CHAPTER IV

DISCUSSIONS

Ovarian Maturity and Spawning.

The higher percentage of gravid females and higher frequency of stage IV ovarian maturity in the groups of large female prawns in this study are similar to other reports. Sakchai Chotikun *et al.* (1987) and Anand Tunsutapanich *et al.* (1989) reported that the optimal size for maturation of the pond-reared prawns should be at least eighteenth-month-old. In the wild, *P. monodon* attained full maturity and spawning at 10 months (Motoh, 1981). The prawn sizes of these reports would be > 100 g and this size was assigned as the large prawns for the present study. Primavera (1978) reported that five-month-old *P. monodon* could mature and spawn after ablation but generated poor quality larvae. This indicated the need for older and larger females which might be more receptive to induced maturation.

As regards the effect of broodstock sources, the present study found that pond-reared and shallow water wild-caught broodstocks performed comparable maturation. This finding is in accordance with the report of Bung-orn Srimukda (1987).

The higher percentage of gravid females and higher frequency of stage IV ovarian maturity in the groups of prawns fed with fresh natural diets and the combined diets in Experiment 2 compared to the group of large prawns in Experiment 1 might be due to a better quality of fresh natural diets used in Experiment 2. Horse mussel was seemed to be an acceptable fresh natural diet for reproduction performance of *P. monodon* in Thailand (Bung-orn Srimuka, 1987) and cow livers which was rich in cholesterol were used for feeding the prawns in Experiment 2. Whereas, squid, ridge venus clam, oyster and sand worm were used as fresh natural diets in Experiment 1. Another reason that the prawns of Experiment 2 performed better ovarian maturity was that these prawn had no disease problem while the prawns in Experiment 1 had the *Fusarium* problem.

The longer elapsed time between eye-stalk ablation and the first stage IV ovarian maturity compared to the elapsed time between two consecutive stage IV ovarian maturities might be due to the fact that the eye-stalk ablated female prawns required a period of time for biochemical and physiological change to mature after eye-stalk ablation.

The shorter elapsed time between two consecutive stage IV ovarian maturities in Experiment 2 compared with Experiment 1 might be due to a better quality of fresh natural diets as already discussed. In addition, the longer elapsed time between two consecutive stage IV ovarian maturities in Experiment 1 might be due to lower temperatures.

The present study indicated that broodstock sources and sizes did not play an important role on the latency period between eye-stalk ablation and the first stage IV ovarian maturity and the elapse time between two consecutive stage IV ovarian maturities. However, it should be noted that wild-caught prawns used in this study were captured from shallow waters, especially in the river estuary. This finding is in accordance with others. Niwes Ruangpanit *et al.*, (1985) reported that shallow water wilds took 20-30 days to mature and spawn after the eye-stalk ablation. For pond-reared prawns, AQUACOP (1983) found that pond-reared prawns took an average of 2-3 weeks to spawn after the eye-stalk ablation.

The results on spawning of the experimental prawns followed the same pattern as ovarian maturity results. The average elapsed time between eye-stalk ablation and the first spawning of Experiment 1 (17 days) was shorter than that of Poernomo and Hamami (1983) who reported the average of 32 days. The average of elapsed time between two consecutive spawnings of Experiment 1 (9 days) was also shorter than Poernomo and Hamami who reported the average of 16 days.

<u>Egg Quantity and Quality.</u>

Statistically the average number of eggs per spawning of the large prawn groups and the small prawn groups in Experiment 1 were similar. Because of this, the greater number of eggs spawned per female of the large prawn groups might be due to the higher frequency of spawning when compared with the small prawn groups.

In the same manner, the greater number of eggs spawned per female of the prawns fed with fresh natural diets and the prawns fed with combined diets in Experiment 2 might be due to the same reason.

The lower number of egg spawned per female in Experiment 1 when compared with Experiment 2 might be due to higher quality of fresh natural diets used in Experiment 2.

The average number of eggs spawned per female of the prawns fed with fresh natural diets (1,002,300 eggs) and the prawns fed with combined diets (848,478 eggs) in Experiment 2 were considered high when compared with those reported by Poernomo and Hamami (1983) which obtained 215,700 eggs spawned per females.

The overall percent fertility obtained during this study was relatively low (35.2% in Experiment 1 and 10.9% in Experiment 2) compared to that reported by AQUACOP (1983) who obtained 60% fertility from eye-stalk ablated pond-reared *P. monodon*. Marked decreases of percent fertility appeared at 22 days and 12 days after ablation of pond-reared and wild-caught prawns in Experiment 1, respectively. This followed the same pattern as reported by Beard and Wickins (1982) where ablated *P. monodon* produced fertilized eggs only during a short period after the eye-stalk ablation.

The absence of fertilization was possibly due to the lack of mating (Primavera *et al.*, 1979). Leung-Trujello and Lawrence (1985) suggested that low mating of *P. vannamei* might had been due to the widely fluctuating environmental conditions. The water temperature of Experiment 1 was lower than the optimal range as reported by Chwang *et al.* (1986). The water temperature of Experiment 1 fluctuated in the range of 24.3^{o}C - 29.9^{o}C.

Mating of prawns depends on overall health and sexual maturity. Chamberlain and Gervais (1984) reported that the low incidence of successful mating of *P. stylirostris* was due to a swollen vas deferens condition observed in the male prawn. Lin and Ting (1985) also reported that low frequency of mating of *P. monodon* was due to diseased males or their short supply in captivity.

Noticeable amount of melanin was observed in the thelycum and ovaries of some females in Experiment 1. This might be related to a disease problem. Melanization occured after prawns were contaminated with crude extracellular products (Lila Ruangpan and Darunee Sae-ui, 1985; Liu, 1989).

The lack of fertilization might also due to sperm quality. Leung-Trujillo and Lawrence (1987) reported that percentage of live sperm in *P. setiferus* decreased steadily during week 4 coupled with an increase of the percentage of morphologically abnormal sperm cells by week 3 of captivity. Bacterial proliferation in the sperm mass of captive male *P. setiferus* and destruction of penaeid sperm occurred in regions of the vas deferens that appeared normal when observed with the dissecting microscope (Talbot *et al.*, 1988).

The lower percentage of egg fertility in Experiment 2 compared with Experiment 1 might be due to the maturation tank size (3 m diameter). Mating was reported to be more successful in larger and deeper tanks although very deep water presented practical difficulties for daily operation. In general, tanks of no less than 4 m in diameter, and 1 m water in depth gave the best mating result with *P. monodon* (Niwes Ruangpanit *et al.*, 1981). The popularity of a 10-12 m^3 tank with 0.8-1.0 m water depth was due to the reasons of easier maintenance and daily monitoring of broodstock for ovarian development as well as satisfactory maturation performances (Primavera, 1985; Bung-orn Srimukda, 1987).

Furthermore, it was found that the remaining prawns of Experiment 2 spawned and produced good percentage of fertilized eggs in the large tanks (15 tons) after the experiment. Whereas, these prawns never produced fertilized eggs during the experimental period in small tanks (3 m diameter). This finding substantiated the effect of tank size, especially the diameter, on mating of the prawns.

The overall hatching rate of eggs obtained from Experiment 1 and Experiment 2 were rather low when compared with other reports, e.g. Simon (1982), AQUACOP (1983) and Anand Tansutapnich *et al.*, (1989). They obtained 39.3-62.0% hatching. This low hatching rate might be due to many fators such as low mating rate and factors influencing fertilization (as already discussed), poor sperms quality, water temperature variation (24.3^{o}C - 28.8 ^{o}C in Experiment 1).

The overall percent metamorphosis from eggs to the first protozoea stage in Experiment 1 and Experiment 2 during this study was lower than that reported by Simon (1982) who reported the average at 48%. This again might be due to the same factors as discussed in a previous paragraph.

Satisfactory maturation, spawning, and metamorphosis of larvae to post-larvae were obtained using eye-stalk ablation and artificial insemination techniques on 6 and 8 month-old pond-reared males and females, respectively. However, the best results were obtained with broodstock prawns that are over a year old (Chwang *et al.*, 1986).

Most hatchery operators believed that wild *P. monodon* spawners are superior for quantity and quality of eggs and larvae production (Primavera, 1985, 1988; Bung-orn Srimukda, 1987). Again, it should be noted that the wild-caught prawns used in this study were from shallow waters. Because of this, they might give unfavorable maturation results as compared to the deep waters wild prawns in other investigations.

Several researchers found that broodstock nutrition and food quality might affect not only prawn maturation and reproductive success, but also the survival of larvae during early development in hatchery. Though the results of Experiment 2 indicated the positive effects of nutritional improvement on ovarian maturity and spawns, the quality of egg produced was rather poor. This might be due to the lack of some nutritional substances in the diets.

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 C20:5W3 at the nauplius stage and C22:6W3 at the nauplius and protozoea stages, were positively correlated to hatching rate (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 enriched with W3-PUFA) resulted in 8.8 times more juveniles with good quality in terms of resistance to temperature shock and starvation than those fed with other diets (Leger, *et al.*, 1989).

Moulting.

The average elapsed time between two consecutive moultings of the small prawn groups in this study (15 days) was comparatively longer than that reported by Poernomo and Hamami (1983) where they took 7-15 days. However, it was similar to that reported by Paitoon Akkayanont *et al.* (1986) who obtained an average of 15 days.

Mortality.

The overall mortality of the prawns in Experiment 1 was relatively high as compared to a report from Poernomo and Hamami (1983) who observed 33% mortality. This might not only be due to handling stress during the monitoring of ovarian maturity but it was also dependent on the low water temperature and diseases which occured during the experiment.

Low water temperature and *Fusarium* sp. found on the prawns gills in this experiment led to poor health condition of the prawns. *Fusarium* sp. appeared to be present in virtually all shrimp culture facilities throughout the world. It had a wide host range or could exist as a free-living saprophyte (Nelson, 1983 and Lightner, 1985). Prawns infected by *Fusarium* sp. died gradually in contrast to those infected with other diseases (Lightner, 1977, 1985). Compared to other factors, *Fusarium* sp. infections might be the most important factor generating prawn mortality in Experiment 1.

The overally mortality of the prawns in Experiment 2 was comparatively lower as compared to Experiment 1 and was similar to a report of Poernomo and Hamami (1983). This might be due to better health condition of the prawns. No infectious diseases were observed in Experiment 2.

the higher of overall mortality of the pond-reared prawns compared with the wild-caught prawns in Experiment 1 might be due to a physiological factor. The pond-reared prawns required more energy to acclimatize themselves to the higher salinity water.

The high mortality of the groups of prawns fed fresh natural diets in Experiment 2 might be due to stress during spawning and handling. The prawns fed fresh natural diets produced higher frequency of spawning than that the others. This incidence was also reported by Primavera *et al.* (1979). The higher mortality of the large females in Experiment 1 might be due to the same reason.

The higher survival of males might be due to a lesser stress reason. The males were not eye-stalk ablated and were not subjected to maturity checking (less handling effect). This finding was similar to the others (Primavera *et al.*, 1979; Poernomo and Hamami, 1983; Somboon Laoprasert and Pitak Polkan, 1984; Piamsak Menasveta *et al.*, 1989).

Water Quality.

In general, most water quality parameters, except water temperature, of the Experiment 1 remained within the suitable ranges for *P. monodon* maturation and spawning as recommended by several investigators (Banchong Tiansongratsami, 1979; Primavera, 1985; Chwang *et al.*, 1986).

All water quality parameters of the Experiment 2 were also within the suitable ranges for *P. monodon* maturation and spawning.

Nutritional Values of the Diets.

The nutritional values of the maturation (artificial) diet used in this study were similar to those reported by the others. AQUACOP (1979) and Lawrence (1985) used artificial diets with excess of 50% protein in reproduction experiments of penaeids. Millamena *et al.* (1986) reported that the diets with 52.8% protein and 12.1% fat content gave the best reproductive performance of *P. monodon* in terms of total number and nature of spawnings, average number of nauplii per spawning and hatching rate.

Horse mussel and squid used as fresh natural food in Experiment 2 were rich in PUFA whereas cow liver is rich in cholesterol. Among these fresh diets, horse mussel was very popular in Thailand and was used for improving reproductive performance of *P. monodon* (Bung-orn Srimuda, 1987).

With respect to the overall results which have already been discussed, it may be concluded that large pond-reared female prawns can be used as the *P. monodon* broodstock, provided that they are reared in a suitable environment, given suitable diets and less rough handling.

Artificial diet can be used as a supplement rather than the replacement of fresh natural diets. Combination of fresh natural diets and artificial diet give a comparable with natural diets. Nevertheless, more researches are required to confirm the optimal nutritional requirements for maturation. The maturation feed with the values and balance of specific amino acids, polyunsaturated fatty acids, phospholipids, sterols, carotenoids, vitamins and minerals require more research.

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