CHAPTER I

INTRODUCTION

The fishery production of Thailand increased rapidly during the past three decades. It increased from 0.3 million tons in 1960 to about 2.6 million tons in 1988 (Department of Fisheries, 1990). The production nevertheless started to level off during the past decade because of the overfishing and the reduction of fishing ground as a result of 200 mile exclusive economic zone (EEZ) declared by neighbouring countries.

In order to compensate the loss, the government tried to promote the fishing joint venture and coastal aquaculture. The fishing joint venture projects, however, faced many problems while coastal aquaculture, especially marine shrimp culture received more attention. Marine shrimp is a fishery product rich in nutritional value, having a good taste and demand high price. During the last decade, the average value of frozen shrimp exported from Thailand was 3,000 million baht per year. The export went up to 9,500 million baht in value in 1988 (Thaifarmer Bank, 1989).

Thailand has a number of marine shrimp species which are suitable for culture. Among these, the giant tiger prawn (*Penaeus monodon*) is perhaps the best species for most situations. During 1985-1988 exported value of frozen shrimp increased about 30 percent compared to the preceding years resulting from increased *P. monodon*

demand of major importers such as Japan, U.S.A. and E.E.C. (Panipa Hanvivatanakit, 1988).

P. monodon culture in Thailand expanded rapidly and this resulted in the higher seed demand. Niwes Ruangpanit (1988) stated that the demand of postlarvae was 500-1,000 million per year. The seed production of *P. monodon* at present is still inconsistent and sometimes can not satisfy the demand. The present technology uses wild-caught spawners which are expensive and available only in certain seasons of the year. The price of wild-caught broodstock, besides feed, is the highest variable cost in *P. monodon* seed production (Niwes Ruangpanit, 1988; Panipa Hanvivatanakit, 1988; Office of Agricultural Economics, 1988).

Because of the forementioned problems, it might be worth to try other methods for the consistent production of *P. monodon* spawners with a lower price. The use of pond-reared *P. monodon* instead of the wild prawns for the broodstock might be an answer to this problem.

Objectives.

The main objectives of this study are as follows :

1. To comparatively study ovarian maturity and spawning of pond-reared and wild-caught *P. monodon*.

2. To determine the effect of the prawn sizes on ovarian maturity and spawning of pond-reared and wild-caught *P. monodon*.

3. To determine the effect of the different diets on ovarian maturity and spawning of *P. monodon*.

4. To determine moulting frequency of unilateral eye-stalk ablated female *P. monodon* rearing in the closed recirculating water system.

Expected Results.

An outcome of this investigation will be a background information for understanding the reproductive biology of *P. monodon*. The results can also be applied for the *P. monodon* broodstock improvement.

Literatures Review.

Taxonomy.

The taxonomy of giant tiger prawn is as follows (Solis, 1988) :

Phylum Arthropoda

Class Crustacea

Subclass Malacostraca

Order Decapoda

Suborder Natantia

Infraorder Penaeidea

Superfamily Penaeoidea

Family Penaeidae Rafinesque, 1815

Genus *Penaeus* Fabricius, 1798

Subgenus *Penaeus*

Species *monodon*

Scientific name : *Penaeus (Penaeus) monodon* Fabricius, 1798.

It has four synonyms :

Penaeus carinatus Dana, 1852

P. caeruleus Stebbings, 1905

P. monodon var. *manillensis* Villaluz and Arriola, 1938

P. bubulus Kubo, 1949

The common names are giant tiger prawn (English), crevette geante tigree (French), camaron tigre gigante (Spanish) and Kung Ku-La-Dum (Thai)

Morphology.

The exoskeleton of *P. monodon* is smooth, polished and glabrous. The rostrum, extending beyond the tip of the antennular peduncle, is sigmoidal shape, and has 6-8 dorsal and 2-4 ventral teeth, mostly 7 and 3, respectively. The carapace is carinated with the adrostral carina almost reaching the posterior margin of the carapace. The gastro-orbital carina occupies the posterior one-third to one-half distance between the post-orbital margin of the carapace and the hepatic spine. The hepatic carina is prominent and the anterior half is horizontal. The antennular flagellum is subequal to or slightly longer than the peduncle. Exopods are present on the first four pereopods but absent in the fifth. The abdomen is cartinated dorsally from the anterior one-third of the fourth to the sixth somites. The telson has a median groove but without dorsal-lateral spines (Motoh, 1981, 1985; Solis, 1988). Figure 1 shows the various parts of *P. monodon*.

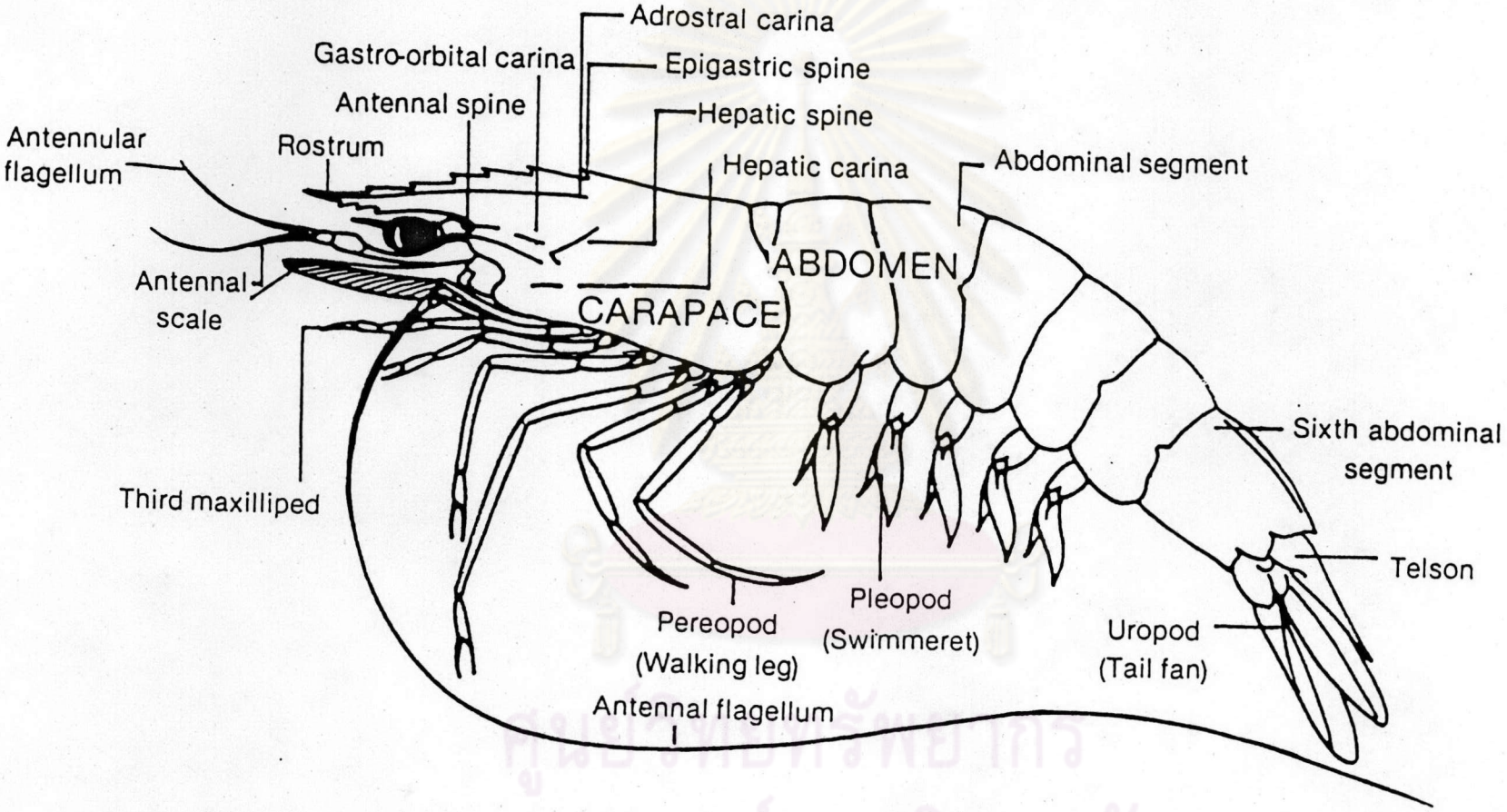

Figure 1. Lateral view of giant tiger prawn showing important parts. (source : Motoh, 1981).

A live giant tiger prawn has the following characteristic coloration. Carapace and abdomen are transversely banded with red and white, the antennae are greyish brown, and the pereopods and pleopods are brown with crimson fringing setae red. Upon entering shallow brackish waters or when kept in ponds, the color changes to dark brown and often to blackish (Motoh, 1981).

Distribution.

The giant tiger prawn can be found throughout the greater part of the Indo-West Pacific region, ranging northward to Japan and Taiwan, eastward to Tahiti, southward to Australia, and westward to Africa (Motoh, 1981, 1985; Solis, 1988).

In general, the distribution area of ranges *P. monodon* distributes from 30° E to 155° E longitude and from 35° N to 35° S latitude with the main fishing grounds located in the tropical countries, particularly Indonesia, Malaysia, and the Philippines.

The fry, juveniles, and adolescents inhabit shore area and mangrove estauries, while most of the adults inhabit deeper waters down to about 160 m.

Reproductive Biology.

A. **Reproductive System.**

P. monodon is dioecious. The sexes can be distinguished by external characters (genital organs); petasma and a pair of appendix masculina in male and thelycum in female. The petasma is

situated between the first pleopods and the appendix masculina on the exopods of the second pleopods, while the thelycum is situated between the fourth and fifth pereopods.

A pair of genital openings in the male is situated on the coxae of the fifth pereopods and in the female on the coxae of the third pereopods.

1. Male Genital Organ.

The internal reproductive organ of the male consists of a pair of testes, vasa deferentia, and terminal ampoules located in the cardiac region dorsal to the hepatopancreas. The testis is translucent and composed of six lobes, each connected in the inner margins leading to the vas deferens. The vas deferens consists of four portions, namely the short narrow proximal vas deferens, a thickened larger median portion or the medial vas deferens, the relatively long narrow tube as the distal vas deferens, and the mascular portion referred to as terminal ampoule. The terminal ampoule contains spermatophore and opens at the base of coxopod of the fifth pereopods (Solis, 1988).

The spermatozoa of *P. monodon* are minute globular bodies composed of a head of about 3 microns in diameter and a short spike.

The petasma is a pair of endopods of the first pleopods formed by the interlocking hook-like structures. The appendix masculina is oval and is located on the endopod of the second pleopod.

2. Female Genital Organ.

The internal reproductive organ of the female consists of paired ovaries and oviducts. The ovaries are bilaterally symmetrical, partly fused, and extend almost the entire length of the mature female. It is composed of the anterior lobe located close to the esophagus and the cardiac region of the stomach, the lateral lobes located dorsal to the hepatopancreas; and the abdominal lobe which lies dorso-lateral to the intestine and ventrolateral to the dorsal abdominal artery. The oviducts originate at the tips of the sixth lateral lobe and lead to the external genital opening at the coxopods of the third pair of pereopods (Solis, 1988).

The thelycum, located between the fifth pair of pleopods, consists of an anterior and a pair of lateral plates. It receives the spermatophores during mating.

B. Sexual Maturity.

Motoh (1981) defined sexual maturity as the minimum size at which spermatozoa are found inside the terminal ampoule of the males and inside the thelycum in the females. The latter indicates that copulation or spermatophore transfer from the male to the thelycum of the female has taken place. On this basis, Motoh stated that wild *P. monodon* males have spermatozoa at 37 mm carapace length (CL) (about 35 g body weight or BW) and females at 47 mm CL (about 67.7 g BW), although pond-reared prawns were mature only at 31 mm CL (about 20 g BW) and 39 mm CL (about 41.3 g BW), respectively.

From the reproduction point of view, Primavera (1985) emphasized the importance of gonadal maturation and the presence of

fully developed spermatozoa with spike. Motoh (1981) found that sperm in wild *P. monodon* males below 37 mm showed only a body (without spike), while Primavera (unpubl.) cited by Primavera (1985) made mention of 10-month old pond-reared *P. monodon* with immature (spikeless) sperm.

The minimum age at first ovarian maturation of captive broodstock is 9-18 months for *P. monodon* (AQUACOP, 1977a; Santiago, 1977; Primavera, Borlongan, and Posadas, 1978; Anand Tunsutapanich *et al.*, 1989). However, Primavera (1978) reported that five-month-old *P. monodon* could mature and spawn after ablation but generated poor quality larvae. This indicates the need for older females that may be more receptive to induced maturation. Pond-reared females should be at least one year old to be able to produce good egg and larval quality after eyestalk ablation (Primavera, 1983; Millamena *et al.*, 1986). Sakchai Chotikun, Kriengsak Padetpai, and Supot N Bangchang (1987) and Anand Tunsutapanich *et al.* (1989) reported that the optimal size for maturation of the pond-reared prawn could be at least eighteenth-month-old. Satisfactory maturation, spawning, and metamorphosis of larvae to post-larvae were obtained using eyestalk ablation and artificial insemination techniques on 6 and 8 month-old pond-reared males and females, respectively, but the best results were obtained with broodstock prawns that are over a year old (Chwang, Chiang, and Liao, 1986).

Spawners from captive broodstock are generally smaller than those from the wild. Ablated female *P. monodon* has a minimum size of 32 g (Poernomo and Hamami, 1983) and 45 g (AQUACOP, 1977a

cited by Primavera, 1985) compared to > 75 g for wild spawners (Primavera, 1978).

Taiwanese and many Philippine hatchery operators believe that wild *P. monodon* spawners are superior for quantity and quality of eggs and larvae production (Primavera, 1985). Prawns with similar physical appearances, but with different origins may respond differently to the maturation technique (Chwang *et al.*, 1986). Prawns from shallow water coastal areas, or from area influenced by freshwater runoff give unfavorable results. High quality *P. monodon* in Thailand typically require males 60 g or larger, and females 80 g or larger (Niwes Ruangpanit *et al.*, 1981; Somboon Laoprasert and Pitak Polkhan, 1984; Somboon Laoprasert, 1985; National Institute of Coastal Aquaculture, NICA, 1985; Sakchai Chotikun *et al.*, 1987; Sakchai Chotikun, 1988). The females captured from the wild may be either gravid, or non gravid. It is believed that larvae of broodstocks obtained from the Andaman Sea, which is deeper and being a real marine environment are better than larvae from other sources (NICA, 1985; Niwes Ruangpanit *et al.*, 1985; Niwes Ruangpanit, 1988).

1. <u>Ovarian Maturation Stages.</u>

The maturation of the ovary has been categorized into five stages (Figure 2), based on ovum size, gonad expansion, and coloration (Primavera, 1983,1985; Motoh, 1981).

Stage I and V (undeveloped and spent stages), ovaries are very small, flaccid, and invisible through the exoskeleton. The ova are covered with a layer of follicle cells and are small, measuring 35 microns on average, and only the largest ones reveal a

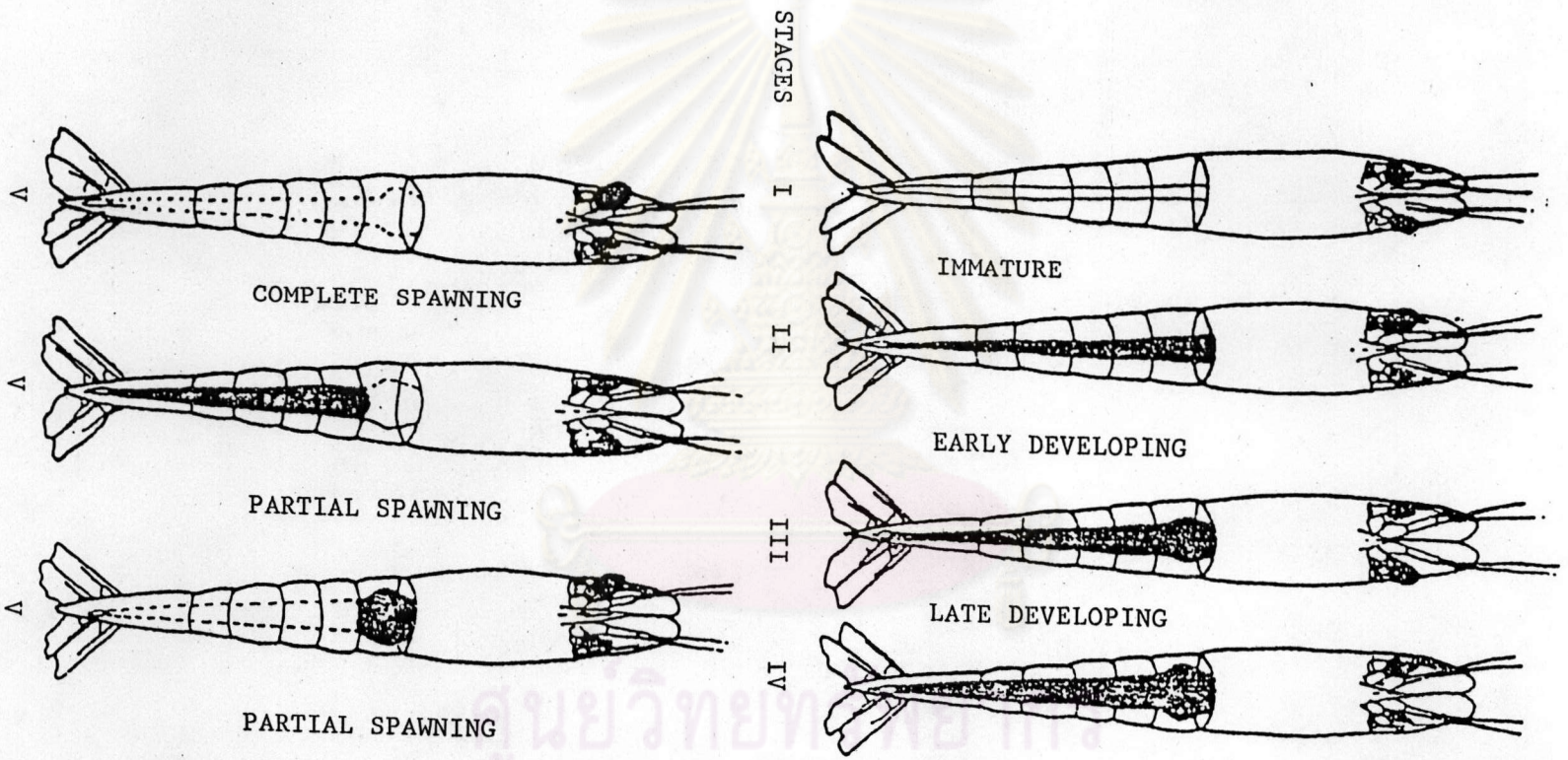

Figure 2. External appearance of the ovaries of giant tiger prawn (source : Primavera, 1983).

nucleus and yolk granules. *P. monodon* with carapace length less than 47 mm, are recognized as undeveloped.

Stage II (developing stage). Developing ovaries can be easily differentiated from other tissues. They are flaccid and white to pale olive puff. Developing ova have yolk granules and cells believed to be nutritive bodies and have an average diameter of 177 microns.

Stage III (nearly ripe stage). Ovaries have glaucous color with the anterior portion thick and expanded. They are very visible through the exoskeleton, particularly at the first abdominal segment, when viewed against the light. The average ova size is 215 microns in diameter.

Stage IV (ripe stage). The ovary classified as ripe (mature) stage is diamond-shaped, expanding through the exoskeleton of the first abdominal segment. The isolated ovary appears dark olive green, filling up all the available space in the body cavity (Primavera, 1983). Motoh (1981) reported the presence of a characteristic margin of peripheral rod-like bodies, the apexes of which radiate from the egg center. The average ova size is 235 microns in diameter.Tan-Fermin and Pudadera (in press) cited by Solis (1988) characterized this stage to consist mostly of yolky oocytes (288-408 microns) with additional rod-like bodies which contain acid and basic mucopolysaccharides but without lipids.

In some cases, ovaries are observed to be discontinuous, i.e., white in color in either the anterior or

posterior portions with olive green color in the opposite ends. This condition is referred to as partially spent ovaries (Solis, 1988).

C. **Mating.**

P. monodon is a closed thelycum penaeid in which the prerequisite to mating or spermatophore transfer is the pre-molting of females.

Courtship (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 over distances of 50-80 cm. 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 female. If successful, the male quickly shifts from parallel position to perpendicular to the female. He curve, his body in a U-shape around the female, flicking head and tail simultaneously, presumably inserting the spermatophores inside the thelycum at this time (Primavera, 1983).

Mating requires a minimum water volume and depth. Limited frequency and success of mating have been experienced in small and shallow tanks (Poernomo and Hamami, 1983). Complete darkness (AQUACOP, 1980 cited by Primavera, 1985) as well as bright floodlights (Primavera, unpubl. cited by Primavera, 1985) can also hinder mating of *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 (Primavera, 1985).

The absence of fertilization and hatching is possibly due to unsuccessful spermatophore transfer in *P. monodon* (Primavera, Lim, and Borlongan, 1979; Emmerson, 1983).

Newly-caught wild or pond-reared broodstock are generally mated when stocked in maturation tanks. Hatching and viability of nauplii from initial captive spawnings are dependent on sperm copulation in the wild or pond environment (Primavera, 1985). Failure of mating in captivity will ultimately lead to unhatched (unfertilized) eggs due to the loss of spermatophores once that female molted. Decrease of hatching rates in *P. monodon* ranging from 96% at 10 days after ablation down to 0% at 12-66 days after ablation may be traced to non-mating of captive broodstock, (Muthu and Laxminarayana, 1977 cited by Primavera, 1988).

D. Spawning.

Spawning in *P. monodon* generally occurs between 8:00 p.m. and 6:00 a.m. and the following description is based on reports by Motoh (1981), and Primavera (1983, 1985).

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

Gravid females that do not spawn for 2-3 successive nights but retain the ovarian outline may have the "milky ovary" disease caused by a microsporidian (Primavera, 1988).

E. Fertilization, Incubation, and Hatching.

The events following spawning have been described by Clark *et al.*, (1984) cited by Primavera (1988) in detail for *Sicyonia ingentis*, a shrimp closely related to penaeids. These include sperm binding, acrosomal reaction, ovum jelly extrusion, fertilization or sperm-egg fusion and hatching membrane formation. Ovum jelly extrusion or the cortical reaction involves the extrusion of cortical rod to form a corona or jelly layer as observed in *P. monodon* (Primavera and Posadas, 1981). Abnormal spawns of *P. monodon* eggs laid in masses on the tank bottom remain unfertilized and unhatched (Villaluz *et al.*, 1972 cited by Primavera, 1988) perhaps due to a failure of the cortical reaction (AQUACOP, 1977a cited by Primavera, 1988).

Development of *P. monodon* eggs has been described by Motoh (1981); hatching time is 12-15 hrs after spawning. Hatching rate from eggs to nauplii is correlated with egg quality which is detertimed following the morphological classification (Primavera and Posodas, 1981; Figure 3).

F. Fecundity and Spawning Success.

The number of eggs spawned varies according to the condition of the spawning female. Fecundity ranges from 248,000 to 811,000 eggs and 57,650 to 550,300 eggs for unablated and ablated wild

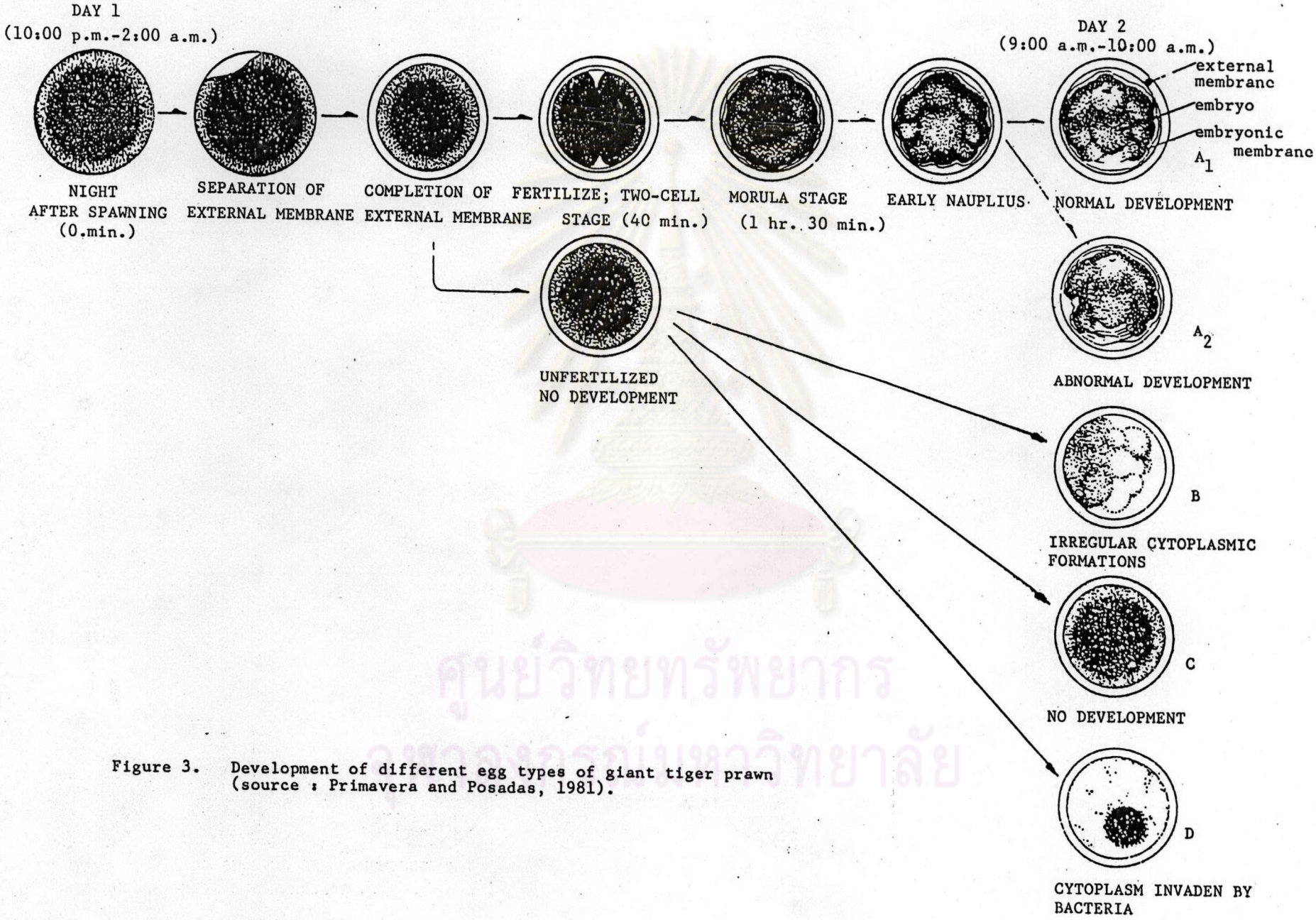

Figure 3. Development of different egg types of giant tiger prawn (source : Primavera and Posadas, 1981).

P. monodon, respectively (Motoh, 1981; Emmerson, 1983; Hillier, 1984; Somboon Laoprasert, 1985; Sakchai Chotikun, 1988); whereas pond ablated female fecundity ranges from 60,000-1,050,000 eggs (Muthu and Laxminarayana, 1977 cited by Primavera, 1985; Primavera, 1978; Aquacop, 1983; Anand Tunsutapanich *et al.*, 1989). The wide range of egg numbers could be due to female size (50 to 200 g) and inclusion of egg counts from both partial and complete spawns. Similarly, hatching rate range from 0 to over 90% depending on nutrition, sex ratio, water depth, and other physiological factors that may affect egg quality and mating efficiency (Primavera, 1988).

G. Artificial Insemination and Fertilization.

Low mating efficiency observed in penaeid prawns is perhaps due to disease or lack of male supply in captivity. Leung-Trujillo and Lawrence (1987) reported a decline in sperm quality of *P. setiferus* after three weeks of captivity. Bacteria proliferate in the sperm mass of captive male *P. setiferus*. Destruction of penaeid sperm is occurring in regions of the vas deferens that appear normal under the dissecting microscope (Talbot *et al.*, 1988). Thus captive males become infertile prior to what would be expected based on the external morphology of the vas deferens.

Artificial insemination has been developed to solve poor mating efficiency problem. Spermatophores are extracted either manually or electrically and inserted in the thelycum of a newly molted female. Mean hatching rate from *P. monodon* females implanted with two spermatophores was 82.35% and 39.11% for the first and subsequent spawnings, respectively, while those implanted with only

one spermatophore were 71.87% and 13.17% respectively (Lin and Ting, 1986). Lin and Hanyu (1990) found that spermatozoa in the vas deferens are functionally mature. Pond-reared *P. penicillatus* females implanted with fragments of vas deferens gave an average hatching rate of 76.2%.

An artificial copulatal fluid (ACF) which contains 62.5 mg/ml of trypsin (1:2,000) can transform the whole spermatophore into spermatic fluid. This method is found to be useful to artificial insemination. Hatching rate is increased in the eye-stalk ablated gravid *P. penicillatus* females implanted with spermatophores immersed previously in ACF and even better by implanting sperm mass of spermatophore together with ACF (Lin and Hanyu, 1990).

Artificial fertilization of *P. monodon* gave 49.4 to 63.1% hatching rate when the sperm homogenate was added right after spawning (Lin and Ting, 1986).

H. Factors Influencing Maturation.

There are 3 main factors influencing maturation and reproduction in marine prawn; hormonal control, environmental factors and nutrition (Lawrence and Bray, 1985; Primavera, 1985).

1. Hormonal Control.

Ovarian maturation and function in decapod crustaceans is apparently inhibited by a gonad-inhibiting hormone (GIH) produced by the neurosecretory cells of the X-organ and then stored and released by the sinus gland, both located in the eyestalks (Adiyodi and Adiyodi, 1970 cited by Primavera, 1985). The GIH

inhibits gonadal maturation, either through direct action on the ovary or fat body (Meusy and Chariaux Cotton, 1984 cited by Primavera, 1985), or through indirect action on the brain and/or thoracic ganglion (Adiyodi and Adiyodi, 1970 cited by Primavera, 1985). Under natural conditions, environmental stimuli act to reduce the production of GIH by the X-organ, and thus activate the production of gonad-stimulating hormone once the GIH titer reaches a certain concentration (Khoo,1988). Artificially induced maturation by eye-stalk ablation destroyes the X-organ by removal or destruction of the eye-stalk,and the GIH titer is thus artificially,reduced. This leads to rapid and repeated gonadal maturation and spawning. *P. monodon* in captivity is difficult to breed, so the eyestalk ablation technique is used to increase maturation and spawning rates (Primavera, 1985).

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 eye-stalk was sufficient to mature *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), Somboon Laoprasert and Pitak Polkhan (1984) but was not observed in AQUACOP (1977a) and by Vincente, Valdez, and Valdez (1979) cited by Primavera (1985).

Synchronization of ablation with the molt cycle has been shown to be an important factor for the prediction of egg

production (Browdy and Samocha, 1985a). Ablation during the postmolt leads to mortality because of added stress on the female and excessive loss of hemolymph (AQUACOP, 1977a cited by Primavera, 1988). 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 or 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 adult from offshore (Primavera and Borlongan, 1978; Simon, 1982). Similarly, wild *P. monodon* from offshore Andaman Sea took only 4-5 days to spawn after ablation in contrast to 20-30 days for female from Brackishwater Songkla Lake (Niwes Ruangpanit *et al.*, 1985). AQUACOP (1983) and Poernomo and Hamami (1983) reported that Pond-reared *P. monodon* took a minimum of 3 and 11 days respectively to spawn after ablation.

The number of spawns per molt cycle is larger from ablated (6 spawns) than in unablated (3 spawns) *P. monodon* (Beard and Wickins, 1980; Emmerson, 1983 and Hillier, 1984). Consequently, the interval between consecutive spawning is reduced to only 3 to 15 days in ablated females (Beard and Wickins, 1980; Emmerson, 1983; Poernomo and Hamami, 1983) compared to a minimum of 10 days up to 2.7 months in the unablated controll and females in the wild (Emmerson, 1983; Primavera, 1985).

This rushing of physiological processes leaves almost no time for adequate replenishment of nutrient stores and tissue building and is in many cases responsible for poor spawns or aborted spawns (Lawrence and Bray, 1985). Therefore, ablated females tend to show a decline in fecundity, hatching rate and egg viability given the greater number of spawns in a molt cycle (Beard and Wickens,1980; Primavera, 1988).

Nevertheless, many commercial hatcheries prefer to do eyestalk ablation because the resulting predictability in spawns and nauplii compensates for the decreased fecundity and spawn success (Primavera, 1985). Also, *P. monodon* broodstocks are replaced 6-8 weeks after ablation (Simon, 1982; Primavera, 1983) so that only the first, or at most, second spawn in a molt cycle is harvested thereby eliminating the less viable later spawns.

2. Environmental Factors.

Most penaeids have a life cycle including an estuarine phase as postlarvae and juveniles; a deep water, offshore phase as adults; followed by offshore spawning of the adults and the inshore migration of larvae and postlarvae. The offshore existence of the adults during reproduction provides some clues about the environmental factors with influence maturation and reproduction (Primavera, 1985). The most successful cases of captive reproduction have duplicated some of these factors.

a) Light.

The deeper offshore waters where adult penaeids breed is characterized by reduced light intensity and a larger penetration of blue and green light compared to other wavelengths (Jerlov, 1970 cited by Primavera, 1985). Unablated *P. monodon* attained only partial maturation under blue and natural light but not under red light (Pudadera and Primavera, 1981). Green light at reduced intensities combined with unilateral ablation induced maturation in wild immature *P. monodon* (Hillier, 1984). Similarly, Primavera (1988) reported that green light gave the best result in terms of nauplii and % hatching rate of ablated and unablated *P. monodon* compared to other wavelengths.

Reduced light levels down to 70 Wcm^{-2} led to faster maturation and spawning in unablated and ablated *P. monodon* (Emmerson, 1983; Hillier, 1984). Dark covers may also reduce light intensities in maturation tanks to around 200 lux or less and minimize disturbance of broodstock (Primavera, 1983).

b) Salinity.

A salinity range of 28 to 32 ppt is required for *P. monodon* hatchery (SEAFDEC, 1984; Chwang *et al.*, 1986). Posadas (1986) cited by Primavera (1988) 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.

c) **Temperature.**

P. monodon spawns at temperatures of 26-32 °C (Primavera and Hamami, 1983; Primavera, 1985). Chwang *et al.* (1986) suggested that optimum temperature for maturation, spawning and incubation for *P. monodon* was about 23-30 °C. Not only was the absolute temperature important, but also the constancy of temperature. Fluctuating temperatures were not common in offshore prawn spawning grounds, and did not enhance reproductive success in captivity.

d) **pH.**

The optimal pH required for warm water prawn hatchery is about 7.5-8.5 (Pinij Kungvankij *et al.*, 1986) 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 cited by Primavera, 1985).

e) **Dissolved Oxygen.**

Lawrence and Bray (1985) and (Pinij Kungvankij *et al.*(1986) suggested that dissolved oxygen should be maintained close to saturation to provide the best culture condition for *P. monodon* broodstock.

f) **Tanks.**

Tank size, shape, roughness and depth are all important considerations. Small tanks can prevent both mating and maturation (Poernomo and Hamami, 1983). Large tanks are better, but if they are too large, it is difficult to attend to the prawn and to maintain the tank without excessive disturbance. In general, tanks of

no less than 4 m diameter, and 1 m water depth give the best mating result with *P. monodon* (Niwes Ruangpanit *et al.*, 1981).

The tank wall should preferably be smooth and circular. This configuration, along with a circular water motion will help to keep the prawn from colliding with the tank sides. The color of the tank walls, color of the bottom, and the presence of a sand substrate are also important in the spawning tanks. Tanks with a white sand bottom gave better results than black sand (Pudadera, Primavera, and Borlongan, 1980). However, Simon (1982) have successfully obtained maturation and spawning in ablated *P. monodon* in tanks with bare substrates. Tanks with black walls were superior to tanks with white walls or unpainted (Emmerson, 1980).

g) Stress.

Stress from noise levels, shadows, handing, tank cleaning and environmental fluctuations should be kept to a minimum during the rearing of prawn broodstocks (Primavera, 1983; Lawrence and Bray, 1985).

3. Nutrition.

The nutrition is considered to be an important factor for broodstock prawn. It may affect not only prawn maturation and reproductive success, but also the survival of larvae during early development in the hatchery. The biochemistry of broodstock nutrition is not well understood, but it is known that food quality has a very important influence on reproductive success. The amount and types of protein, lipids and cholesterol all play an important role.

a) Protein.

Ablated decapods procure additional energy to sustain frequent molt, accelerated growth efficiency and larger egg output (Pandian and Kumari, 1985 cited by Marian *et al.*, 1986). Marian *et al.* (1986) reported that ablated prawn *Macrobrachium lamarri* required higher dietary protein (40%) than non-ablated prawn (36%). Most prepared diets used in reproduction experiments of penaeids are in excess of 50% protein (AQUACOP, 1979; Lawrence and Bray, 1985; Millamena *et al.*, 1986).

b) Lipid.

Ovarian lipid concentration in the crab, *Parathelplusa hydrodromous* increased rapidly in late first vitelogenesis whereas lipid levels in the hepatopancreas declined (Adiyodi and Adiyodi, 1970 cited by Lawrence and Bray, 1985). Chamberlain and Lawrence (1983) stated that accumulative lipids in the hepatopancreas of decapod crustaceans were used during vitellogenesis.

The destalked prawns *P. japonicus* are conceived to meet the lipids for the vitellogenesis mainly by the transfer of body lipid reverse, particularly hepatopancreatic lipids, to the ovaries which accumulated triglycerides (TG) and phosphatidylcholines (PC) as the major lipid classes (Teshima *et al.*, 1988a, b). Wild immature *P. monodon* females showed an increase in ovarian lipid levels upon reaching full maturity 5.8 to 17.0% and from 7.5 to 21.9% in unablated and ablated females, respectively (Millamena, Pudadera, and Catacutan, 1985).

c) Fatty Acids.

Ovarian lipids, especially neutral lipids, of destalked prawns *P. japonicus* contain higher proportions of monoenes such as 16:1 and 18:1 and 22:6 W 3 and lower proportion of 20:4 W 6 and 20:5 W 3 than those of non-destalked prawns (Teshima *et al.*, 1988a). Since *P. japonicus* is incapable of synthesizing *de novo* W 3 fatty acids but capable of elongating carbon chains, the increase in proportion of 22:6 W 3 and concomitant decrease in proportion of 20:5 W 3 in the ovarian neutral lipid of destalked prawns suggest the conversion of 20:5 W 3 to 22:6 W 3 in the body during the ovarian maturation and assume some role of 22:6 W 3 in the elaboration of ovaries.

Teshima *et al.*(1988b) stated that major ovarian phosphatidylcholines accumulated during the induced ovarian maturation of *P. japonicus* are rich in W 3 - fatty acids, possibly 18:3 W 3, 20:5 W3 and 22:6 W 3 rather than 16:0 and its metabolites, whereas the major ovarian triglycorides consists of 16:0 and its metabolites rather than W 3 - fatty acids as acyl groups.

The fatty acid profile of ovarian lipid in *P. monodon* showed 12.14-24.87% and 11.81-21.50% total fatty acids in wild unablated and ablated females, respectively, consisting of 20:4 W 6, 20:5 W 3, and 22:6 W 3 fatty acids. Similar proportion of the same polyunsaturated fatty acids (PUFA) were found in the eggs spawned, indicating their importance in the reproductive process (Millamena *et al.*, 1985).

Fatty acid profiles of early development stages, eggs through protozoea, of laboratory matured *P. stylirostris* showed that individual fatty acids appeared to be utilized at differential rate throughout early life stages. Level of C14 at the early eggs, C18 at the egg-nauplius, C16 at the nauplius, and C20 : 4 at the protozoea stages, were inversely correlated to hatch rate. Conversely, level of C20:5 W 3 at the nauplius stage and C22:6 W 3 at the nauplius and protozoea stages, were positively correlated to hatching rate in spawns (Araujo and Lawrence, 1989).

P. setiferus and *P. monodon* broodstock performance appeared to be related to the fatty acid pattern of the diet (Middleditch, Missler, Hines, Mc Vey *et al.*, 1980; Millamena *et al.*, 1985). Recently, *Mysidopsis bahia* fed with Marila (commercial maturation diet enrich with W 3-PUFA) got 8.8 times more juveniles with good quality in terms of resistance to temperature shock and starvation than those fed with other diets (Leger, Ferraz de Quairoz, and Sorgeloos, 1989). This confirms that the content of W 3-highly unsaturated long chain fatty acids is one of the factors determining the quality of the diet for marine crustacean broodstocks.

d) Cholesterol.

Cholesterol synthesis does not occur in decapod crustaceans, so prawns need lipids, cholesterol and its related compounds to supply precursors for synthesis of yolky material which is required for their ovarian maturation (Lawrence and Bray, 1985; Khoo, 1988). Clark and Lawrence (1988a) stated that the minimum cholesterol requirement of *P. vannamei* for good growth is 0.5%.

e) Lecithin.

Lecithin is a questionable essential lipid in prawn diets, especially because of its varying fatty acid composition. Clark and Lawrence (1988b) suggested that lecithin requirement of *P. vannamei* is between 2% and 8%.

f) Food Sources.

A variety of fresh diet items have been used for captive maturation of penaeids, including crustaceans (*Penaeus, Crangon, Mesopodopsis, Artemia*); squid (*Sepia, Loligo and Logiguncula*); polychaete worms (mainly Glycera); mollusc (squid, mussel, oyster, cockle and clams); and various fish; among these, marine invertebrates give good results (Lawrence and Bray, 1985; Primavera, 1988). Missler, Hines, Chan *et al.* (1980) found that lipid profiles of various marine invertebrate species were very similar and the unsaturated fatty acids were the major fatty acids in most marine invertebrate especially annelids. The C20 compounds are particularly abundant but relatively low concentration of the C18 compounds are observed, whereas cholesterol is predominated sterol.

Lytle, Lytle and Ogle (1988) reported that several food materials provided to equal or exceed bloodworms in absolute quantities of PUFAs and may alter to bloodworms which provide nutritional components in diets of captive *P. vannamei* that were essential for maturation. He also found that bloodworm could be stored at least one year at 4 °C with little degradation in fatty acids.

A composite diet of squids, polychaete worms, prawns and clams has been found to produce significantly higher level of maturation and ovary size than any single item (Chamberlain and Lawrence, 1981). The food organisms for maturation should have ripe gonads for ingestion by the broodstock (AQUACOP, unpubl. cited by Khoo, 1988).

Many researchers (Beard, Wickins, and Arnstein, 1977; Santiago, 1977; AQUACOP, 1979; Brown *et al.*, 1979; Primavera *et al.*, 1979; Beard and Wickins, 1980; Lawrence *et al.*, 1980; Chamberlain and Lawrence, 1981) found artificial diets useful as supplements to, rather than replacements of, fresh food items. Bray and Lawrence (1988) got the result that gonad tissue long chain fatty acid levels in *P. stylirostris* fed with fresh diet (squid, bloodworms, shrimps, and brine shrimp in a 4:2:2:1 ratio) were most similar to those of wild matured female, and significantly lower in those fed with combination diets of 40% squid and 60% prepared dry feed.

However, Millamena *et al.*, (1986) found that reproductive performance of pond-reared *P. monodon* in terms of total number of spawnings, eggs and nauplii production, average hatching rate of eggs and larval quality were superior in females given practical diet with cod liver oil as lipid source, compared to those given practical diet with soybean lecithin as lipid source and an natural diets (squid and marine annelids).