34.1			natural	mussel, pellet, and fish
28±2	30±2		40-70 lux	19 hr	mussel and shrimp
26-8	30-31	7.8-8.0		natural	squid, cockle and prepared feed
28-31				natural	green mussel and cow liver
26.5-30.5	28-32		fluorescent light	14 hr	squid and clams
26-28	25-30			natural	squid, cockle and prepared feed
27±2.2	30±3		70 uW (reduced)	cm <sup>2</sup>	pellet, prawn
26-31			green	natural (12 hr)	
27.0-30.8	15, 25, 32	7.25-8.8	reduced	natural	pellet and mussel
26-31	30-32.5			natural	pellet, squid and annelids

removed totally or only partially retaining the outer corneal layer. The objective is to prevent the loss of body fluids and infection by the use of cautery or by retention of the corneal layer which eventually forms a scar (Primavera 1978).

The incision-pinching method commonly used for *P. monodon* and other penaeids has been described in detail by Primavera (1978). Females selected for ablation should be of a minimum size (80-90 g) or age (8-10 months for pond stock), healthy, and hard-shelled. The thelycum must be uninfected and appear slightly bulging or convex with whitish vertical streaks indicating the presence of spermatophores (Primavera 1983). Ablation is performed on either eye but an already diseased or damaged eye should be ablated to leave one healthy (unablated) eye. Santiago (1977) observed that ablation of a single eyestalk was sufficient to induce maturation in *P. monodon*.

Survival rates of 0%, 38% and 49% for bilaterally ablated, unilaterally ablated, and unablated *P. monodon*, respectively, were obtained after 196 days (Santiago 1977). Ablation-related mortality in *P. monodon* was observed by Primavera et al (1978) but not in Aquacop (1977a) or by Vicente et al (1979).

With reference to the molt cycle, ablation during the postmolt leads to mortality because of added stress on the female and excessive loss of hemolymph (Aquacop 1977a). Ideally, ablation should be undertaken during the intermolt for maturation to follow. Ablation during the premolt leads to molting with a subsequently longer latency period of 2-4 weeks before maturation in *P. monodon* (Aquacop 1979, Primavera et al 1979).

The latency period represents the interval between ablation and maturation/spawning and is affected by molt cycle stage, age, and source of broodstock and other factors at the time of ablation. Wild subadult *P. monodon* caught in mangroves took 40 days to mature and 69 days to spawn after ablation (Hillier 1984) compared to a minimum of only 3 days for wild adults from offshore (Primavera and Borlongan 1978, Simon 1982). Similarly, wild *P. monodon* from offshore Indian Ocean took only 4-5 days to spawn after ablation in contrast with 20-30 days for females from brackishwater Songkhla Lake



(Ruangpanit et al 1985). Salinity may therefore affect the latency period.

The great majority of captive maturation in *P. monodon* has been from ablated females. Very few workers have reported maturation in unablated females (Santiago 1977, Primavera et al 1978, Aquacop 1980, and Emmerson 1983) with successful spawns (16.7 to 82.0% hatch rate) only from Emmerson (1983).

The maximum number of spawns/molt cycle is greater from ablated (6) compared to unablated (3) *P. monodon* (Beard and Wickins 1980, Hillier 1984, and Emmerson 1983). Similarly, the proportion of first spawns in the molt cycle is higher for unablated (67%) compared to ablated (36%) *P. monodon*, meaning the latter have spawns mostly from rematurations (Emmerson 1983). Therefore, ablated females will tend to show a decline in fecundity, hatch rates, and egg viability given the greater number of spawns in a molt cycle. Nevertheless, many commercial hatcheries prefer to do eyestalk ablation because the resulting predictability in spawns and nauplii supply compensates for the decreased spawn sizes and spawn quality (Primavera 1985). Also, *P. monodon* broodstock are replaced 6-8 weeks after ablation (Simon 1982, Primavera 1983) so that only the first, or at most, second spawns in a molt cycle are harvested thereby eliminating the less viable later spawns.

## Nutrition

*Food sources.* Molluscs including squid and mussel (Primavera et al 1979), cockle and clams are commonly fed to *P. monodon* broodstock either alone or in combination with pellets. Aquacop (1979) obtained best results using a squid-containing pellet with 60% protein.

In the wild, 85% of ingested food of adult *P. monodon* consisted of small crabs and shrimps and molluscs (Marte 1980). The more frequent occurrence of molluscs and other non-crustaceans during months when *P. monodon* showed a higher feeding index may reflect changes in dietary requirements related to gonad development during the spawning season (Marte 1982).

Feeding of broodstock is at a daily rate of 3.5% for dry feed and 10-30% for wet feeds (Primavera 1983) or *ad libitum*

once to three times a day. Marte (1980) observed that feeding activity of female *P. monodon* was significantly higher than that of males.

*Lipid requirement.* Wild immature *P. monodon* females showed an increase in ovarian lipid levels upon reaching full maturity from 5.8 to 17.0% and from 7.5 to 21.9% in unablated and ablated females, respectively (Millamena et al 1985). The fatty acid profile showed 12.14-24.87% and 11.81-21.50% total fatty acids in unablated and ablated females, respectively, consisting of 20:4w6, 20:5w3, and 22:6w3 fatty acids. Similar proportions of the same polyunsaturated fatty acids (PUFA) were found in the spawned eggs, indicating their importance in the reproductive process.

Another experiment assessed the effect of different lipid sources on maturation and spawning of ablated pond-reared *P. monodon* (Millamena et al 1986). Reproductive performance in terms of number of spawns and production of eggs and nauplii was superior in females given pellets with cod liver oil (longer chain C<sub>20</sub> and C<sub>22</sub> PUFA and lower w3:w6 ratio).

In Aquacop (1979), *P. monodon* matured on pellets alone spawned unfertilized eggs and therefore early maturing females were fed fresh troca univalves to improve egg viability. Conversely, an all-natural food diet (squid and marine worms) gave the lowest spawning and hatching rates from ablated *P. monodon* (Primavera et al 1979). In contrast, wild *P. monodon* broodstock gave satisfactory results with only natural food in the absence of pellets.

*Feeding rhythm.* Wild adult *P. monodon* were given two feed types (squid and pellet) at three daily feeding frequencies over a one-week period to determine feeding rhythm (Primavera et al 1987). The prawns consumed significantly greater amounts of squid per feeding as frequency decreased (3.2 g every 3 h, 6.7 g every 6 h, and 10.2 g twice daily). There was no significant difference in amount of feed consumed at one feeding time for either feed type at any feeding frequency. Nevertheless, there was a general trend for greater amounts of squid consumed in the afternoon and evening hours compared to morning - the 3-h feeding frequency showed a major peak at 1800 h (dusk) and a minor peak at 1200 h (noon).

## Environment

Even under ideal conditions, eyestalk ablation causes some degree of stress to the female prawn. It should therefore be considered a stop-gap measure until less stressful methods, along environmental and dietary manipulation, are developed. Among various environmental parameters, the control of light appears to be the most promising.

*Light.* Maturation tanks may depend on natural light under a roofed structure or an artificial light inside a completely enclosed building with walls and ceiling.

*Light intensity.* Reduced light levels down to  $70 \text{ uW cm}^{-2}$  led to faster maturation and spawning in unablated and ablated *P. monodon* (Emmerson 1983, Hillier 1984). Dark covers may also reduce intensities in maturation tanks (Primavera 1983) to around 200 lux or less. Wurts and Stickney (1984) recommend that light intensities for captive maturation should simulate levels in the natural spawning grounds of the species based on observations that light intensities used for *P. setiferus* have reached up to 4000 times those in the natural spawning grounds.

*Light quality.* Unablated *P. monodon* attained only partial maturation under blue and natural light but not under red light (Pudadera and Primavera 1981). More recently, Primavera (unpubl.) obtained maturation in unablated *P. monodon* subjected to different wavelengths in tanks. Green light gave best results ( $2.7 \times 10^6$  nauplii, 63.1% hatch rate) followed by natural light ( $0.78 \times 10^6$  nauplii, 86.7% HR) with poor results under cool white light and blacklight blue light. In a follow-up study using only green light, ablated females produced  $8.25 \times 10^6$  nauplii with 61.8% HR compared to  $0.54 \times 10^6$  nauplii and 68.4% HR from unablated females (Primavera, unpubl.). Green light at reduced intensities combined with unilateral ablation induced maturation in wild immature *P. monodon* (Hillier 1984).

*Photoperiod.* Increased photoperiod of 19 h failed to induce maturation in unablated *P. monodon* (Beard and Wickins 1980). *P. monodon* broodstock are maintained either under a natural photoperiod (Aquacop 1977b, Primavera 1983) or a 14 h photoperiod (Simon 1982).

*Salinity.* Ruangpanit et al (1985) observed a higher maturation rate and proportion of spawns with fertile eggs after

ablation in *P. monodon* females collected from the Indian Ocean compared to those from Songkhla Lake. A spawning ground, the Indian Ocean has 33 ppt salinity compared to 22-28 ppt in Songkhla Lake. However, the differences in other environmental factors make this observation inconclusive.

Posadas (1986) showed that ablated *P. monodon* can mature and spawn at 15, 25, and 32 ppt but require full seawater salinity for incubation and hatching of eggs. Moreover, females could not tolerate a net shift of 17 ppt from maturation salinity of 15 ppt to spawning salinity of 32 ppt and therefore eggs did not hatch.

*Substrate and tank color.* A comparison of black and white sand substrates showed significantly greater nauplii production and hatch rates from ablated *P. monodon* in tanks with white sand (Pudadera et al 1980a). Other workers (Simon 1982; Millamena, pers. comm.) have obtained maturation and spawning in ablated *P. monodon* in tanks with bare substrates.

Another recent study (Primavera et al, in prep.) determined the effect of tank color and female (eyestalk and/or carapace) tags on reproductive performance and survival of ablated *P. monodon*.

## OTHER ASPECTS OF BROODSTOCK AND MATURATION

### Spawner Monitoring and Retrieval

Monitoring ovarian maturation in dark-shelled species such as *P. monodon* requires a light source. This can be done by scooping out females and holding against the light or, with less stress, by flashing a beam from an underwater light against the female (Primavera 1983).

End-products of maturation may be retrieved as gravid females, eggs, or nauplii (Primavera 1985). Spawner retrieval is more manageable in 10-20 m<sup>3</sup> tanks than in tanks larger than 20 m<sup>3</sup>, and more efficient with frequent monitoring of broodstock. Nightly checking of a 12 m<sup>3</sup> tank yielded 48 spawners producing  $6.8 \times 10^6$  nauplii compared to only  $3.0 \times 10^6$  from 29 spawners with thrice weekly monitoring (Pudadera et al 1980a). Although egg collectors (Simon 1982) and nauplii collectors have

been tried for *P. monodon*, retrieval of spawners gives the advantages of individual records of female measurements, fecundity and hatch rates, and easier processing of eggs.

### **Spawning Behavior**

Spawning in *P. monodon* generally occurs between 2200 and 0200 h and the following description is based on reports by Villaluz et al (1969), Motoh (1981), and Primavera (1983).

A ready-to-spawn female becomes restless and actively swims upwards in circles with the last three pairs of pereopods held tightly together in flapping movement. Eggs are released through the gonopores (simultaneous with sperm release from the thelycum) over a period of 2-7 min. Movements of the pleopods keep the female actively swimming and also disperse the eggs and nonmotile sperm. The pink-orange scum associated with spawning is not so abundant in tanks provided with gentle aeration (Primavera 1985).

Gravid *P. monodon* that do not spawn for 2-3 successive nights but retain the ovarian outline may have the "milky ovary" disease caused by a microsporidian.

### **Fertilization, Incubation, and Hatching**

The events following spawning are described for *Sicyonia engentis* which is closely related to penaeids. These include sperm binding, acrosomal reaction, ovum jelly extrusion, fertilization or sperm-egg fusion and hatching membrane formation (Clark et al 1984). Ovum jelly extrusion or the cortical reaction involves the extrusion of cortical rods to form a corona or jelly layer as observed for *P. monodon* by Primavera and Posadas (1981). Abnormal spawns for *P. monodon* eggs laid in masses remain unfertilized and unhatched (Villaluz et al 1969) perhaps due to a failure of the cortical reaction (Aquacop 1977a).

Development of *P. monodon* eggs has been described by Motoh (1981); time of hatching is 12-15 h after spawning. To estimate the number of nauplii that will hatch out, the proportion of good eggs from a random sample is determined following the morphological classification of Primavera and

Posadas (1981). Hatch rate is correlated with egg quality and its determination gives hatchery personnel enough lead time to prepare the required larval rearing tanks.

Salinity in spawning tanks has ranged from 28 to 35 ppt for *P. monodon* (Villaluz et al 1969, Primavera and Borlongan 1978, Simon 1982, Hillier 1984). Among various temperature-salinity combinations, Reyes (1981) obtained highest mean hatch rate in *P. monodon* eggs incubated at 33 ppt at temperatures of 23°C and 33°C whereas 23 ppt and 28 ppt at any given temperature level produced weak larvae.

A temperature range of 26-29°C has been recorded for incubation of *P. monodon* eggs (Villaluz et al 1969, Primavera and Borlongan 1978, Hillier 1984). Increasing temperature levels of 23, 28, and 33°C had no effect on hatch rate of *P. monodon* eggs but significantly decreased incubation period (Reyes 1981).

Aside from the required temperature and salinity, spawning tank water should be chlorinated to remove all microorganisms. The chelating agent EDTA (ethylenedinitrotetraacetic acid) may also be added (Simon 1982, Hillier 1984). Gentle aeration must be maintained at a low rate of 4 bubbles/s (Emmerson 1980).

Maximum egg density should be 3 000/l or 600 000 eggs for a spawning tank with 200 l of water. Wild spawners of 150-200 g body weight producing more than a million eggs should have at least 400 l of spawning water.

After a spawning, the eggs are cleaned by removing any scum and passing the eggs through a series of meshes to remove both coarse and fine dirt particles (Primavera 1983).

### **Spawn Size (Fecundity) and Spawn Quality (Hatch Rate)**

Several researchers have reported a wide range of 57 650 to 550 300 eggs per spawn from wild ablated *P. monodon* (Emmerson 1983, Hillier 1984) and 60 000 to 747 500 from pond ablated females (Alikunhi et al 1975, Muthu and Laxminarayana 1977, Primavera 1978, Aquacop 1980). The wide range of egg numbers could be due to varying female sizes (50 to 200 g) and inclusion of egg counts from both partial and complete spawns. Similarly, hatch rates range from 0 to over 90% depending on

nutrition, sex ratio, water depth, and other physiological factors that may affect egg quality and/or mating efficiency.

Primavera (1982) reported higher mean fecundity and hatch rate of 270 000 eggs and 36%, respectively, from wild ablated *P. monodon* (n=213) compared to only 204 400 eggs and 20% hatch rate from ablated pond stock females (n=111). For wild, unablated spawners, Motoh (1981) reported greater egg numbers of 248 000 to 811 000 eggs per complete spawning. The smaller spawn sizes from ablated females may be due to the inadequacy of present maturation systems in providing the full nutritional and environmental requirements for captive maturation of *P. monodon*. It could also be due to the GIH secreted by the neurosecretory cells of the remaining unablated eyestalk in unilaterally ablated females.

Both greater egg numbers and (perceived) higher hatch rates explain the preference of many Philippine *P. monodon* hatchery operators for wild spawners over ablated broodstock.

## MATURATION TANKS, PENS, AND CAGES

By far the most popular and convenient means of holding captive *P. monodon* broodstock is the land-based broodstock or maturation tank. Marine offshore pens are site-specific (requiring a protected cove or bay), offer less security against poachers, are inconvenient for broodstock maintenance and monitoring, and have not progressed beyond the experimental stage (Primavera and Gabasa 1981).

Maturation tanks are usually incorporated within the hatchery complex due to the continuous sea-water supply requirement for the tanks and the convenience in immediate transfer of ripe females or newly hatched larvae to larval rearing tanks (Primavera 1983).

Tanks may be made of cement, ferrocement, fiberglass, and plastic or canvas-lined aluminum or wooden tanks. Tank size is based as much on the biological requirements of the broodstock as on the convenience of the hatchery personnel, hence the popularity of a 10-12 m<sup>3</sup> circular tank with 0.8 to 1.0 m water depth (Primavera 1983, 1985). Large tanks yield better maturation and mating performance based on past trials comparing 40-1 aquaria, 1-m<sup>3</sup>, 4-m<sup>3</sup>, 50-m<sup>3</sup>, and 200-m<sup>3</sup> tank by

Primavera (1979) and other workers (Vicente et al 1979, Poernomo and Hamami 1983, Hillier 1984).

Ideally, maturation tanks should have a flow-through water system with 100-400% daily exchange rate to achieve excellent results. However, when flow-through is not feasible as when sea water is polluted, turbid (during typhoons), or heated (when water temperature is low), sea water may be recirculated through filters.

Filters may be biological and/or mechanical and installed externally or inside the tanks as in a sand-gravel substrate with the aid of air-water-lifts. Aside from flow-through and recirculating water, simple aeration together with regular replacement of water may be used in maturation tanks. The disadvantage with aeration, however, is that the tank is prone to fouling. Ablated *P. monodon* achieved maturation and spawning only after air stones were replaced with a subgravel filter and air-lift recirculation inside plastic lined pools (Muthu and Laxminarayana 1977).

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