A Review of Maturation and Reproduction in Closed Thelycum Penaeids

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Abstract Commercially important penaeids of the closed thelycum group belong to five subgenera of the genus *Penaeus* — *Penaeus, Fenneropenaeus*, *Marsupenaeus* and *Melicertus* that are almost exclusively Indo-West Pacific and *Farfantepenaeus* that is predominantly Western Atlantic. Since the ablation of *Penaeus duorarum* more than a decade ago, the first for any penaeid, around 23 species have been matured in captivity, 17 of them belonging to the closed thelycum subgenera (*P. aztecus*, *P. brasiliensis*, *P. californiensis*, *P. duorarum*, *P. esculentus*, *P. indicus*, *P. japonicus*, *P. kerathurus*, *P. latisulcatus*, *P. merguiensis*, *P. monodon*, *P. notialis*, *P. orientalis*, *P. paulensis*, *P. penicillatus*, *P. plebejus*, and *P. semisulcatus*).

The complete spectrum of controlled reproduction in penaeids covers maturation, spawning, hatching of eggs into viable larvae, and the production of postlarvae to constitute the next batch of broodstock. The full closing of the cycle has been achieved in at least six closed thelycum species whereas gaps, e.g. inability of mature females to spawn or nonhatching of eggs, remain for the others.

Spawners or mature females used in commercial hatcheries and research laboratories are either wild-caught or matured in captivity with human control ranging from nil to a regular closing of the cycle. Wild spawners may be spawned directly after capture and transport or subjected to environmental manipulation, e.g. thermal control to induce or inhibit spawning. Females matured in captivity may come from wild broodstock (adults and subadults caught from estuaries or "sourced" by trawlers from offshore waters) or captive (pond- or tank-reared) broodstock. Introduced or exotic penaeid species must depend on a pond- or tank-reared broodstock whereas indigenous prawns and shrimps may be constituted from wild or captive broodstock.

There are three basic approaches employed singly or in combination to induce ovarian maturation in penaeids — endocrine, dietary or nutritional and environmental. Endocrine manipulation has so far been synonymous with unilateral eyestalk ablation, a technique with far-reaching impact on penaeid aquaculture. Closed thelycum penaeids may be classified into those that require ablation in order to mature and those that do not. To a third group belong species that have been experimentally induced to mature with and without ablation.

Diets for maturation include fresh and frozen animal sources (mussel, clam, oyster, squid, marine worms, shrimps, fish) and formulated pellets given in any combination. The choice of marine worms and mollusks is based on their high levels of arachidonic, eicosapentaenoic and docosahexaenoic acid, the dominant fatty acids found in mature ovaries and testes. Environmental parameters studied in relation to maturation include light (intensity, quality and photoperiod), temperature, salinity and pH.

Although a regular closing of the cycle has been achieved for some, the state-of-the-art for most penaeids is the successful production of larvae and postlarvae from either wild spawners or wild immature/spent females matured/rematured in captivity. The improvement of reproductive performance including larval quality from captive broodstock remains a major area for future research and includes the determination of minimum age and size for maturation. The complete description of the nutritional and environmental requirements for maturation should lead to the development of alternatives to ablation such as photoperiod manipulation or the use of reproductive hormones.

The present focus on characterizing the physicochemical and dietary requirements for maturation should be extended to other phases of reproduction: mating, spawning, fertilization and hatching. Studies on biology (molting, mating, fertilization including the cortical reaction) and biochemistry (maturation stages) provide baseline information for designing maturation tanks and formulating broodstock pellets. Investigations of wild stocks complement laboratory studies in elucidating the interrelationships among molting, mating, maturation and spawning.

Manual spermatophore transfer is being developed to solve the problem of nonmating in closed (and open) thelycum species. This technique will also be useful in future hybridization work, together with *in vitro* fertilization.

Introduction

Seed supply in the culture of penaeids had its beginnings in the tidal entry of wild fry into milkfish ponds as in the Philippines or in the *pokkali* (paddy) fields of Kerala, India (Fig. 1). This progressed to the stocking, first, of wild-caught fry from the coastline or estuaries, and then of hatchery-reared fry. The spawners used in hatcheries are either wild-caught or matured in captivity from wild "broodstock" or immature females. The final stage in this evolution — the

regular closing of the cycle with the use of spawners from broodstock reared in ponds or tanks — completely eliminates dependence on the wild.

In penaeids, the thelycum or structure that receives the spermatophores during mating may be of the open type with ridges and protruberances for spermatophore attachment or closed featuring lateral plates that lead into a seminal receptacle where the spermatophores are inserted. Commercially important species of the genus *Penaeus* belong to six subgenera: a single open thelycum subgenus, *Litopenaeus.*
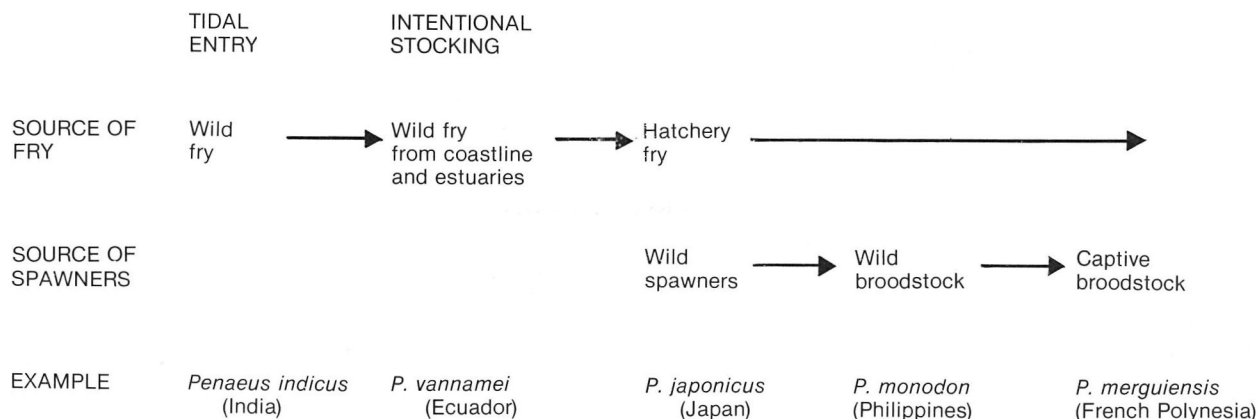
	TIDAL ENTRY	INTENTIONAL STOCKING			
SOURCE OF FRY	Wild fry	→ Wild fry from coastline and estuaries	→ Hatchery fry	————————————→	
SOURCE OF SPAWNERS			Wild spawners	→ Wild broodstock	→ Captive broodstock
EXAMPLE	*Penaeus indicus* (India)	*P. vannamei* (Ecuador)	*P. japonicus* (Japan)	*P. monodon* (Philippines)	*P. merguiensis* (French Polynesia)

Fig. 1. Evolution of fry supply in pond culture of penaeid prawns and shrimps.

and five closed thelycum subgenera. Among the latter, *Penaeus, Fenneropenaeus, Marsupenaeus* and *Melicertus* are almost exclusively Indo-West Pacific in distribution while *Farfantepenaeus* is predominantly Western Atlantic (Table 1).

Although the first captive spawning of a penaeid (a wild *Penaeus japonicus* spawner) was by Fujinaga in 1934 (Hudinaga, 1942), it was not until 1970 that maturation was first obtained in ablated *P. duorarum* (Caillouet, 1972) and unablated *P. latisulcatus* (Shokita, 1970). Almost 30 years earlier, Panouse (1943, cited by Adiyodi and Adiyodi, 1970) observed precocious maturation and egg deposition in a palaemonid that had undergone ablation. Since 1970, around 23 penaeid species have been matured (and 14 spawned) in captivity, 17 of them belonging to the closed thelycum group (Table 6). Two interesting points are highlighted in Table 1 — the worldwide interest in *P. monodon* as a species for culture based on number of studies, and the introduction of penaeids outside their natural range of distribution, e.g. *P. japonicus* to France and Italy and *P. monodon* to the U.K.

Ovarian maturation

Studies on reproduction have predominantly focused on female maturation. Stages of ovarian maturity described for various closed thelycum species generally fall into five groups — immature, early maturing, late maturing, mature and spent (Tuma, 1967; Rao, 1968; Villaluz et al., 1972; Brown and Patlan, 1974; Gehring, 1974; Duronslet et al., 1975; Aquacop, 1977a).

Ovaries have been classified *in vivo* or dissected using such criteria as size, outline, texture, color and gonadosomatic index (GSI); measuring lipid and fatty acid levels; and under light and electron microscopy describing oocytes (diameter, staining, nuclear appearance, cortical rods, etc.) and follicle cells. Given the need to keep female prawns alive for spawning, most penaeid workers use the *in vivo* classification by looking at the ovaries externally through the dorsal exoskeleton.

There are three basic approaches employed singly or in combination to induce ovarian maturation in penaeids — endocrine, nutritional and environmental.

Endocrine

Reproductive hormones. It was Panouse (1943, cited by Adiyodi and Adiyodi, 1970) who first demonstrated that removal of the eyestalk during sexual quiescence in *Leander serratus* led to ovarian development and egg deposition. In the eyestalk of decapod crustaceans, a gonad-inhibiting hormone (GIH) is produced by the neurosecretory cells of the X-organ and transported to the sinus gland for storage and release (Adiyodi and Adiyodi, 1970). In *Parapeneopsis hardwickii*, the activity of the ovary-(gonad-)inhibiting hormone was highest in the eyestalk of females with inactive and spawned ovaries whereas it was negligible in those at full vitellogenesis (Kulkarni and Nagabhushanam, 1980).

Earlier, the target organs of the GIH were postulated to be the brain and/or thoracic ganglionic mass with the GIH preventing their synthesis of a gonad-stimulating hormone (Adiyodi and Adiyodi, 1970). More recent studies however, suggest the fat body or the ovary (Meusy and Charniaux-Cotton, in press).

The ovaries of the amphipod *Orchestia gamarella* have been demonstrated to produce the vitellogenin-stimulating ovarian hormone (VSOH) which controls the synthesis of vitellogenin (Meusy, 1980). Vitellogenin is a lipoprotein complex found in the hemolymph during reproduction, synthesized outside the ovaries but used by the oocytes to constitute the yolk during secondary vitellogenesis in malacostracans (Meusy, 1980; Meusy and Charniaux-Cotton, in press). During reproductive rest, the GIH inhibits the synthesis of vitellogenin (Meusy and Charniaux-Cotton, in press). Ongoing trials in CNEXO-COB (Centre Oceanologique du Brest) in France are testing the effect of ovarian extract injections on immature *P. japonicus* and *P. vannamei* (C. Cahu, pers. comm.).

In *Parapeneopsis stylifera,* Joshi (1980, cited by Kanazawa, 1982) observed a greater GSI and egg diameter in

females injected with the mammalian hormone progesterone (4.74 and 106.48 μm, respectively) compared to ethanol-injected females (2.46 and 48.59 μm, respectively) and uninjected controls (0.70 and 18.26 μm, respectively).

Eyestalk ablation. Other than the few studies mentioned above, endocrine manipulation has so far been synonymous with unilateral eyestalk ablation, a technique first performed in penaeids on *P. duorarum* (Caillouet, 1972) and with far-reaching impact on crustacean aquaculture.

Penaeids in captivity may be divided into a difficult-to-breed group that requires ablation to mature, e.g. *P. aztecus, P. duorarum, P. monodon,* and *P. orientalis* and those that have matured without ablation, e.g. *P. californiensis, P. indicus. P. japonicus,* and *P. merguiensis* (Table 3) among the closed thelycum species. *P. monodon* is classified as a difficult species because the proportion of females that have matured without ablation is so far very low (Santiago, 1977; Primavera et al., 1978; Aquacop, 1979; Emmerson, 1983).

For either easy- or difficult-to-breed species, the effect of ablation is to increase maturation and spawning rates. Unablated controls did not mature or had a lower maturation rate than ablated females of *P. aztecus* (Aquacop, 1975), *P. esculentus* (P.J. Crocos, pers. comm.), *P. monodon* (Aquacop, 1977b; Santiago, 1977; Primavera and Borlongan, 1978; Hillier, 1984) and *P. plebejus* (Kelemec and Smith, 1980). Ablated *P. indicus* produced 10 times the number of spawns, 8 times the number of eggs and 6 times the number of nauplii as unablated controls (Primavera et al., 1982). Ablation also increased the spawning rate up to 8-17 spawns/♀ in one run for *P. kerathurus* (Lumare, 1979) and 4-6 spawns/♀/molt cycle for *P. monodon* and *P. orientalis* (Arnstein and Beard, 1975; Beard and Wickins, 1980; Hillier, 1984) (Table 2). In contrast, unablated females have a rate of 0.38 spawns/♀/month for *P. japonicus* (Laubier-Bonichon and Laubier, 1979), 0.38 spawns/9/month and 1 spawn/♀/molt cycle for *P. merguiensis* (Beard et al., 1977; Crocos and Kerr,

Table 1. List of closed thelycum subgenera and species of the genus *Penaeus* matured in captivity including distribution.

Distribution[a]	Subgenus	Species	Country[c]	References
Indo-West Pacific	*Penaeus*	*esculentus*	Australia	P. Crocos, pers. comm.
		monodon	French Polynesia, India, Indonesia, Kenya, Philippines, South Africa, Taiwan, Thailand, U.K.	Liao, 1973; Alikunhi et al., 1975; Arnstein and Beard, 1975; Aquacop, 1977a, b, 1979, 1980, 1983; Muthu and Laxminarayana, 1977, 1984; Santiago, 1977; Haider, 1978; Primavera, 1978, 1982, 1983; Primavera and Borlongan, 1978; Primavera and Gabasa, 1981; Primavera et al., 1979; Vicente et al., 1979; Beard and Wickins, 1980; Pudadera and Primavera, 1981; Pudadera et al., 1980a, b; Ruangpanit et al., 1981, 1984; Emmerson, 1983; Poernomo and Hamami, 1983; Hillier, 1984; Lin and Ting, 1984; Millamena et al., 1984
		semisulcatus	French Polynesia, Israel	Aquacop, 1975; Browdy and Samocha, 1985, in press
Indo-West Pacific	*Fenneropenaeus*	*indicus*	French Polynesia, India, Philippines, South Africa	MSU-IFRD, 1975; Muthu and Laxminarayana, 1977; Muthu et al., 1984, Emmerson, 1980; Emmerson et al., 1983; Primavera et al., 1982; Aquacop, 1983a
		merguiensis	French Polynesia, Indonesia, U.K.	Alikunhi et al., 1975; Aquacop, 1975, 1983; Nurjana and Won, 1976; Beard et al., 1977
		orientalis[b]	China, U.K.	Arnstein and Beard, 1975: Liang et al., 1983
		penicillatus	Taiwan	Liao, 1973
Indo-West Pacific	*Marsupenaeus*	*japonicus*	France, French Polynesia, Italy, Japan	Aquacop, 1975: Caubere et al., 1979; Laubier-Bonichon and Laubier, 1979; Lumare, 1981; Kanazawa, 1982; Yano 1984
Indo-West Pacific	*Melicertus*	*latisulcatus*	Japan	Shokita, 1970
		plebejus	Australia	Kelemec and Smith. 1980. 1983
Eastern Atlantic and Mediterranean Sea		*kerathurus*	Italy, Spain	Lumare, 1979
Western Atlantic	*Farfantepenaeus*	*aztecus*	French Polynesia. U.S.A.	Aquacop, 1975; Duronslet et al., 1975
		brasiliensis	Brazil	Martino, 1981; Barros et al., 1982
		duorarum	U.S.	Idyll, 1971; Caillouet, 1972
		paulensis	Brazil	Martino, 1981; Marchiori and Boff, 1983
Eastern and Western Atlantic		*notialis*	Cuba	Ramos and Gonzales, 1983
Eastern Pacific		*californiensis*	Honduras, U.S.A.	Broom. 1972; Moore et al., 1974

[a]Holthuis, 1980
[b]*P. chinensis* is the accurate but less familiar name (Holthuis, 1980)
[c]Where maturation work was done in alphabetical order

1983 and a maximum of 3 spawns/♀/2 months for *P. indicus* (Primavera et al., 1982).

Consequently, the interval between consecutive spawnings is reduced to only 3 to 15 days in ablated females compared to a minimum of 10 days up to 2.7 months in unablated controls and females in the wild (Table 2). This gap of 1-2 months (Fig. 2) probably represents the length of time for eggs to fully mature during a reproductive season (Rao, 1968). By reducing it to as short as three days with rapid maturation and overstimulation of spawning, ablation may lead to insufficient reserves in the hepatopancreas (Aquacop, 1977b; Lumare, 1979; Beard and Wickins, 1980). A decline in fecundity, hatch rate and egg viability has been observed with successive spawns in a single intermolt or with successive molt cycles in *P. monodon* (Beard and Wickins, 1980) and *P. indicus* (Primavera et al., 1982) and an increase in the proportion of partially developed ovaries and partial spawnings in successive spawnings of *P. kerathurus* (Lumare, 1979).

Given this reproductive decline, *P. monodon* broodstock are replaced 6-8 weeks after ablation (Simon, 1982; Primavera, 1983). Emmerson (1980) obtained viable spawns from ablated *P. indicus* for up to 7 months and from unablated females for one year. Ablated females gave lower fecundity and hatch rates compared to nonablated females due to a greater number of poor spawns which may be traced to a rapidity of egg development. However, spawning frequency of ablated females (2.24 spawns/molt cycle of 19.1 days) was not much higher than for unablated *P. indicus* (1.98 spawns/molt cycle of 20.1 days). Therefore, the lower hatch rates may be due to inherently poor egg quality in ablated females and not the rapidness of ovarian development.

In addition to the exhaustion of female reserves, this decline in reproductive performance could also be attributed to a decrease in quantity if not quality of sperm (Beard and Wickins, 1980) considering that sperm from a single mating

will need to fertilize up to six spawns within a single molt cycle (Fig. 2). However, Emmerson (1980) concluded that decreased hatchability of successive spawns within a molt cycle from ablated *P. indicus* females was not due to insufficient sperm but a decline in egg quality because the two spermatophores deposited during a single mating could fertilize up to three successive spawns.

Many commercial hatcheries that use broodstock for part or all of their spawner requirements prefer to do eyestalk ablation even for penaeid species demonstrated to mature unablated in captivity. Ablation leads to predictable peaks of maturation and spawning which facilitates the setting up of production schedules, in contrast to scattered spawns from unablated females. For production purposes, this predictability in availability and number of nauplii compensates for the trend towards decreased fecundity and hatch rates with successive spawns from ablated broodstock.

The various techniques used to ablate penaeids may be classified into two. The first method results in the total removal of the eye and the partial/total removal of the eyestalk by cutting with scissors; cautery using clamps or soldering iron or electrocautery; tying with a string; or manual pinching (Caillouet, 1972; Arnstein and Beard, 1975; Duronslet et al., 1975; Aquacop, 1977a). The second method partially destroys the eyestalk but retains the outer (corneal) layer of the eye. It is performed by first incising the eye, pressing the contents outwards then crushing the eyestalk (Primavera, 1978). The important thing is to prevent excessive loss of fluids and infection either by cauterizing the open wound and applying antibiotics as in the first method or by means of the remaining corneal layer that contains the bleeding and also forms a scar in the second method.

The term "ablation" meaning removal of a part especially by cutting strictly applies to the first method, so with "extirpation" meaning the rooting out or complete destruction.

Table 2. Effect of ablation on maturation and spawning in closed thelycum penaeids.

Species	Spawning rate		Interval between spawnings	
	Unablated	Ablated	Unablated	Ablated
P. japonicus	0.37 sp./♀/mo[a]		2.7 mo[a]	
P. merguiensis	0.38 sp./♀/mo[b] 1 sp./♀/molt cycle[c]		2.6 mo[b]	
P. indicus	3 sp./♀/2 mo (max.)[d] 1.98 sp./♀/molt cycle[e]	7 sp./♀/2 mo (max.)[d] 2.24 sp./♀/molt cycle[e]	2 mo[l] 10.2 days[e]	
P. kerathurus		8 sp./♀[f]		
P. paulensis		17 sp./♀[g]		
P. monodon		1 sp./♀/mo[h] 1-6 sp./molt cycle[i,j,q]		5 days[d,e,f,h,j,m, n,o,p,q,r]
P. orientalis		4 sp./molt cycle[k]		
P. latisulcatus			30-40 days[m]	
P. semisulcatus	1.13 sp./♀[s] 2-4 sp./♀/molt cycle (max.)[s,t]	4.46 sp./♀[s] 4-6 sp./♀/molt cycle (max.)[s,t]		

[a]Laubier-Bonichon and Laubier, 1979; [b]Beard et al., 1977; [c]Crocos and Kerr, 1983; [d]Primavera et al., 1982; [e]Emmerson, 1980; [f]Lumare, 1979; [g]Marchiori and Boff, 1983; [h]Aquacop, 1977a; [i]Beard and Wickins, 1980; [j]Hillier, 1984; [k]Arnstein and Beard, 1975; [l]Rao, 1968; [m]Penn, 1980; [n]Nurjana and Won, 1976; [o]Primavera and Borlongan, 1978; [p]Primavera et al., 1979; [q]Aquacop, 1980; [r]Poernomo and Hamami, 1983; [s]Browdy and Samocha, in press; [t]Browdy and Samocha, 1985.
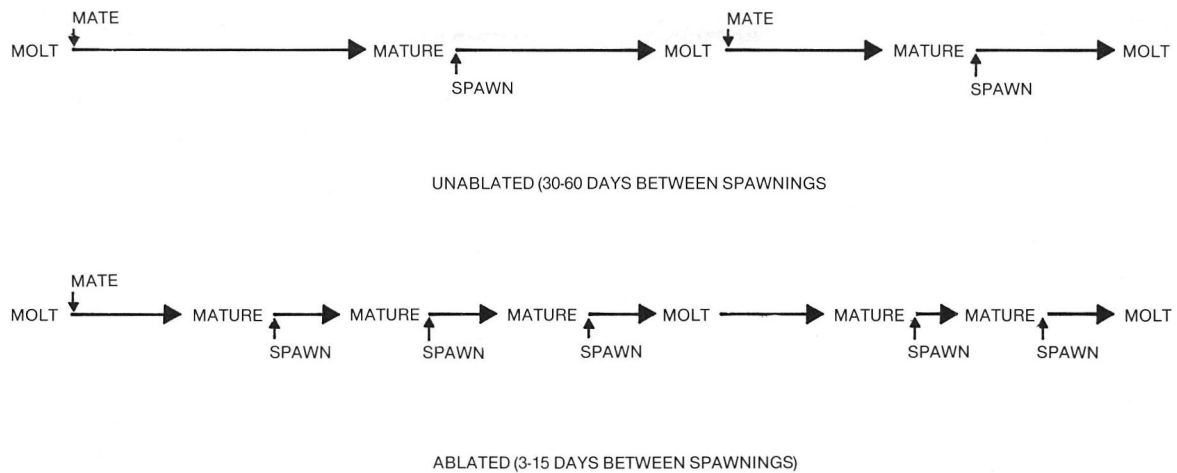
Fig. 2. Frequency of maturation and spawning in unablated and ablated closed thelycum penaeids.

"Enucleation" or the removal from a sac or capsule more appropriately describes the second method, although ablation has become the generally accepted term for most penaeid workers. Other terms used are the French *ablatíon* and *épédonculatíon* and the Spanish *ablacíon* and *oculotomía.*

At present, the standard procedure is unilateral ablation of either left or right eyestalk. Arnstein and Beard (1975) and Santiago (1977) observed that ablation of a single eyestalk was sufficient to induce maturation in *P. orientalis* and *P. monodon,* respectively, contrary to the findings of Alikunhi et al. (1975). In addition to the high mortality rates experienced in these species, bilateral ablation also leads to a loss of balance, swimming in spiral motion near the water surface, and other abnormal behavior in *P. duorarum* (Caillouet, 1972) and *P. merguiensis* (Alikunhi et al., 1975) and to lack of copulation in *P. paulensis* (Marchiori and Boff, 1983).

The latency period from ablation to the onset of maturation and subsequent spawning ranges from three days up to two months depending on the age and source of broodstock, stage of the molt cycle, 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 three days for wild adults from offshore waters (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 to 20-30 days for females from brackishwater Songkhla Lake (Ruangpanit et al., 1984). Lumare (1979) observed a longer latency period for captive *P. kerathurus* compared to wild stock.

Correlating events in the reproductive cycle with the molt cycle in *P. indicus* and *P. merguiensis,* respectively, Emmerson (1980) and Crocos and Kerr (1983) conclude that ovarian maturation proceeds through the intermolt and early premolt followed by spawning during the intermolt or premolt and that mating occurs immediately after ecdysis when the females have undeveloped ovaries. Ideally, ablation should be undertaken during the intermolt for maturation to follow in less than a week. 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). On the other hand, ablation during the postmolt leads to mortality because of added stress on the female and excessive loss of hemolymph (Aquacop, 1977a).

Mortality associated with ablation may be immediate or of a long-term nature. Ablation did not affect survival of *P. monodon* (Aquacop, 1977a; Vicente et al., 1979) and *P. plebejus* (Kelemec and Smith, 1980) whereas initial mortality due to ablation was observed in *P. kerathurus* (Lumare, 1979) and *P. monodon* (Primavera et al., 1978). Survival rates of 0, 38 and 49% for bilaterally ablated, unilaterally ablated and unablated *P. monodon,* respectively, were obtained after 196 days (Santiago, 1977). Higher survival of unablated females was also observed in *P. duorarum* (Caillouet, 1972) and *P. indicus* (Primavera et al., 1982). Other causes of broodstock mortality could be nutritional deficiency, stress due to molting, spawning, handling, etc. *P. monodon* males generally show a higher survival rate due to the absence of ablation, spawning and handling stress (Primavera et al., 1979; Pudadera et al., 1980b).

Environmental

The life cycle of most penaeids consists of an estuarine phase with the shoreward movement of postlarvae and a marine phase with the offshore migration of adolescents and subadults. The postlarvae grow into juveniles in mangroves, rivers and other brackishwater nurseries. Ovarian development may start in the estuaries but it is only after returning to the sea that full maturation and spawning take place. Attendant to this return migration are changes in physicochemical factors that may provide the stimuli for the lowering of GIH levels. Among the parameters so far studied, temperature and light appear to play a role in maturation. Tables 3 and 4 give details on various physicochemical parameters in maturation tanks.

Light. The deeper offshore waters where adult penaeids breed is characterized by reduced light intensity and greater

Table 3. Physicochemical parameters, stocking, feeding and water management in maturation tanks for closed thelycum penaeids.

Species	Abla-tion[a]	Brood-stock source	Age	Stocking density (no/m²)	Sex ratio (♀:♂)	Daily water exchange rate	Water manage-ment	Temp. (°C)	Sali-nity (ppt)	pH	Light intensity & quality	Photo-period (hr light)	Food	Reference
Penaeus aztecus	+	captive	9 mo	16-24	1:1	200-300%	flow-through	25-29	34.5	8.2	10-40%	natural	art. diet & bonito	Aquacop, 1975
P. brasilien-sis	− −	wild		2	1:1		flow-through	23.5-27	34-35	7.6-8.1	reduced		fresh mussel	Barros et al., 1982
P. califor-niensis		wild		6			water spray	22-28	35	8-8.3	20%	natural	commercial flake, shark & art. food	Moore et al., 1974
P. duorarum	+	wild & captive							30-35				commercial trout food & squid	Caillouet, 1972
P. esculen-tus	±	wild						26				14.5 hr		P. Crocos, pers. comm.
P. indicus	+	pond & wild					recircu-lating	24.5-30.2	26.8-38.6			natural	fresh clam, live mysids	Muthu & Laxminara-yana, 1977
	±	wild		2.5/ton	1.5:1	30%/2 days	replace-ment	19.0-29.1	20-35	7.1-8.6	70 µW cm⁻² (covers)	natural	prawn & dry pellet	Emmerson, 1980
	±	pond	3 mo	35	1:1	200-400%	flow-through	26-31.8	26.4-31.9	7.8-8.1	reduced	natural	brown mus-sel & pellet	Primavera et al., 1982
	−	pond		16-24	1:1	200-300%	flow-through	25.5-30	34	8.2	10%, 100 lux	natural	squid, mus-sel, troca & pellet	Aquacop, 1983a
	−	wild		2.5/ton	1.5:1		recircu-lating	24.1-26.6		7.8-8.1	45-50 µW cm⁻² blue (480 nm) green (510 nm)	natural, artificial	prawn & dry pellet	Emmerson et al., 1983
	+	pond		1-1.6/ton	1:1		recircu-lating			8.2-7.2; 8.2	500-3,600 lux; natural light		clam	Muthu et al., 1984
	+	pond	3 mo	21/ton	1.3:1	100%/5 days	replace-ment	24.9-29.4	32	6.5-8.6	dark cover	natural	squid, mus-sel & marine worms	A. Openiano, pers. comm.
P. japonicus	+	captive	11 mo	16-24	1:1	200-300%	flow-through	25-29	34.5	8.2	10-40%	natural	art. diet & bonito	Aquacop, 1975
	−	captive	1 & 2 yr	7	1:1		recircu-lating	24-26		8.1-8.3	500-1,500 lux	14.75-16 hr	mussel	Laubier-Bonichon & Laubier, 1979
	−	captive	7 & 18 mo		1:1		recircu-lating	24-28	30.5-36	7.5-8.5	4,000 lux	8→16 hr		Caubere et al., 1979
	+	pond		20	1:1		recircu-lating	18-26	34-38	7.6-8.4			mussel, crab & fish	Lumare, 1981
	+	wild & captive						15-25			black cover	8→16 hr	clam	Kanazawa, 1982
	−	wild spent		12/1.5 ton	0.7:1	0.3 ton/hr	flow-through	25±1	32-34		1,000-3,000 lux	15 hr	pellet, clam & krill	Yano, 1984
P. kerathu-rus	+	wild & captive		10-15	1:1	1/3 daily	recircu-lating	24-26	35-37	7.7-8.1			mussel	Lumare, 1979
P. merguien-sis	−	pond	4-5 mo	16-24		200-300%	flow-through	25.5-30	34	8.2	10%, 100 lux	natural	squid, mus-sel, troca & pellet	Aquacop, 1975, 1983a

[a]+ with eyestalk ablation; — without eyestalk ablation

Table 3. (continued).

Species	Abla-tion	Brood-stock source	Age	Stocking density (no/m²)	Sex ratio (♀:♂)	Daily water exchange rate	Water manage-ment	Temp. (°C)	Sali-nity (ppt)	pH	Light intensity & quality	Photo-period (hr light)	Food	Reference
P. merguiensis	—	pond			1:1			27.8-30.6	24-31				mysid, pellet & shrimp	Alikunhi et al., 1975
	—	wild		1.5	1.6:1	60%	replace-ment	26-30	30-34				mysid	Nurjana & Won, 1976
		pond	6-7 mo	5-15	1-2:1	50%/wk	recircu-lating	25-31	30-36	7.5-8.5			mussel & shrimp	Beard et al., 1979
P. monodon	+	pond			1:1		recircu-lating	27.8-30.6	24-31				mysid, shrimp & art. pellet	Alikunhi et al., 1975
	+	pond	9-12 mo	5	1:1	200-300%	flow-through	25.5-30	34	8.2	10%, 100 lux	natural	squid, mus-sel troca & pellet	Aquacop, 1977a, b 1979, 1980 1983a
		pond	15 mo	0.8	1:1			22.5-33.2	33 ± 1.6		± outdoor pens			Santiago, 1977
	+	pond	5 mo	4	1:1	30%/3 days	replace-ment	23.8-26.2	30-34	7.8-8.1		natural	salted mussel	Primavera, 1978
		pond	1-2 yr	5-6	1-2:1	30%/3 days	replace-ment				±	natural	salted mussel	Primavera et al., 1978
	+	pond		6	2:1	200-400%	flow-through	24-34	27.6-30.2		60%	natural	pellet, mus-sel, squid	Primavera et al., 1979
	+	wild		4-5	2:1	200-400%	flow-through	28.6-33.6		7.0-8.2	40-60%	natural	brown mus-sel & pellet	Pudadera et al., 1980a
	+	wild		5	1-4:1, 1:0	200-400%	flow-through	25-30.5	15.4-34.8	7.2-8.1	60%	natural	brown mus-sel & pellet	Pudadera et al., 1980b
	+	pond		6	1:1	200-400%	flow-through	21.3-33.6	28-36	7.9-8.1	1,210-3,500 lux; blue, red, natural	natural	mussel & pellet	Pudadera & Primavera, 1981
	+	wild & pond		7.5/ton	3.3:1			26-28.5	32.3-34.1			natural	mussel, pellet & fish	Vicente et al., 1979
	+	captive		1.6	1:12	15%/week	recircu-lating	28±2	30±2		40-70 lux	19 hr	mussel & shrimp	Beard & Wickins, 1980
	+ +	wild		13/ton	1:1			26-28	30-31	7.8-8.0		natural	squid, cockle & prep. feed	Ruangpanit et al., 1981
		wild		4	1:1	100-200%	flow-through	28-31	30-31			natural	green mus-sel & cow liver	Ruangpanit et al., 1984
	+	pond & wild	5-8 mo	7	1:1	100-250%	flow-through	26.5-30.5	28-32		fluorescent light	14 hr	squid & clams	Simon, 1982
	+	pond		2.7	1:1	20-50%/ 2-3 days	replace-ment	26-28	25-30			natural	squid, cockle & prep. feed	Poernomo & Hamami, 1983
		wild		2.2/ton	1:1	30%/2 days	replace-ment	27± 2.2	30± 3		± 70 μW cm⁻² (reduced)		pellet, prawn	Emmerson, 1983
	+	wild		2.3	3:1			26-31			green	natural (12 hr)		Hillier, 1984
P. notialis	+	wild			1:0			24-30	35-37.3	7.5-7.8			fish	Ramos and Gonzales, 1983
P. orientalis	+	tank	8 mo	6.4	1:1	50%/wk	recircu-lating	20 ± 2	28-30		subdued	8 hr	mussel & shrimp	Arnstein & Beard, 1975
P. paulensis	—	wild					flow-through	25.5-27.5	34-35	7.6-8.2	outdoor		mussel & white fish	Agarez & Barros, n.d.

Table 3. (continued).

Species	Abla-tion	Brood-stock source	Age	Stocking density (no/m²)	Sex ratio (♀:♂)	Daily water exchange rate	Water manage-ment	Temp. (°C)	Sali-nity (ppt)	pH	Light intensity & quality	Photo-period (hr light)	Food	Reference
P. paulensis	+	wild				10-15%	recircu-lating	24.5-27	33-35	7.8-8.2	2,500 lux	16 hr	clam, fish & annelid worms	Marchiori & Boff, 1983
P. plebejus	+	wild		11-17	7:1	2.5-5%	recircu-lating	26-28	32.5-33.5	8.0-8.5	dim, blue	12 hr	prawn tail	Kelemec & Smith, 1980
P. semisul-catus	?	captive		16-24	1:1	200-300%	flow-through	25-29	34.5	8.2	10-40%		art. diet	Aquacop, 1975
	±	wild pond	2 yr	12-15/m³	1.8-2.5:1	250%	flow-through	19.5-27.3	40		0.1-0.3 μE/m²/sec		*Artemia* biomass, fish, shrimp & squid	Browdy & Samocha, 1985
		pond	17 mo	18/3m³	2.6:1	250%	flow-through	22.4-27.5	40	7.82-8.08	0.1-0.3 μE/m²/sec		green mussel	Browdy & Samocha, in press

penetration of blue and green light compared to other wavelengths (Jerlov, 1970). Various studies have tried to approximate light conditions in the natural habitat inside maturation tanks.

Light intensity. Decreasing levels to 10-60% of incident light through the use of covers made of plastic, cloth, etc. discourages algal growth in tanks and decreases the solar energy which may inhibit maturation in nonburrowing species such as *P. merguiensis* (Aquacop, 1983a). Reduced light levels led to fast maturation in nonablated and ablated *P. monodon* (Emmerson, 1983; Hillier, 1984). Covered tanks also minimize disturbance of broodstock (Primavera, 1983).

Light quality. Unablated *P. duorarum* did not mature in tanks provided with blue, green and white light (Caillouet, 1972). Similarly, unablated *P. monodon* attained only partial maturation under blue and natural light but not under red light (Pudadera and Primavera, 1981). However, nonablated *P. indicus* kept under dim green and blue light showed improved growth and condition and increased spawning activity after a depression of all three during the initial 'settling down' period (Emmerson et al., 1983). In contrast, initially high spawning levels of females in the control (natural light) tank decreased after the second month as a consequence of a steady decline in prawn condition.

Photoperiod. Unablated *P. duorarum* failed to mature under varying light-dark combinations including continuous light and continuous darkness (Caillouet, 1972). Similarly, increasing photoperiod to 19 hr (light) failed to induce maturation in unablated P. *monodon* (Beard and Wickins, 1980). In contrast, both ablated and unablated *P. plebejus* produced more maturations and spawnings with day length of 14.5 hr compared to 12 hr (P.J. Crocos, pers. comm.).

Unablated *P. japonicus* produced the greatest number of spawnings over a 7-month period when daylength was gradually increased from 12.75 to 14.75 hr compared to shorter and longer photoperiods (Laubier-Bonichon and Laubier, 1979). Slightly different results were reported by Caubere et al., (1979) with nonablated *P. japonicus* producing more

nauplii when photoperiod was gradually increased to 16 hr over a 6-month period compared to an abrupt increase to 16 hr over 3 months. However, for the duration of both studies, temperature was also increased from 15°C to 28°C so that maturation cannot be attributed solely to photoperiod. Lumare (1979) observed a longer latency period in ablated *P. kerathurus* maintained at a photoperiod of 12 hr compared to those in natural daylight (9 hr) but noted that the former could have been stressed by abrupt exposure to the artificial photoperiod.

Most maturation tank systems rely on natural photoperiod (Table 3). Controlled photoperiod in tanks may have longer daylengths of 12-16 hr for *P. monodon* (Simon, 1982), *P. paulensis* (Marchiori and Boff, 1983) and *P. plebejus* (Kelemec and Smith, 1980) and shorter periods of 8 hr for subtropical and temperate species such as *P. japonicus* and *P. orientalis* (Arnstein and Beard 1975; Kanazawa, 1982). The role of photoperiod in the control of maturation is probably not as critical for species distributed along the equator and therefore not exposed to significant differences in daylight hours as it is for subtropical penaeids.

Temperature. A comparison of temperature regimes maintained in maturation tanks shows an upper range of 26-32°C for most penaeid species and a lower one of 16-28°C for subtropical species such as *P. japonicus* and *P. orientalis* (Tables 3 and 4). For the latter group, ablated females mature even at the lower temperature limits, suggesting a greater need for fine tuning of environmental parameters in the absence of ablation. Nonablated *P. orientalis* had a mean ovarian weight of 1.56 g at a higher temperature of 18°C in contrast to ablated females with 10.31 g mean ovarian weight at only 11°C (Liang et al., 1983). Similarly, ablated *P. japonicus* started to mature even at 18°C (Kanazawa, 1982) whereas unablated females produced more spawnings and more eggs and larvae per spawning at higher temperatures of 24°C and 26°C compared to 20°C (Laubier-Bonichon and Laubier, 1979). In the latter study, however, photoperiod was also manipulated so the effect of temperature on maturation is not clearcut. In reproductively active ablated *P. japonicus, a*

rest period was induced by decreasing temperature to below 17.5°C Lumare, 1979). In both ablated and nonablated *P. esculentus*, more maturations were obtained at 26°C compared to 21°C (P.J. Crocos, pers. comm.).

Salinity. There were no significant differences in maturation rates of unablated *P. indicus* kept in tanks at 22, 32 and 42 ppt although females maturing at 32 ppt showed significantly higher fecundity and hatch rates (A. Openiano, pers. comm.). Similarly, manipulation of salinity did not induce maturation in unablated *P. duorarum* (Caillouet, 1972). On the other hand, Ruangpanit et al. (1984) observed a higher maturation rate and proportion of *P. monodon* females with fertile eggs after ablation in prawns collected from the Indian Ocean which is a spawning ground with 33 ppt salinity compared to those from Songkhla Lake, a nursery area of the species with 22-28 ppt salinity. However, the differences in depth (20-30 m in the Indian Ocean and 1-1.5 m in Songkhla Lake) and other environmental factors make this observation inconclusive.

Most maturation systems depend on available seawater with ambient salinity of 24-36 ppt (Table 4) with lower levels experienced during typhoons or heavy rains.

pH. Ablated *P. indicus* females reached early maturation then resorbed their ovaries when pH of recirculated water was allowed to decline from 8.2 to 7.2 in plastic-lined pools (Muthu et al., 1984). Successful maturation, spawning and hatching of viable nauplii were obtained only from ablated females kept in pools where pH was maintained at around 8.2 by daily addition of sodium carbonate.

Nutrition

Recent studies on nutritional requirements for penaeid maturation have focused on lipids which provide energy, as well as essential nutrients such as sterols and phospholipids.

Nutritional studies. In wild *P. japonicus,* Teshima and Kanazawa (1983) observed an increase in ovarian lipid concentration from slightly mature to yellow ovarian stages, reaching constant levels in mature ovaries and declining after spawning. In contrast, lipid levels in the hepatopancreas declined in mature ovaries after reaching a maximum in the yellow ovaries suggesting a possible movement of lipids from the hepatopancreas to ovaries during maturation. Ovarian lipid concentration in wild *P. aztecus* showed an increase from early developing to ripe stages and a decline in spent females (Chamberlain and Lawrence, 1983). There was also an increase in ovarian carbohydrate levels from nearly ripe to ripe stages but no changes in protein concentration for all maturation stages.

Ovarian lipid concentration in immature *P. monodon* increased upon reaching full maturity from 5.8 to 17.0% in wild (unablated) females (Millamena et al., 1984) and from 7.5 to 21.9% in wild ablated females (O. Millamena, pers. comm.). The fatty acid profile showed 12.14-24.87% and 11.81-24.50% for total fatty acids in wild (unablated) and wild ablated females, respectively, to consist of 20:4ω6 (arachidonic acid), 20:5ω3 (eicosapentaenoic acid) and 22:6ω3 (docosahexaenoic acid). The same polyunsaturated fatty acids (PUFA) were reflected in the spawned eggs, indicating their importance in the reproductive process. Simi-

larly, high levels of these PUFA's were found in wild *P. setiferus*, *P. stylirostris* and *P. vannamei* (Middleditch et al., 1979, 1980).

Food sources. Mollusks including mussel, clam, cockle and squid are the most common food sources for penaeid broodstock (Tables 3 and 4). Other food items used are fresh or frozen marine worms, mysids, shrimp and fish, and dried pellets. These various items may be given alone or in combination. The broodstock are fed *ad libitum* or according to a daily feeding rate of approximately 3-5% for dry feed (pellets) and 10-30% for wet (fresh or frozen) feed. Feed is given once up to four times a day and the daily ration divided accordingly.

A mussel-pellet and an all-mussel feeding combination gave better maturation and hatching rates than a squid-pellet or all-pellet feeding for ablated P. *monodon* (Primavera et al., 1979). Aquacop (1979) obtained best results using a squid-containing pellet with 60% protein. However, the feeding of fresh troca univalves to early maturing ablated P. *monodon* has a positive effect on maturation and egg viability (Aquacop, 1977b). Females that mature on pellets alone spawn unfertilized eggs although they undergo successful mating and are positive for sperm.

These findings point to the need for natural food sources, particularly those rich in PUFA's, e.g. mollusks and marine worms, for penaeid maturation.

Maturation systems

Majority of penaeid maturation systems use tanks incorporated within the hatchery complex whereas a few have experimented with pens and cages (Fig. 3). The advantages offered by land-based tanks include easy monitoring and

BROODSTOCK SOURCE	MATURATION	RETRIEVAL OF:
		Mature females
WILD	TANK	Eggs
		Nauplii
CAPTIVE (pond/tank)	PEN	Mature females
	CAGE	Mature females

Fig. 3. Broodstock source, maturation system and retrieval in penaeid species.

spawner retrieval, convenience in cleaning and maintenance, and better security against poachers (Primavera and Gabasa, 1981).

Tanks

Construction. Tank size ranges from 500 ℓ to 50 m³ and construction materials include cement, ferrocement, fiberglass and aluminum lined with plastic sheets. Size of the tanks is a compromise between biological requirements of the animals and convenience of the hatchery staff.

Maturation rates of various penaeids were higher in bigger tanks (Vicente et al., 1979; P.J. Crocos, pers. comm.) although Beard and Wickins (1980) obtained maturation in *P. monodon* in only 125 ℓ of water (0.83 × 0.72 × 0.2 m). Better maturation of ablated *P. monodon* in large round tanks compared to smaller rectangular tanks may have been due to less disturbance in the large tanks during routine procedures (Hillier, 1984).

Similarly, mating is more successful in larger and deeper tanks (section IV, C) although very deep water presents practical difficulties for daily operations. The popularity of a 10-12 m³ circular tank with 0.8-1.0 m water depth is due to the convenience in maintenance and daily monitoring of broodstock for ovarian development as well as satisfactory maturation performance.

Physicochemical parameters. Except for manipulation of

Table 4. Summary of maturation requirements in tanks for closed thelycum penaeids.

Eyestalk ablation	Required for some species, optional for others
Light intensity	Reduced: 10-60% of incident (natural) light 100-500 lux, 50-70 μW/cm² (artificial)
Light quality	Blue or green light
Photoperiod	Natural Artificial: 12-16 hr light
Temperature	Tropical spp.: 26-32°C Subtropical and temperate spp.: 16-28°C
Salinity	24-36 ppt
pH	7.5-8.5
Water exchange rate	Flowthrough water: 100-400%/day Recirculated and/or replaced water: 5-50%/l-7 days
Stocking density (= 300-400 g/m²)	50-150 g body wt.: 2-7/m² 10-60 g body wt.: 10-35/m²
Sex ratio	1-2♀:1♂
Feed	Mussel, clam, squid and other mollusks; marine worms; shrimp; mysids; fish; cow liver; formulated diets Fresh, fresh/frozen or dry (pellets) In combined or single diets given 1-4X/day
Daily feeding rate	a) Wet feeds: 10-30% of total biomass Dry feeds: 3-5% of total biomass b) *Ad libitum* or to satiety

light and temperature, physicochemical parameters in maturation tanks are dependent on incoming seawater (Tables 3 and 4). Ranges are 24-36 ppt salinity; 26-32°C and 16-28°C temperature for tropical and subtropical/temperate species, respectively; 7.5-8.5 pH; and dissolved oxygen at saturation levels of 5-7 mg/ℓ with flowthrough water or continuous aeration.

As discussed earlier (section II, B), most maturation tanks depend on a natural light source with intensity reduced to 10-60% of incident light through the use of dark covers. If artificial light is used, photoperiod and spectral quality can also be controlled in addition to intensity. Tanks are generally located inside a roofed structure, with or without walls.

Water quality. Good water quality with excellent maturation results can be achieved with a flowthrough water system which gives a daily exchange rate of 100-400% of total water volume. However, it needs an unlimited supply of clear, unpolluted seawater. Where natural sources are limited, during typhoon months when seawater is turbid or when heated water is used for temperature control, there is a need to recirculate water through filters, often with the aid of air-water-lifts.

Filters may be biological and/or mechanical to remove metabolites, e.g. ammonia and particulate matter, respectively. They are often installed external to the tank but they may also be built-in as a sand-gravel substrate. The water quality and exchange rate in recirculating tanks will depend on the efficiency of these filters.

In addition to flowthrough and recirculating water, maturation tanks may use simple aeration to circulate water in conjunction with regular or periodic water replacement (20-50% of total volume every 1-7 days). Of the three management systems, the last requires the least input but is the most vulnerable to fouling.

Muthu and Laxminarayana (1977) had negative results with ablated *P. monodon* and *P. indicus* in plastic-lined pools with airstones. Only after seawater quality improved with the addition of a subgravel filter with air-lift recirculation were ovarian maturation and spawning observed. In another experiment, however, ablated *P. indicus* failed to attain full maturation in recirculating pools where pH was allowed to decline to 7.2 in contrast to successful maturation, spawning and hatching when pH was maintained at 8.2 (Muthu et al., 1984). Aquacop (1975) stresses the importance of oceanic water with a low level of organic and inorganic particles for the successful maturation of various penaeid species in flowthrough maturation tanks.

Prophylactics such as 2.5 ppm furanace, 50-25 ppm formalin, and 1.5-2 ppm streptomycin are used initially and/or regularly after stocking to disinfect tanks and broodstock, control disease and reduce mortality (Simon, 1982; Poernomo and Hamami, 1983).

Substrates. As earlier discussed, a sand-gravel substrate in flowthrough and recirculating tanks can improve water quality by acting as filter. A substrate is also required for burrowing species such as *P. japonicus* whether the water is static, flowing or recirculating.

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). Moreover, white sand provides greater contrast and is therefore more convenient for regular monitoring of females and daily cleaning of tanks. On the other hand, non-ablated *P. indicus* kept in tanks with inner walls painted black (without substrate) produced more spawns, greater average fecundity and hatch rates than females in white tanks (Emmerson, 1980).

For maturation of nonburrowing penaeids, substrates are optional. However, necrosis and injuries to appendages as a result of crawling and other benthic activities may be more frequent on a bare tank particularly if broodstock are maintained for a long time. Such damage can be minimized if the tank bottom has a smooth finish.

Stocking density. In general, lower stocking densities produce better maturation and survival rates in broodstock. Stocking density depends on water quality and exchange rate and on the size of the animals.

Larger species such as *P. monodon* weighing 50-150 g are stocked at 2-7/m^2 (Aquacop, 1977a, 1983a; Primavera, 1978, 1983; Simon, 1982; Poernomo and Hamami, 1983; Hillier, 1984). Smaller-sized species such as *P. indicus, P. japonicus, P. merguiensis* and *P. plebejus* with a body weight of 10-60 g have a higher density of 10-35/m^2 (Aquacop, 1975, 1983a; Lumare, 1979, 1981; Kelemec and Smith, 1980; Primavera et al., 1982). Whether few or many animals, the biomass should not exceed 300-400 g/m^2 (Table 4).

Tank area is more important than volume because of the benthic nature of penaeids. However, water depth may be critical for such activities as mating (section IV, C).

Sex ratio. In penaeid hatcheries, males are required for mating and spermatophore transfer but not for maturation. All-female ablated populations of *P. monodon* (Beard and Wickins, 1980; Pudadera et al., 1980b) and *P. notialis* (Ramos and Gonzales, 1983) matured in the absence of males. However, the spawned eggs of *P. monodon* were not fertilized and did not hatch indicating a lack of sperm (Pudadera et al., 1980b).

Generally, sex ratios are maintained at 1 ♀:1 ♂ to ensure mating success in tanks. However, a 2 ♀:1 ♂ ratio produced the highest spawning rate, fecundity and total number of nauplii compared to 1:0, 1:1 and 4:1 female to male ratios (Pudadera et al., 1980b). Higher sex ratios in favor of females (1.5-3♀:1♂) are more economical because they maximize egg and larval production per tank. If female broodstock mortality is high, the ratio gradually evens out.

Tank monitoring and retrieval. The end-products of maturation may be retrieved from the tank as gravid females, spawned eggs or hatched nauplii (Fig. 3). The main advantage of female retrieval is that it allows individual records including data on fecundity, hatching and spawner measurements which are important for experimental work.

Monitoring light-colored species such as *P. indicus* for ovarian maturation can be done during the day by directly looking at the prawns if the water is clear enough. In the case of *P. monodon* and other dark-shelled species, a source of light is needed to show off the outline of the ovaries for mature females. This can be done by scooping out the females and holding against the light or by the less stressful method of flashing the beam of an underwater light against the female thereby avoiding unnecessary handling.

Efficiency in spawner retrieval depends on tank size and frequency of sampling. Smaller tanks of less than 20 m^3 are more manageable and more frequently sampled than larger tanks of 20-50 m^3. Also, nightly monitoring of a 12 m^3 tank yielded 48 spawners producing 6.8 × 10^6 nauplii of *P. monodon* compared to only 3.0 × 10^6 nauplii from 29 spawners with thrice weekly monitoring (Pudadera et al., 1980a).

Egg collectors have been installed in tanks for *P. japonicus* (Laubier-Bonichon and Laubier, 1979; Lumare, 1981) and *P. monodon* (Simon, 1982) either alone to minimize the tedium of retrieving numerous females particularly in commercial hatcheries, or to retrieve the eggs of any stray spawners missed out during regular tank monitoring. Nauplii collectors devised for *P. indicus* were found unsatisfactory due to proliferation of bacteria and sea anemones (A.T. Young, pers. comm.).

The observation of individuals through various tags and marks has yielded important information on the maturation and molt cycles of penaeids. Tags of brass, silicone or cellophane around the unablated eyestalk of *P. monodon* (Rodriguez, 1976; Primavera, 1978; Aquacop, 1983a; Poernomo and Hamami, 1983) and aluminum bands around the unablated or ablated eyestalk of *P. plebejus* (Kelemec and Smith, 1980) with coded numbers, letters and colors have allowed the monitoring of maturation in individual females. To chart the molt cycle, however, additional tags attached to the cephalothorax (Aquacop, 1983a) and a coded system of cutting the uropods (Hillier, 1984) have been used for *P. monodon.*

Pens and cages

The offshore maturation pen requires a cove or bay protected from wind and wave action and seawater free from industrial and agricultural pollution (Primavera and Gabasa, 1981). The SEAFDEC Aquaculture Department prototype consists of a 16 × 16 × 4 m framework of bamboo posts, braces and mattings and an inner net which holds the *P. monodon* broodstock. Maturation cages made of nylon netting and installed inside a pond (Haider, 1978; B. Pudadera, pers. comm.) are so far experimental.

As earlier mentioned, tanks are preferred over pens and cages because of the greater security and convenience in broodstock monitoring and tank maintenance. Moreover, tanks can be located anywhere a prawn hatchery is put up unlike pens which are site-specific.

Other aspects of reproduction

In addition to maturation, the complete spectrum of controlled reproduction in penaeids includes spawning, hatching of eggs into viable larvae and the production of postlarvae to constitute the next batch of broodstock (Fig. 4). In turn, hatching of eggs presupposes incubation, fertilization, mating or spermatophore transfer, and male maturation.

Constitution of broodstock

The source of broodstock may be wild immature adults/subadults caught from estuaries or "sourced" by trawlers from offshore, or spent wild spawners recycled from
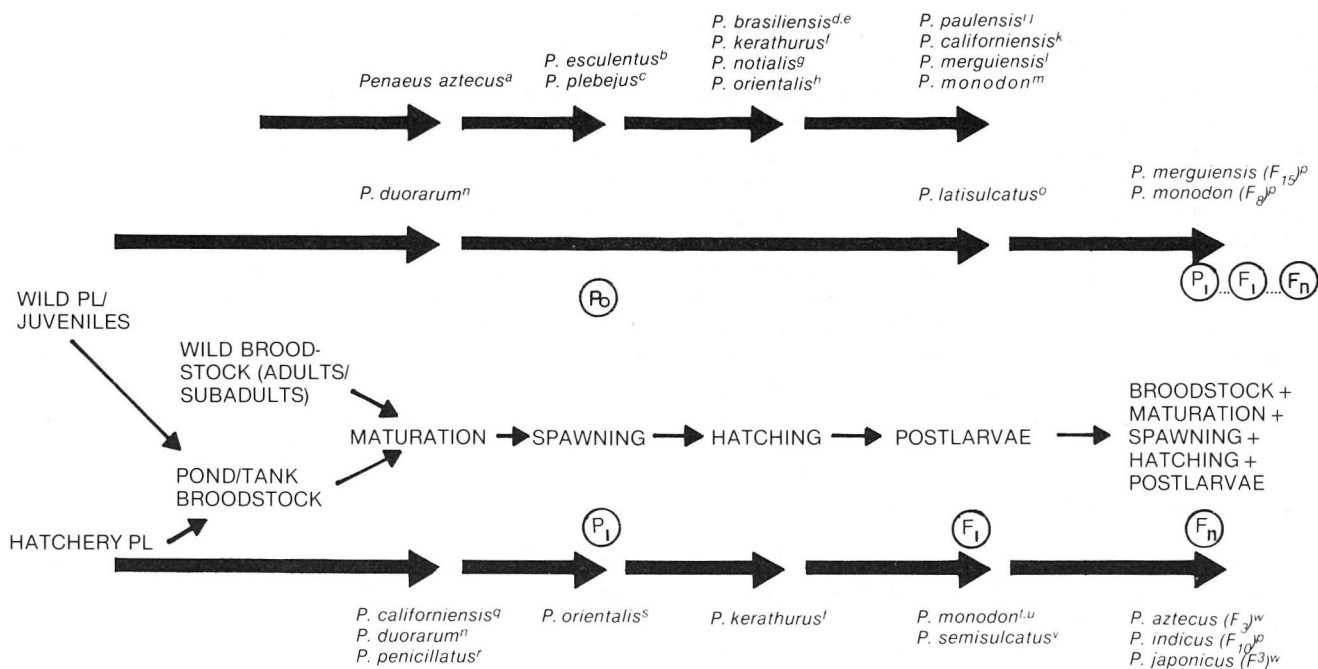
Fig. 4. Reproductive phase achieved in captivity in closed thelycum penaeids ([a]Duronslet et al., 1975; [b]P. Crocos, pers. comm.; [c]Kelemec and Smith, 1980; [d]Martino, 1981; [e]Barros et al., 1982; [f]Lumare, 1979; [g]Ramos and Flores, 1983; [h]Liang et al., 1983; [i]Marchiori and Boff, 1983; [j]Agarez and Barros, n.d., [k]Moore et al., 1974; [l]Nurjana and Won, 1976; [m]Ruangpanit et al., 1981, 1984; [n]Caillouet, 1972; [o]Shokita, 1970; [p]Aquacop, 1983a; [q]Broom, 1972; [r]Liao, 1973; [s]Arnstein and Beard, 1975; [t]Santiago, 1977; [u]Beard and Wickins, 1980; [v]Browdy and Samocha, 1985; [w]Aquacop, 1977a).

the hatchery. Or they may be captive broodstocked reared in ponds and tanks from hatchery or wild fry (Fig. 3).

Hatchery postlarvae grown to broodstock constitute the first parental (P_1) generation completely reared in captivity when they mature and spawn (Fig. 4). In turn, their offspring become the first filial (F_1) generation that grow to be the P_2 generation when they reproduce. The wild spawners or wild broodstock (matured in captivity) that produce the original hatchery postlarvae are referred to as the P_0 generation, same as wild fry that are reared to broodstock in captivity, because part of their life has been spent in the wild.

In French Polynesia, the rearing of *P. monodon* broodstock from young postlarvae to 60 g body weight over 9-12 months involves a series of 3-stage pond transfers during which density is gradually decreased from $20/m^2$ to $1-2/m^2$ and various artificial pellets and fresh feeds are given (Aquacop, 1983a). During the last stage (6 to 9 months of age), the supply of fresh feeds to the broodstock in ponds is critical to future maturation performance. *P. monodon* given fresh feed during this time show adequate serum protein concentration and a high spawning index whereas those fed pellets alone have a low serum protein level and few or no spawnings (Aquacop, 1983b). For *P. indicus* and *P. merguiensis,* standard pond grow-out technology is employed at lower stocking densities (MSU-IFRD, 1975; Aquacop, 1983a).

The minimum age at first ovarian maturation of captive broodstock is 4-8 months for *P. indicus* and *P. merguiensis* (Aquacop, 1975; Beard et al., 1977; Primavera et al., 1982; Emmerson, 1983), 8-9 months for *P. aztecus* (Aquacop, 1983a) and *P. orientalis* (Arnstein and Beard, 1975), 7-12 months for *P. japonicus* (Aquacop, 1975; Caubere et al., 1979;

Laubier-Bonichon and Laubier, 1979) and 9-15 months for *P. monodon* (Aquacop, 1977a; Santiago, 1977; Primavera et al., 1978). Five-month-old *P. monodon* matured and spawned after ablation (Primavera, 1978) but the poor quality of larvae produced points to the need for older females that may be more receptive to induced maturation. In the wild, *P. monodon* attain full maturity and spawning at around 10 months (Motoh, 1981).

Spawners from captive broodstock are generally smaller than those from the wild. Ablated *P. monodon* had a minimum size of 32 g (Poernomo and Hamami, 1983) and 45 g (Aquacop, 1977a) compared to 75 g for wild spawners (Primavera, 1978). On the other hand, only female *P. monodon* with carapace length (CL) of at least 52 mm out of a range of 42-70 mm matured after ablation (Muthu and Laxminarayana, 1977) which is greater than the minimum CL of 48 mm for wild spawners (Motoh, 1981). Minimum size at first ovarian maturation is 23 mm CL for *P. semisulcatus* (Thomas, 1974) and *P. merguiensis* (Crocos and Kerr, 1983) and 130.2 mm total length for *P. indicus* (Rao, 1968). *P. indicus* with 24-44 mm CL were ablated but only females with 30 mm CL and above matured (Muthu and Laxminarayana, 1977).

The choice of spawner source (wild spawner vs. wild broodstock vs. pond broodstock) depends on a number of factors foremost among which is expense. In the Philippines, a wild *P. monodon* spawner fetches from P100 to P1,000 (P18:US $1) apiece compared to only P10 to P40 each for wild immature females (Primavera, 1984). Hatchery production target is also important with large hatcheries relying on wild or captive broodstock to fill part or most of their spawner needs while small hatcheries may depend solely on wild spawners.

Also, hatcheries rearing native species can rely on wild spawners or wild broodstock whereas introduced or exotic species will have to depend on captive sources, e.g. *P. japonicus* in France and *P. monodon* in Brazil. Hatcheries with proximity to sources of wild spawners or wild broodstock may not need captive broodstock. With no abundant natural populations of *P. monodon*, Taiwan has had to import up to 40% of its spawner needs (Liao and Chao, 1983) mostly from the Philippines (Primavera, 1984). Taiwanese and many Philippine hatchery operators spare no expense to obtain wild *P. monodon* spawners in the belief they are superior to ablated females in terms of quantity and quality of eggs and larvae produced (section IV, F).

Male maturation

As with females, male maturity has two aspects — functional maturity or the ability to mate with the completion of the secondary sexual organs and physiological maturity or the ability of sperm to fertilize eggs with the development of the gonads. Male penaeids are functionally mature when their petasmata (accessory structures on the first pair of pleopods) are joined to each other by means of interlocking hooks.

However, more important is gonadal maturation and the presence of fully developed spermatozoa with spikes. These spikes are non-motile with an ultrastructure different from flagella (Clark et al., 1973) and are characteristic of other penaeid species. Because checking for spikes requires microscope work and sacrificing the male, many workers prefer to look for the swelling and whitish coloration of the terminal ampoules near the fifth pair of pereiopods as an indicator of gonadal maturity.

Ruangpanit et al. (1984) noted immature males without sperm from both pond and wild *P. monodon*. Alikunhi et al. (1975) reported maturation of male *P. monodon* from ponds 7-8 days after ablation but did not specify criteria for determining maturation. Primavera (1978) reported the presence of mature (spiked) sperm in *P. monodon* of 40 g and more from both wild and ponds although more recently, 10-month-old pond *P. monodon* were observed with immature (spikeless) sperm (Primavera, unpub.). Motoh (1981) reports that sperm from wild *P. monodon* with CL below 37 mm have no spikes. Among wild *P. merguiensis*, the joining of petasmata occurs between 20 to 25 mm CL (Tuma, 1966).

Male maturation should not be taken for granted because nonhatching of eggs may sometimes occur even when mating has taken place with the failure traceable to immature sperm.

Mating (spermatophore transfer)

Courtship and mating (precopulatory and copulatory) behavior has been described for *P. japonicus* (Hudinaga, 1942) and *P. monodon* (Primavera, 1979). In the latter, an elaborate series of stages is involved including parallel swimming, male turning ventral side up, then perpendicular to, and finally making a U-shape around, the female during which the sperm sacs are presumably inserted inside (transferred to) the thelycum. ("Spermatophore transfer" more ap-

propriately denotes mating or copulation among penaeids than the terms "impregnation" or "insemination" which are better applied to mammals.)

The prerequisite to mating in closed thelycum penaeids is the molting of the female (Fig. 2) in contrast to open thelycum species which require ovarian maturation and imminent spawning of the female (Aquacop, 1977a).

In both cases, mating probably depends on one or more pheromones released by the female to attract males (Table 5).

Complete darkness (Aquacop, 1980) as well as bright floodlights (Primavera, unpub.) are reported to hinder mating in *P. monodon*. Salinity may have no effect on copulation judging from the presence of sperm in the thelycum of *P. monodon* caught from estuaries and brackishwater ponds.

Mating requires a minimum water volume and depth with success and frequency limited in small tanks for *P. monodon* (Primavera, 1979; Poernomo and Hamami, 1983), *P. japonicus* (Lumare, 1981) and *P. esculentus* (P.J. Crocos, pers. comm.). The absence of (fertilization and) hatching possibly due to unsuccessful spermatophore transfer was observed in *P. monodon* (Liao, 1973; Primavera et al., 1979; Emmerson, 1983). Such failure of mating could be traced to absence of males in *P. monodon* (Pudadera et al., 1980b), shallow water depth (25 cm) in *P. orientalis* (Arnstein and Beard, 1975), too few males (7 ♀:1 ♂) and/or crowding due to numerous air-lift pipes and high stocking density (18/m²) in *P. plebejus* (Kelemec and Smith, 1980). Flowthrough water may also dilute pheromonal levels and decrease mating frequency.

Table 5. Role of various factors in controlled reproduction in closed thelycum penaeids.

	Mating	Maturation	Spawning	Fertilization, incubation and hatching
Hormonal/ pheromones	✓	✓	?	?
Nutritional	×	✓	×	×
Environmental (temperature, salinity, light)	✓	✓	✓	✓

Newly-caught wild or pond broodstock are generally mated when stocked in maturation tanks. Hatching and viability of nauplii from initial captive spawnings are dependent on sperm from copulation in the wild or pond environment. However, unsuccessful mating in captivity will eventually lead to nonhatching of eggs due to loss of spermatophores once the female molts as observed in *P. plebejus* (Kelemec and Smith, 1980) and *P. notialis* (L. Ramos, pers. comm.). *P. monodon* spawnings averaged 96% hatch rate up to 10 days after ablation and decreased to 0% 12-66 days afterwards (Muthu and Laxminarayana, 1977).

Completeness of male and female genitalia (minimum age/size), molting of female, light intensity, tank size and depth all appear to play a role in spermatophore transfer. The effect of other factors needs to be studied.

Spawning

The spawning behavior of *P. monodon* has been described by various workers (Villaluz et al., 1972; Aquacop, 1977b; Motoh, 1981). The presence of pinkish to orange scum along the walls of spawning tanks is generally an indication of spawning in penaeids with *P. kerathurus* a notable exception (Lumare, 1979). Very little or no scum has been observed with spawns from ablated *P. monodon* in tanks provided with gentle aeration (Primavera, unpub.) and may be associated with reduced stress on the ready-to-spawn females. Trays or plates are installed on the bottom of spawning tanks to prevent the females of *P. japonicus* (Lumare, 1981), *P. indicus* (Primavera et al., 1982), and other species (Aquacop, 1975) from eating their eggs.

Non-spawning and regression of ovaries due to stress and "overripe" ovaries invaded by haemocytes have been reported for *P. merguiensis* (Beard et al., 1977). On the other hand, regression of ovaries has been observed in both stressed and undisturbed *P. monodon* females (Aquacop 1977b, 1980). Gravid *P. monodon* that do not spawn for 2-3 successive nights but retain the outline of apparently ripe ovaries may have the "milky ovary" disease caused by a microsporidian.

White light and low temperature were found to inhibit spawning in *P. plebejus* (Kelemec and Smith, 1984) whereas temperature shock (abrupt increase) has been used to induce spawning. Spawning activity of wild *P. latisulcatus* appears to be related to water temperature (Penn, 1980). Although Aquacop (1975) mentions a lunar periodicity in spawnings of *P. merguiensis* broodstock in tanks, asynchronous spawning relative to the moon phase has been observed for wild populations of *P. latisulcatus* (Penn, 1980) and *P. merguiensis* (Crocos and Kerr, 1983).

Fertilization, incubation and hatching

The events following spawning have been described by Clark et al. (1984) in detail for *Sicyonia ingentis*, a shrimp closely related to penaeids. These include primary and secondary binding of sperm, a biphasic acrosomal reaction which is Ca^{++}-dependent, ovum jelly extrusion, fertilization or sperm-egg fusion and hatching membrane formation. During ovum jelly extrusion, also called the cortical reaction, a stratified corona around the egg is formed with the dehiscence of the cortical rods as observed in *P. aztecus* (Clark et al., 1982), *P. japonicus* (Hudinaga, 1942) and *P. monodon* (Primavera and Posadas, 1981).

The jelly layer or corona formed by the cortical reaction supposedly facilitates capture of the non-motile sperm by the egg in *P. orientalis* (Oka, 1967 cited by Wickins, 1976). In *P. aztecus,* ovum jelly extrusion is Mg^{++}-dependent and in penaeid eggs in general, the reaction is stimulated by exposure to seawater and not by fertilization (Clark and Lynn, 1977 cited by Clark et al., 1984). Abnormal spawnings of *P. monodon* eggs laid in masses on the tank bottom remain unfertilized and unhatched (Villaluz et al., 1972) perhaps due to a failure of the cortical reaction (Aquacop, 1977a).

Salinity in spawning tanks has ranged from 28 to 35 ppt for eggs of *P. monodon* (Villaluz et al., 1972; Primavera and

Borlongan, 1978; Simon, 1982; Hillier, 1984) and P. *semisulcatus* (Tseng and Cheng, 1981). Among various temperature-salinity combinations, Reyes (1981) obtained highest mean hatch rate of *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. Similarly, *P. indicus* eggs showed a significantly higher hatch rate and shorter incubation period at 33 ppt compared to 22 ppt and 42 ppt (A. Openiano, pers. comm.). At 20-25 ppt, the eggs of *P. indicus* and *P. semisulcatus* show retarded development and swelling to the point of bursting at 10-15 ppt (Tseng and Cheng, 1981) whereas they shrink at 50 ppt.

A temperature range of 26-29°C has been recorded for incubation of *P. monodon* eggs (Villaluz et al., 1972; 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).

In *P. semisulcatus*, incubation water pH of 7-8 gave 40-70% hatch rate while pH of 6 and 9 led to abnormal development with less than 20% hatch rate (Tseng and Cheng, 1981). Ethylene dinitro tetraacetic acid (EDTA), an agent which chelates heavy metals, is added to spawning tanks at 10 ppm (Simon, 1982; Hillier, 1984). Spawning or incubation tank density should not exceed 2,500-3,000 eggs/ℓ otherwise hatching will be poor (Primavera, 1983). A low aeration rate of 4 bubbles/sec increased spawn quality, fecundity and hatch rate of wild *P. indicus* spawners (Emmerson, 1980).

Comparison of fecundity, egg and larval quality

Lower fecundity in females matured in captivity compared to wild spawners has been observed for *P. californiensis* (Moore et al., 1974), *P. indicus* (Emmerson, 1980) and *P. japonicus* (Lumare, 1981). Similarly, ablated *P. merguiensis* produced a mean of 91,000 nauplii/spawn compared to 210,000-446,000 nauplii/spawn from wild spawners (Nurjana and Won, 1976). A range of 60,000 to 600,000 eggs/spawn has been observed for ablated *P. monodon* (Santiago, 1977; Vicente et al., 1979; Aquacop, 1983a; Poernomo and Hamami, 1983; Primavera, 1983) compared to 250,000-800,000 eggs/spawn for wild spawners (Motoh, 1981).

The lower fecundity of captive females may be due to the generally smaller sizes of broodstock compared to wild spawners (section IV, A) and uneven development of right and left ovarian sides in unilaterally ablated females as observed for *P. monodon* (M.N. Lin, pers. comm.). Even with adjustment made for female size, the lower fecundity of domestic unablated *P. indicus* is due to narrower ovary width compared to wild spawners, and to breaks in the ovary caused by collisions with tank walls (Emmerson, 1980).

More important than quantity is the quality of eggs and larvae. Aquacop (1977b) has classified *P. monodon* eggs into unfertilized, normal fertilized and abnormal fertilized eggs. These groupings approximate the egg types described by Primavera and Posadas (1981) based on morphology and hatch rates. A highly linear relationship was established between the proportion of good (A_1) eggs and hatch rate for

P. monodon. Although many Philippine hatchery operators tend to believe that fry from ablated females is weaker than from wild spawners, others have observed both good and poor quality eggs from wild spawners (SEAFDEC, 1984). Half-spent or partial spawnings of *P. semisulcatus* produced poor eggs with irregular cytoplasmic formation and autolysis (Tseng and Cheng, 1981). On the other hand, Primavera and Posadas (1981) found that wild *P. monodon* spawners had the highest proportion of good eggs followed by ablated wild females with ablated pond females producing many bad eggs.

Ruangpanit et al. (1984) observed relatively low survival rates (4-8.5%) from nauplii to postlarvae which may indicate a greater susceptibility to bacterial and fungal infection of *P. monodon* larvae from ablated wild stock. All these point to the need to improve egg and larval quality in both wild and pond broodstock.

Artificial spermatophore transfer; in vitro fertilization

Compared to closed thelycum species, the failure of mating and consequent absence of spermatophores is more frequent in open thelycum penaeids from which spermatophores are more easily dislodged. However, low frequency of mating may also be observed in closed thelycum species, e.g. *P. monodon* (Lin and Ting, 1984) perhaps due to diseased males or their short supply in captivity.

The artificial transfer of spermatophores developed to solve this problem consists of two processes — extraction and insertion. Extraction of spermatophores may be done by means of manual pressure on the base of the fifth pair of pereiopods; forceps inserted through the genital pores; siphoning out; or by the use of low electrical charges as tried on *P. japonicus* (Laubier-Bonichon and Ponticelli, 1981; Lumare, 1981; Ponticelli, 1981) and *P. monodon* (Lin and Ting, 1984; Muthu and Laxminarayana, 1984). The use of electricity prevents injury to the male and permanent damage to the seminal vesicles (Lumare, 1981; Muthu and Laxminarayana, 1984).

The structure of the open thelycum and the pouch-like closed thelycum of *P. japonicus* makes the insertion of spermatophores easier than with other closed thelycum species. With *P. monodon,* treatment must be on newly-molted females in contrast to *P. japonicus* which can be at any molt cycle stage. To reduce stress and mortality, females may be placed in a continuous gill irrigator that allows gas exchange (Tave and Brown, 1981) or anesthetized by lowering the temperature to 10°C for 5 min (Laubier-Bonichon and Ponticelli, 1981). Tave and Brown (1981) report a spawning rate of 80% for various species while Laubier-Bonichon and Ponticelli (1981) claim a fertilization rate of 80% and more for *P. japonicus.* Out of five *P. japonicus* tested, four females retained the spermatophores, two spawned and one hatched viable nauplii (Ponticelli, 1981).

In contrast, Lumare (1981) produced very poor results with 7.5% mean fertilization rate and 3.3% mean hatch rate from artificially tested female *P. japonicus* compared to 67.7% mean fertilization rate and 40.1% mean hatch rate from naturally mated females. Similarly, Muthu and Laxminarayana (1984) obtained nauplii with a low hatch rate of

2.4% from only one out of 10 artificial spermatophore transfers performed on three ablated *P. monodon* females. Higher hatch rates of 71.87% and 82.35% were obtained by insertion of one and two spermatophores, respectively, in *P. monodon* by Lin and Ting (1984).

In vitro fertilization has also been tried to solve the problem of lack of mating. Clark et al. (1973) obtained a hatch rate of 10% by mixing ampoules of mature males with gravid ovaries of female *P. aztecus.* Lin and Ting (1984) obtained successful fertilization with 49.4-63.1% hatch rate only when the sperm homogenate was added right before, and not right after or two hours before, spawning in *P. monodon.*

Future directions

Out of some 109 penaeid species of present or potential commercial value (Holthuis, 1980), almost a third have been reared in grow-out ponds and tanks (Table 6). Twenty-three species have been matured in captivity but a full closing of the cycle has been achieved for only seven species. This is because the state-of-the-art in most hatcheries is the successful production of penaeid larvae and postlarvae from either wild spawners or wild immature/spent females matured/re-matured in captivity (Fig. 4).

Table 6. Comparison of total number of commercial and cultured penaeid species.

	Total	Closed thelycum
A. No. of penaeid species of present or potential commercial value (Holthuis, 1980)	109	
B. No. of penaeid species cultured		
Grow-out (ponds and tanks)	34 (?)	—
Larval rearing	30	18
Maturation	23	17
Full closing of cycle (F_1 generation)	7	5

The improvement of reproductive performance including egg and larval quality from captive pond broodstock remains a major area for future research and includes the determination of minimum/optimum age and size for maturation. The complete characterization of the hormonal, nutritional and environmental requirements for maturation should lead to the development of alternatives to ablation, e.g. photoperiod manipulation or the use of hormones, or should at least enhance the eyestalk ablation technique.

Aside from maturation, the other major bottleneck in controlled reproduction of penaeids is successful spermatophore transfer. The present emphasis on female maturation should be extended to other aspects, particularly mating (Table 5). Studies on biology (molting, fertilization including cortical reaction) and biochemistry provide baseline information for the broodstock and maturation aquaculturist. Investigations of wild stocks complement laboratory studies in elucidating the interrelationships among molting, mating, maturation and spawning.

Lastly, the techniques of artificial spermatophore transfer and *in vitro* fertilization are useful not only in solving the immediate problem of lack of copulation but also for future genetic studies and hybridization work.

Addendum

Since the December 1984 conference, substantial data on maturation and spawning in *P. semisulcatus* have been reported by Browdy and Samocha (1985, in press) bringing to 8 and 6 the total number and the number of closed thelycum species, respectively, whose life cycle has been completed in captivity (Table 6). The P_2 generation was achieved with both ablated and unablated broodstock of *P. semisulcatus* maintained in 3 m^3 tanks with 40 ppt flowthrough water and fed with frozen *Artemia*, fish, shrimp and squid.

The average daily numbers of spawns and eggs produced by an ablated female was double that of unablated controls. Egg production in ablated females was consistent for 70-80 days followed by a decline while that of unablated females was more erratic with a decline after 100-110 days. Ablated females had fewer eggs in an average spawn than unablated ones but the quality as measured by rates of fertilization, hatching and metamorphosis to zoea remained the same.

There was no significant difference in spawn size or quality over the first three spawnings of both ablated and unablated females. There was a reduction in spawn size but not in quality of successive spawns in a single molt cycle. Similarly, there was a reduction in fecundity of pond broodstock over successive generations. Ablation did not affect survival of broodstock with relatively high rates attributed to the use of electrocautery, ablation of females during the intermolt, application of prophylactics, and reduced light intensity. Relatively successful spermatophore transfer (84-90%) was achieved at 1.8-2.5 ♀:1♂. Ablation significantly reduced the success of mating and molt cycle duration.

The maximum number of spawns in one molt cycle was 6 and 4 for ablated and unablated females, respectively. A single mating was sufficient to fertilize up to four spawns in a single molt cycle indicating that closed thelycum penaeids have the physiological capability to fertilize several spawns over the molt cycle.

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