CHAPTER II

MATERIALS AND METHODS

Experiment 1 : Effects of Broodstock Sources and Sizes on Ovarian Maturity and Spawning of *P. monodon*.

Broodstock Preparation.

The experiment was conducted at Sichang Marine Science Research and Training Station (SMaRT), Chulalongkorn University, Chonburi Province during September - December 1988.

Over ten-month-old pond-reared prawns were collected from semi-intensive prawn farms in Chantaburi Province, while the wild-caught prawns weighing at least 60 g (total length 19.0 cm) were collected from shallow water of the coastal area in Trat Province.

The prawns were transfered to the Marine Shrimp Maturation Laboratory at SMaRT in seawater with aeration and low temperature of about 19-22 ^{o}C. Each prawn was confined in a porous PVC tube. Transportation of prawns in the tubes could minimize injury, stress, and strain.

Upon arrival, the prawns were first gradually acclimated to laboratory condition by adjusting the water temperature up to the same level as in the laboratory. Then they were disinfected by 100 ppm formalin and 3 ppm malachite green for 10-15 minutes. Pond-reared

prawns and wild-caught prawns were acclimated for 15 - 20 days before the beginning of the experiment. The acclimation was done in two 3.1 x 6.5 m^2 concrete ponds with a daily change of seawater. The prawns were fed 3 times daily (9:00-9:30 a.m., 3:30-4:00 p.m. and 11:00-11:30 p.m.) with fresh natural diets (ridge venus clam and squid) and an artificial diet (pellets) at 5-8% and 1.2-1.5% of their body weight, respectively.

After acclimatization, weight and length of prawns were recorded. Each female was double-tagged to identify individually and to establish time of molting and ovarian maturity. The double tags consisted of a plastic strip with number, fastened around the occular peduncle of the unablated eye-stalk by means of a small rubber ring and another corresponding 1x1 cm^2 thin plastic sheet with the same number, glued on the carapace of the same prawn, using an epoxy resin adhesive. When an individual female molted, the carapace segment of the exuvia retained the carapace-tag while the eye-stalk-tag remained fastened around the occular peduncle of the prawn; so it was easy to ascertain which prawn had molted.

The experimental prawns were 60 pond-reared males, 30 small size pond-reared females, 30 large size pond-reared females, 60 wild-caught males, 28 small size wild-caught females and 31 large size wild-caught females. The lengths and weights of the experimental prawns were shown in Table 1.

Table 1. Size of giant tiger prawn broodstocks used in Experiment 1.

Group	Ave. carapace length (cm) (1)	Ave. weight (g)
Pond-reared male	5.2 ± 0.3	90.7 ± 14.4
Small pond-reared female	5.3 ± 0.2	86.6 ± 8.2
Large pond-reared female	6.2 ± 0.2	135.7 ± 15.9
Wild-caught male	5.1 ± 0.3	84.6 ± 13.0
Small wild-caught female	5.6 ± 0.2	102.9 ± 10.4
Large wild-caught female	6.3 ± 0.8	140.2 ± 19.6

(1) carapace length = distance between the post-orbital margin and the medial posterior border of the carapace (Motoh, 1981)

Data are given as mean ± standard deviation

Rearing System.

Closed recirculating seawater system was used in this study. It had a circular shape with a rearing capacity of 38 m^3. It was the same system described by Piamsak Menasveta *et al.* (in Press). Schematic drawing is shown in Figure 4.

Stocking Density.

Pond-reared prawns and wild-caught prawns were separately raised in the maturation tanks for 60 days with stocking density at

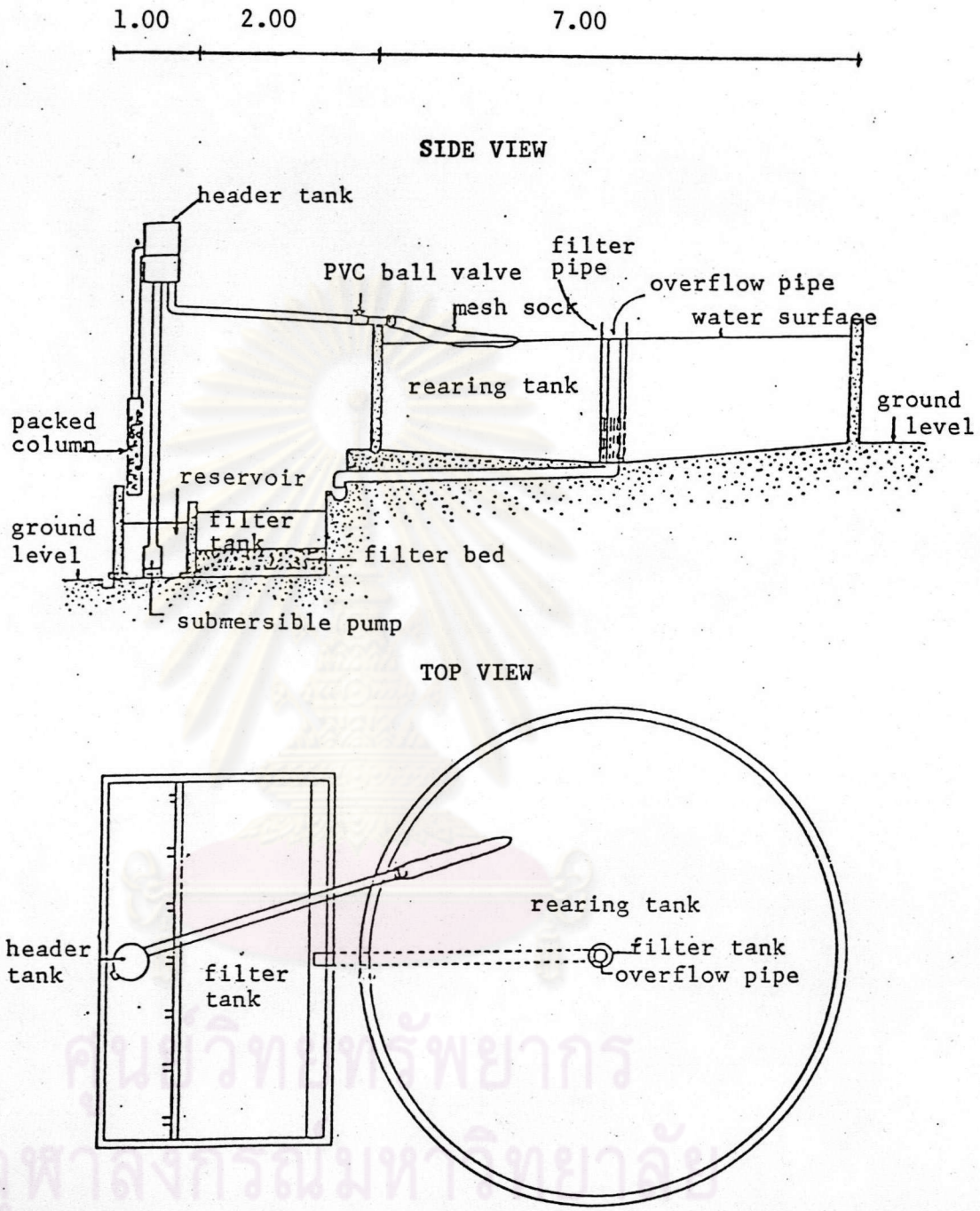


Figure 4. Schematic illustration of the maturation tank for Experiment 1. The scale is in metres. (source : Piamsak Menasveta *et al.*, in press)

3.4 individuals/m^2 and sex ratio at 1 male : 1 female. An untagged eyestalk of each female was ablated by cutting with scissor.

Feeding.

Prawns were fed 3 times daily (9:00-9:30 a.m., 3:30-4:00 p.m. and 11:00-11:30 p.m.) with artificial diet modified from Millamena *et al.* (1986) and fresh natural diets at 1.2-1.5% and 5-8% of their body weights, respectively. Composition of this artificial diet was shown in table 2. Squid meal, shrimp head meal,fish meal and soybean meal supplied protein to give a total content of approximately 50%. The major carbohydrate source was rice bran. Lipid sources were cod liver oil and lecithin. Squid, ridge venus clam, oyster, sand worm were used as fresh natural diet. Remained food and waste were taken off two times daily before the first and third meal.

Ovarian Maturity and Spawning.

Two days after being ablated, female prawns were netted out and checked for ovarian maturity by exposing the flash light beam through the abdominal part of the females. This procedure was done underwater at 6:00-7:00 pm once every three days.

Stage III and IV matured (gravid) females were recorded and transferred individually to a circular fiberglass spawning tanks with 150 l of new filtered seawater of 28-30 ppt with moderate aeration. The females with other stages of maturity were left in the maturation tanks.

Table 2. Composition of maturation diet used for giant tiger prawns in Experiment 1 and Experiment 2.

Ingredients	% Wet weight of pellets
Squid meal	30
Shrimp head meal	20
Fish meal	13
Soybean meal	13
Rice bran	5.5
Cholesterol	0.5
Cod liver oil	5.5
Lecithin	0.5
Vitamin mix(1)	3
Mineral mix(2)	6
BHT	0.03
Sodium hexametaphosphate	1
Sodium alginate	2

(1) Vitamin mix mg/kg diet) : Inositol, 2040.8; Vitamin A/D3 (500/100), 10; Vitamin E. (500), 1,000; Vitamin K3 (98%), 102; Vitamin B1 (99%), 121.2; Vitamin B2 (96%), 208.3; Calcium. D. Pantothenate (98%), 255.1; Nicotinamide (99%) 505.1; Vitamin B6 (99%), 121.2; Folic Acid (98%), 5.1; Vitamin B12 (0.1%), 300; Biotin (2%), 100; Vitamin C (96% coat), 5,208.3; Paraaminobenzoic Acid, 204.1; BHT, 10.2; Choline chloride (50%), 11,520.7; Filler (Zeolite Alumina Silicate), 8,287.8.

(2) Mineral mix (g/kg diet) : $CaCl_2.6H_2O$, 0.14, KCl, 2; $MgSO_4.7H_2O$, 6.35; $NaH_2PO_4.H_2O$, 14; $AlCl_3.6H_2O$, 0.0255; $CaCO_3$, 10.3; Dicalcium phosphate (Rock), 15; K_2HPO_4, 5, $CoSO_4$ (33.6%Co.), 0.0081; $CuSO_4$ (25%Cu), 0.022; $FeSO_4$ (30.9%Fe), 0.5; KI (23.9%K-76.1%I), 0.025; $MnSo_4$ (32.1%Mn), 0.15; ZnO (78%Zn), 0.153; Filler (Zeolite Alumina Silicate), 6.3264.

The following morning, females in spawning tanks were checked for spawning by observing the presence of floating proteinaceous scum released during spawning and the water was sampled in a glass container for egg observation.

External visual examination of the ovaries of spent females was done again to determine nature of spawning. Completely spawning females were returned to their respective tank. Partial spawning females and stage III or IV matured females that did not spawn were still kept in the spawning tanks for another 1-2 nights and they were returned to the tank after spawning or when ovaries were reabsorped.

Eggs Quantity and Quality.

Spawned eggs of individual spawner were gently siphoned from the spawning tank into an egg washer with a series of 2 net, 1. Coarse net (0.4 mm mesh): retained large dirt particles but allowed eggs to pass through. 2. Fine net (0.15 mm mesh): retained the eggs but allowed finer particles to pass through. The eggs were always kept immersed in sea water and processed gently to avoid mechanical damage.

Cleaned eggs of individual spawner were collected separately in 500-ml seawater in a beaker. Three 1-ml aliquot eggs were sampled in a counting chamber after stirring the beaker to ensure uniform distribution. The number of fertilized and unfertilized eggs were determined microscopically by the method described by Primavera and Posadas (1981). The average egg count of the three samples (=no. eggs/ml) multiplied by 500 (=no. eggs/beaker) gave the estimated total number of eggs spawned. Percent fertility was therefore computed as :

$$\% \text{ Fertility} = \frac{\text{Total fertilized eggs}}{\text{Total eggs spawned}} \times 100$$

Remaining eggs were transferred to hatch in the spawning tank with continuous aeration. Hatching rate was determined 36 hrs after spawning by counting the number of nauplii in three 50-ml aliquot samples.

$$\text{Hatching Rate} = \frac{\text{Total nauplii}}{\text{Total eggs spawned}} \times 100$$

The nauplii in the spawning tanks were incubated to develop for 48 hrs, at which time the number of protozoea metamorphosed was determined as the same manner as nauplii. Percent metamorphosis from egg to the first protozoea stage was therefore computed as followed :

$$\% \text{ Metamorphosis from egg to protozoea} = \frac{\text{Total protozoea}}{\text{Total eggs spawned}} \times 100$$

Moulting and Mortality.

Dead prawns and their splited old exoskeleton in the culture tanks were taken off every morning. Number of moulting and dead prawns were recorded. Dead prawns were determined visually and microscopically. Retagging of moulting prawn was done every 3-4 days.

Water Quality Analysis.

Water temperature, dissolved oxygen, pH and salinity in the experimental tanks were monitored daily : Water temperature and dissolved oxygen were determined using a YSI oxygen meter, pH was

determined by using pH meter and salinity was determined using in American Optical salinity refractometer. Nutrients : ammonium-N(NH_4^+), nitrite-N(NO_2^-), nitrate-N(NO_3^-) and phosphate-P(PO_4^{3-}) were monitored weekly by spectrophotometric methods (Grasshoff *et al.*, 1983).

Proximate Analysis.

Proximate analysis of the nutritional values such as moisture, protein, fat, ash and fiber of the diets were done by using AOAC methods (AOAC, 1980).

Statistical Analysis.

The statistical analysis used in the experiment was descriptive statistics, analysis of variance, analysis of covariance and regression analysis. SAS programme was used for all statistical analysis.

Experiment 2 : Effects of Diets on Ovarian Maturity and Spawning of Large Size Pond-reared *P. monodon*.

Broodstock Preparation.

The experiment was conducted at Rayong Brackishwater Fisheries Station, Rayong Province during February-June 1989.

The broodstocks of two hundred prawns were collected from semi-intensive prawn farms in Chantaburi Province. Females weighing at least 88 g(Total length 19.6 cm) and males at least 69 g(Total length 18.8 cm) were selected,transported and quarantined as described in Experiment 1. The broodstocks were reared for 5-14 days in six 4.7 m^2 circular concrete tanks with seawater at 0.8 m water level. Water in the tanks were replaced twice dialy. Prawns were fed twice a day in the morning and evening with commercial diet (pelleted) and horse mussle at 1-2% and 15-20% of their body weights, respectively.

Strong and healthy acclimatized prawns were individually weighed, lengthed and tagged with the eye-stalk-tag in the same manner as Experiment 1. The average sizes of the experimental prawns were as follows :

Sex	Ave. carapace length (cm)	Ave. weight (g)
Female	6.2 ± 0.4	136.5 ± 22.7
Male	5.1 ± 0.2	80.2 ± 8.8

Data are given as mean ± standard deviation.

Experimental Design.

Complete Randomized Design was used for this experiment which consisted of 3 treatments and 2 replications as follows :

Treatment 1 : Prawns were fed with fresh natural diets consisted of cow liver, squid and horse mussel.

Treatment 2 : Prawns were fed with combined diets of artificial diet and fresh natural diets.

Treatment 3 : Prawns were fed merely with artificial diet.

Artificial diet used in this experiment was the same as the artificial diet used in Experiment 1.

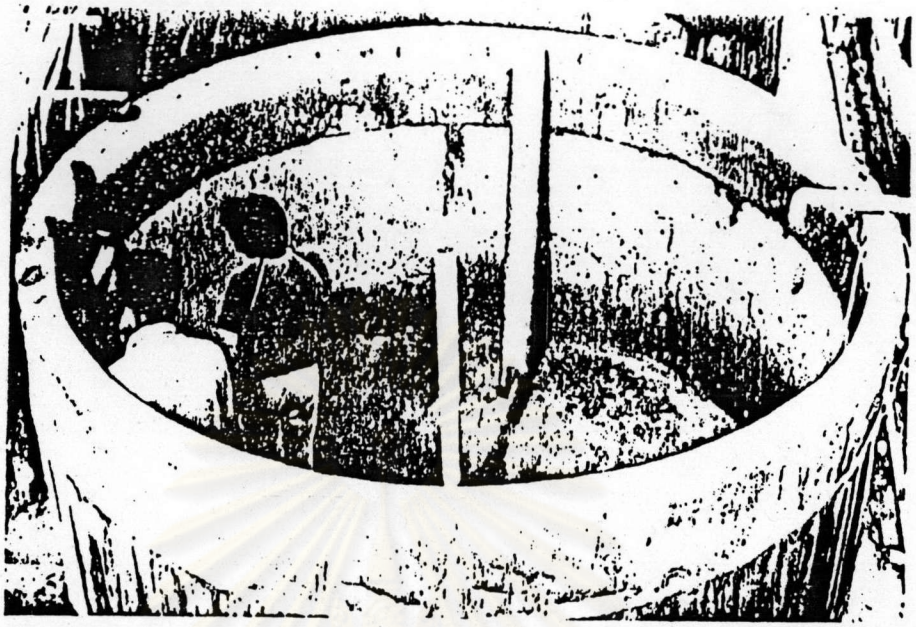
The experiment was conducted for 60 days after the prawns were completely acclimatized to the diets. Day zero started when the female prawns were unilaterally ablated.

Rearing System.

Six outdoor circular concrete tanks were used for rearing the prawns. Measurements of each tank were : diameter-3.0 m; height-1.65 m; water level-1.4 m; water capacity-9.8 m^3 (Figure 5).

The water supply system was 10-hrs flowthrough system allowing a daily exchange rate of 200% of the total water volume in each tank at flow rate 33 l/min. Four airstones was provided on the bottom of each tank. The tanks were covered with dark cloth to reduce light intensity and to minimize the disturbance of the broodstocks.

TOP VIEW



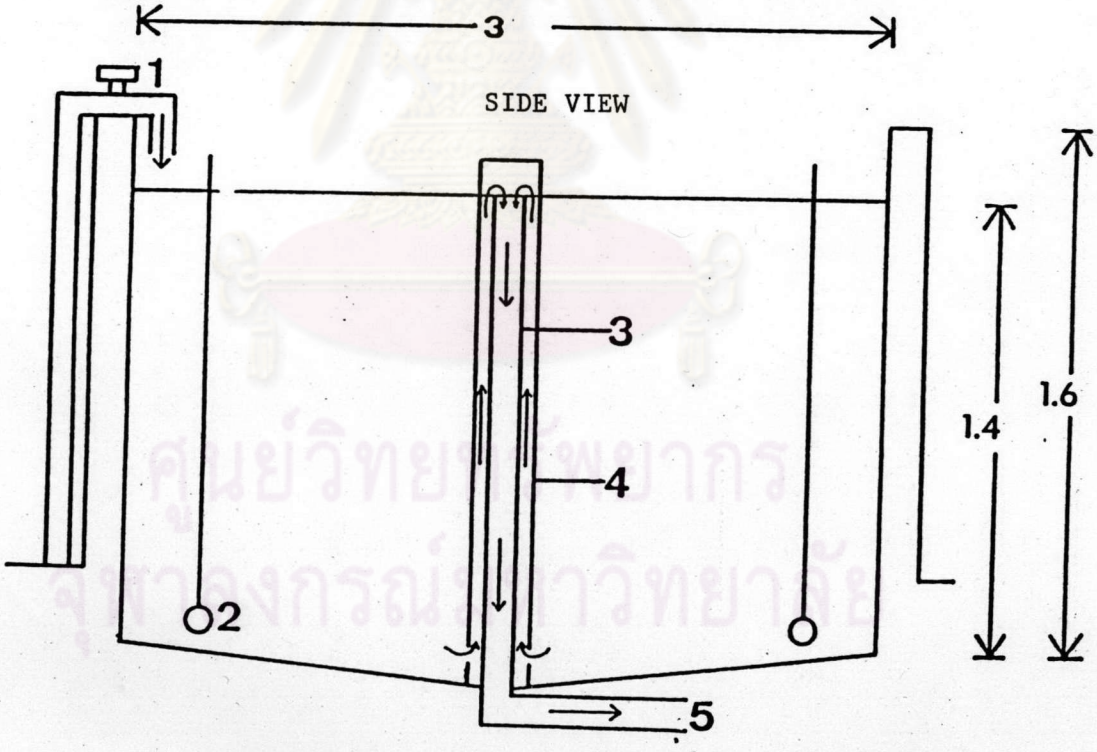


Figure 5. Schematic illustration of the maturation tank for Experiment 2. The scale is in metres (1 = inlet, 2 = air stone, 3 = overflow pipe, 4 = filter pipe, 5 = outlet).

Stocking Density.

Eighty four females and the same number of males were divided into 6 groups and each group was reared in each experimental tank. Stocking density in each tank was 4 individuals/m^2 with ratio of male to female at 1:1.

Feeding.

Prawns were fed 3 times daily with diets shown in table 3. This feeding regime was done throughout the experiment. Remained food and wastes in the experimental ponds were taken off every morning.

Table 3. Feeding regime of giant tiger prawn broodstocks in Experiment 2.

Group	Feeding Feed type & Feeding schedule		
	9:00-10:00 a.m.	3:00-4:00 p.m.	9:00-10:00 p.m.
Prawns in treatment 1 (tank no.1 & 5)	Cow liver at 7-10% BW	Squid at 10-15% BW	Horse mussel at 30-35% BW
Prawns in treatment 2 (tank no.3 & 6)	Cow liver at 3-5% BW + Prepared diet at 0.4-0.5% BW	Squid at 5-8% BW + Prepared diet at 0.4-0.5% BW	Horse mussel at 15-20% BW + Prepared diet at 0.4-0.5% BW
Prawns in treatment 3 (tank no.2 & 4)	Prepared diet (pelleted) at 0.8-1% BW/meal		

BW = body weight

Ovarian Maturity and Spawning.

Three days after being ablated, female prawns were checked for ovarian maturity and spawning, and the routine monitoring program on maturity and spawning were conducted in the same manner as Experiment 1.

Eggs Quantity and Quality.

The methods for determining egg quantity and quality of Experiment 2 was slightly different from Experiment 1. Two hundred fifty litre conical fiber glass spawning tanks, micropipette, slide and hatching cone were used in experiment 2. The procedure is as follows:

Cleaned eggs of individual spawner were collected separately in 500-ml seawater in a beaker as the same manner as Experiment 1. Taking three 100 ul aliquot eggs samples in a slide were done by using micropipette after stirring the beaker to ensure uniform distribution. The number of fertilized and unfertilized eggs were determined microscopically. The average egg amount of the three samples (no. eggs/ml) multiplied by 500 (=no. eggs/beakers) gave the estimated total number of eggs. Percent fertility was computed by the same method as described in Experiment 1.

One ml of remaining eggs in the beaker were sampled to hatch in the hatching cone with continuous aeration for determining nauplii and protozoea. Hatching rate and percent metamorphosis from egg to the first protozoea stage were computed by the method described in Experiment 1.

Mortality.

Dead prawns were determined visually and microscopically in the same manner as Experiment 1.

Water Quality Analysis.

Water quality in the experimental tanks were monitored by the same procedure as described in Experiment 1. Whereas, nutrients were monitored biweekly.

Proximate Analysis.

Proximate analysis of nutritional values of the diets were analysed by the same procedure as described in Experiment 1.

Statistical Analysis.

The statistical analysis procedure for this experiment was similar to Experiment 1.