CHAPTER IV

DISCUSSION

Basic data from pond-reared and wild-caught *P. monodon* female broodstock.

A total of 185 prawns was collected from three different stocks during July 1989 to December 1990. The average body weight was 100±30 g and average carapace lenght was 5.8 ± 0.6 cm. The variance showed that, there was no signficant differences ($P>0.05$) among stocks for all treatment such as control, sham I, P 0.1 and E 0.1. The first and second group were collected from Samut Sakorn province and Khlong Cone respectively. The average age of prawn is 6-7 months. The third was collected from nearly shore Chon Buri province. The average weight is 70-120 g. There are difficulty to find pond-rared broodstock size 70-120 g because most of the farm reared prawn to the marketing size about 20-40 g or 3-4 months. So this study must be a small sample size and collect prawn from three sources.

Effect of Progesterone on ovarian development in *Penaeus monodon*.

The ovarian development (quantitative gonad index) of *Penaeus monodon* were observed after 1, 2 and 3 weeks injected with progesterone 0.01, 0.1, 0.2 and 0.4 µg/g body weight, compared with control (no injected) and sham (solvent without hormone 0.1 µl/g body weight). The results were concluded in Table 11, showing no significant effect on ovarian development of the prawn either single injection or twice injection.

Yano (1985) was able to induce ovarian development, stage III and IV, with injection of progesterone 0.1 µg/g body weight in greasy back prawn, *Metapenaeus ensis*. In 1987, Yano studied the effect of 17 a-hydroxy-progesterone or vitellogenin synthesis in Kuruma prawn, *Penaeus japonicus*, by use dose 0.01 µg/g body weight. His results indicated that 17 α-hydroxy-progesterone stimulates vitellogenin synthesis and/or release into the hemolymph in *Penaeus japonicus* within 48 hours. After injecting Vitellogenin (Vg), a blood serum precursor of the egg yolk protein, is secreted into hemolymph or blood and accumulated in the developing oocytes. Yano and Chinzei (1987) demonstrated that the ovary is the site of vitellogenin synthesis in *Penaeus japonicus*. Vitellogenin was found to occur for the first time in the follicle cells on the oil globule stage I oocytes in the early developing ovaries. They concluded that 17 α-hydroxy-progesterone may be induce ovary development in *Penaeus japonicus*.

Table 11. Effects of progesterone and β-estradial17 on ovarian development of giant tiger prawn, *Penaeus monodon* Fabricius.

Treatments	No. of Injection	Carapace lenght (cm)	Body weight (g)	No. of shrimp	No. of females showing various stages of ovarion development at each time																				
					Day 0[a]					Day 7					Day 14					Day 21				
					0	I	II	III	IV[b]	0	I	II	III	IV	0	I	II	III	IV	0	I	II	III	IV
Control	1	5.2 - 5.9	82.7 - 111.8	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	1	-	-	-	1
Sham control I	1	5.3 - 6.2	82.0 - 162.3	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	2	-	-	-	-
Sham control II	2[c]	5.0 - 5.6	76.9 - 102.8	6	6	-	-	-	-	1	1	-	-	-	2	-	-	-	-	2	-	-	-	-
P 0.01	1	5.5 - 6.5	97.2 - 148.5	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	1	1	-	-	-
P 0.1	1	5.1 - 6.1	70.2 - 111.4	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	2	-	-	-	-
PII 0.1	2	5.2 - 6.3	78.4 - 135.8	6	6	-	-	-	-	1	-	1	-	-	2	-	-	-	-	2	-	-	-	-
P 0.2	1	5.2 - 6.6	82.5 - 144.6	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	2	-	-	-	-
P 0.4	1	4.9 - 5.5	73.0 - 98.9	6	6	-	-	-	-	2	-	-	-	-	1	-	1	-	-	2	-	-	-	-
E 0.01	1	5.1 - 6.4	80.7 - 135.9	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	2	-	-	-	-
E 0.1	1	5.4 - 6.0	86.6 - 155.7	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	2	-	-	-	-
EII 0.1	2	5.4 - 6.4	82.0 - 127.5	6	6	-	-	-	-	2	-	-	-	-	1	1	-	-	-	2	-	-	-	-
E 0.2	1	5.2 - 6.7	85.7 - 137.9	6	6	-	-	-	-	2	-	-	-	-	2	-	-	-	-	2	-	-	-	-
E 0.4	1	5.2 - 6.4	71.8 - 137.3	6	6	-	-	-	-	1	1	-	-	-	2	-	-	-	-	-	1	1	-	-

[a] Day after injection.

[b] Stages of ovarian development.

[c] Injected 2nd times 1 week after 1st injection.

Our observation, suggested that progesterone seemed to have no effect on ovarian development of *Penaeus monodon*. This may happen because the prawn used in the experiment are immatured due to average age of the prawn is less than 7 months. The minimum age at first ovarian maturation of captive broodstock is 9-18 months for *P. monodon* (AQUACOP, 1977; Santiago, 1977; Primavera *et al.*, 1978). Nevertheless, Primavera (1978) reported that five-month-old *Penaeus monodon* could mature and spawn after ablation but generated poor quantity larvae. In this study the prawns were not ablated eyestalk. This study may indicate that only progesterone injection should not induce ovarian development. The need for older females or accommulated with eyestalk ablation may be more receptive to induced maturation.

Effect of β-estradiol17 on ovarian development in *Penaeus monodon*.

Exogenous treatment with β-estradiol17 by injection of dose 0.0, 0.01, 0.1, 0.2 and 0.4 µg/g body weight were observed after injection 1, 2 and 3 weeks compared with control and found the same that result as in progeoterone and β-estradiol17 do not affect ovarian development in female *P. monodon*.

Presently, a study of effect of β-estradiol17 on marine shrimp are very rare. Caillovet (1972) tested estradiol-17β (the major follicular hormone) by added it into the diets of *P. duorarum* and found that there was no positive effect on ovarian development.

Effect of progesterone and β-estradiol17 on ovarian development of *Penaeus monodon*.

In crustaceans, the ovarys are capable of producing steroids similar to sex steroid hormones of vertebrates (Burns *et al.*, 1984a). Donahue (1940, 1948) found estrogens in the ovary of *Panulirus argus* and testosterone in eggs of *Homarus americanus*. Fairs, Quinlan and Goad (1990) detected the titers of estrone, 17β-estradiol and progesterone from ovary tissue at various stage during ovarian maturation of *Penaeus monodon* by Gas chromatography/mass spectrometry with selected ion monitoring (GC/MS(SIM)) the result is shown in Figure 23. The titers of conjugated 17β-estradiol and estrone in both unconjugated and conjugated form were found to be maximal at stages II and III of the vitellogenic cycle. Unconjugated progesterone was found in ovary at the last two stages (stages III and IV) of maturation.

Moreover, human chorionic gonadotropin stimulates oogenesis in *Cragon cragon* (Bomirski and Klek-Kawinska, 1976). Yano (1985, 1987) used progesterone and 17α-hydroxyprogesterone for inducing maturation of *Metapenaeus ensis* and *Penaeus japonicus* resulting in increased vitellogenin in the haemolymph. In this context, introducing steroid hormone to *Penaeus monodon* may also help control or manipulate the development of the ovary at the right period.

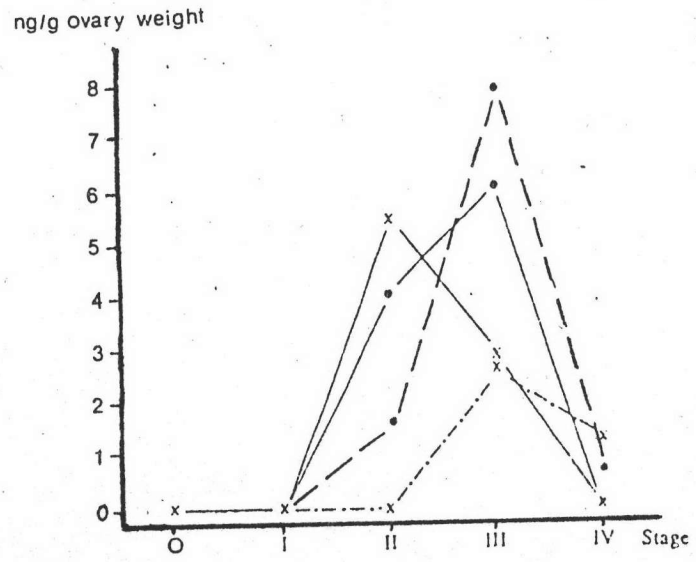

Figure 23. Concentrations of unconjugated (X) and conjugated (•), estrone (——), 17β-estradiol (— —) and progesterone (-•-) in the ovary of *P. monodon* during ovarian development (source: Fairs, Quinlan and Goad, 1990).

The present study suggestes that progesterone (4-Pregnene 3, 20 dione) or β-estradiol17 (β-D-Glucuronide) injection seems to have no effect ovarian development in *P. monodon*. Moreover, the observation of ovarian development by calculated the gonad index is not clear. So, the study of histology of ovary is necessary to understand the stage and the alteration of oocyte in control, sham and hormones treated. However, Fairs, Quinlan and Gonad (1990) detected the titers of 17β-estradiol and progesterone from ovary tissue at stage II and stage III, respectively. Therefore, the stage of ovarian development in experimental prawns were less to induced the higher stage.

Moulting cycle and effect of 2-deoxyecdysone and β-ecdysone on moulting in *Penaeus monodon*.

The uropods of *Penaeus monodon* used for moulting observation in laboratory, are very helpful for destermining the stage of moulting. In *P. monodon* the duration of stage D is approximately 12 days (66.7 % of whole moulting cycle). The duration of stage C, B and A is approximately 3 days (16.7 %), 2 days (11.1 %) and 1 day (5.5 % of whole cycle), respectively.

The moulting cycle of penaeids prawn, may vary by the effect of age, size, temperature, salinity and nutrition or level of moulting hormone (Crocos and Kerr, 1986; Okumura *et al.*, 1989; Putth and Sumeth, 1988; Smith and Dall, 1985). The duration of moulting cycle in the tiger prawn, *Penaeus esculentus*, is approximately 20

days (Smith and Dall, 1985) while the *Penaeus merguiensis* is approximately 18.6 days (Putth and Sumeth, 1988) and *P. monodon* was 18 days (present study).

The present study demonstrates that the premoult stage is the longest duration in *P. monodon*. The premoult stage is controlled by moulting hormone. Ecdysone, the steroid hormone known to control moulting cycle and metamorphosis of insects and crustaceans, is produced in the thoracic gland of many insects and from the Y-organ in crustaceans. Skinner (1985) suggested that the moult inhibiting hormone, which released by X-organ-sinus gland complex, is decrease in premoult stage and the Y-organ can produce moulting hormone into the hemolymph.

Ecdysteroids (ECD) are one of the major steroids that have been investigated in crustaceans and play an important role in controlling the moult cycle. Okumura *et al.* (1989) seperated ecdysteroids by high performance liquid chromatography (HPLC) and measured by radioimmunoassay (RIA) to trace the changes in hemolymph ecdysteroids during the moult cycle in *Penaeus japonicus*. Generaly, levels of hemolymph ecdysteroids rise during premoult stage, peak just before ecdysis, and decline to basals soon after ecdysis (Figure 24). The predominant immunoreactive ecdysteroid is 20-hydroxyecdysone in all moult stage. Small amounts of ecdysone, 2-deoxyecdysone and ponasterone A were also detected (Figure 25 and 26). The 2-deoxyecdysone was detected in postmoult stage, especially.

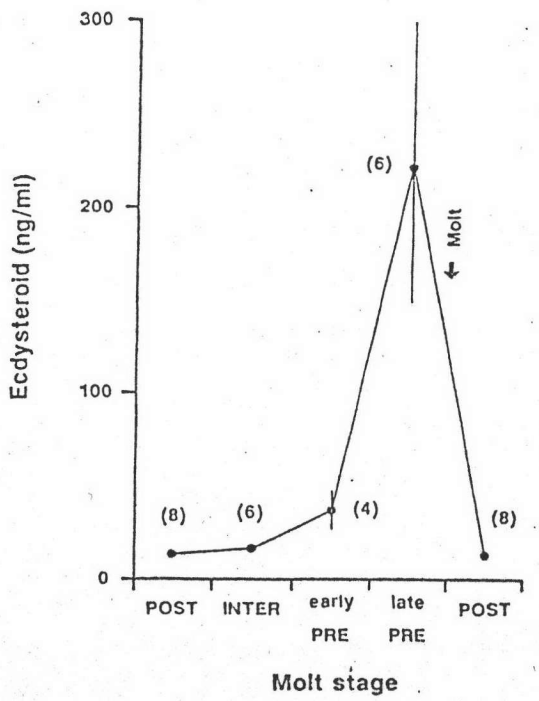

Figure 24. Whole ecdysteriod levels during the moult cycle in the Kuruma prawn. The number of animals assayed is given in parentheses. Ecdysteroid was calculated as 20-hydroxyecdysone equivalents. The data for the postmoult stage ecdysteroid levels are shown twice (Source: Okumura *et al.*, 1989).

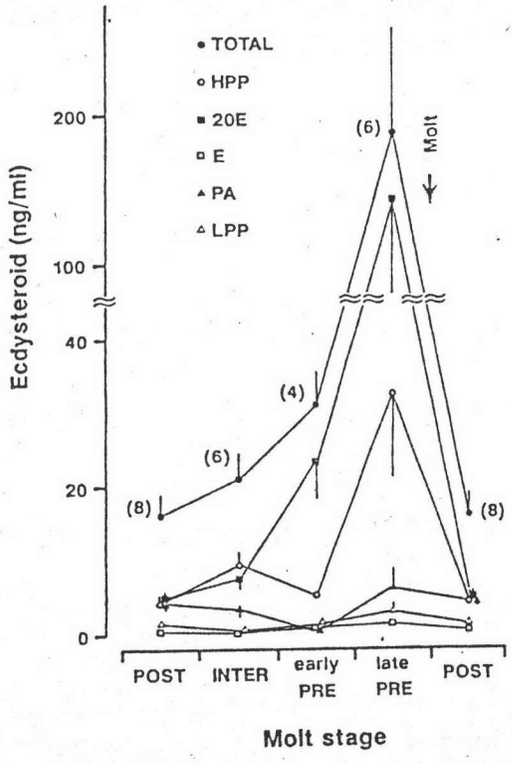

Figure 25. Changes in total ecdysteriod levels, and in the levels of five HPLC-separated ecdysteroid groups from the hemolymph of the kuruma prawn during the moult cycle. The number of animals assayed is given in parentheses. Ecdysteroid was calculated as 20-hydroxyecdysone equivalents. The data for the postmoult stage ecdysteroid levels are shown twice. 20E, 20-hydroxyecdysone; E, ecdysone; PA, ponasterone A; HPP, high polarity products; LPP, low polarity products (Source: Okumura *et al.*, 1989).

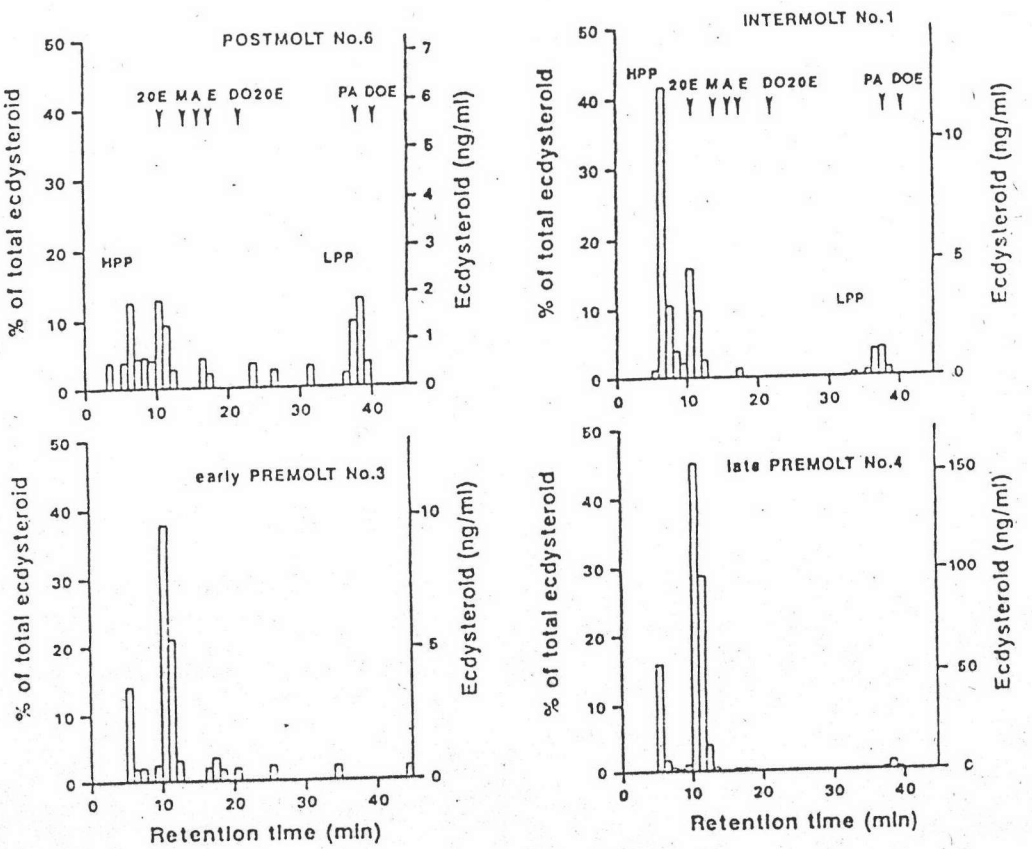

Figure 26. Immunoreactive ecdysteroids detected by RIA in fractions of kuruma prawn hemolymph separated by HPLC on methanol-water solvent system. Results are expressed as both the percent of the total ecdysteroid present in the hemolymph, and concentration (ng/ml) (Source: Okumura *et al.*, 1989).

Using doses of 2-deoxyecdysone and β-ecdysone (0.01, 0.1, 0.2, 0.4 µg/g body weight), moulting cycle of *Penaeus monodon* is reduced approximately 4.9 days (27.0 %) comparing with normal moulting.

The moulting duration of stage B, C and D_1'' to ecdysis is reduced approximately 5.5 days (from 15 days), 4.9 days (from 12 days) and 4.2 days (from 8 days), respectively (Figure 17).

The duration of moulting suggest, that 2-deoxyecdysone (which resembles the prawn steroid 2-deoxycrustecdysone) extracted from marine crayfish, *Jasus lalendei* (Highnam and Hill, 1978) and β-ecdysone (thus extracted from barks of *Vitex glabrata* R.Br.) have ability to activate ecdysis in *Penaeus monodon*.

It is clear that either 2-deoxyecdysone or β-ecdysone produce a same or equal effect on the moulting of *P. monodon*. The shorten of moulting duration is a benefit for controlling moulting of the prawns. This may make artificial in semination in *P. monodon* easily to manupulate and control, especially, in hatchery management and seed production.