CHAPTER III

MATERIALS AND METHODS

1. Experimental design

Completely Randomized Design was obtained in this experiment. The experiment is divided into 4 treatments; each treatment composes of 5 replications. Four levels of *Butea superba* were used in the experiment, namely 0, 0.05%, 0.5% and 5% *Butea superba* in feed. The density of rearing unit was 100 prawns per replication. The culture period of the experiment was carried out 20 weeks (20 September 2003 – 7 February 2004).

2. Proximate analysis of the experimental diets

The proximate analysis of the major dietary ingredients was analyzed before the particulate diet obtains to evaluate the fitness of the major nutrient requirement. The instant particulate feed of all treatments were analyzed the major contents of protein, fat, fiber, ash and moisture with the method as shown in Appendix A.

3. Particulate diet preparation

The feed in 4 treatments were prepared more than 40% crude protein. One treatment feed served as control (0% *B. superba* powder in feed), and the three others were formulated to contain 0.05%, 0.5%, 5% *B. superba* in particulate feed as shown in **Table 3-1.** There similarly was 95% basal diet in each experimental treatment. The

remaining 5% ingredients were whole wheat and/or *B. superba* in each formulation. The powder of *B. superba* was kindly provided by Associate Professor Dr. Wichai Cherdshewasart and the formulation of the ingredients in pelleted diet was illustrated in **Table 3-2**.

The ingredients' mesh was mixed with the premix (vitamin mixture, mineral mixture, shrimp head meal, lecithin, cholesterol, wheat bran and/or *B. superba*) in the feed mixer for 20 minutes. The homogenized mixer was extruded though the pelleting machine (CPM, California Pelleting Machine, California, USA) into the size of 2 mm in diameter and 5 mm in length. The pelleted diet was streamed for 5 minutes and dry in hot air oven at 60 °C for 2 hours. The pelleted diets were aliquot and frozen at -20 °C.

Table 3-1. Four experimental groups with different levels of *B. superba* treatment.

Experiment groups	Levels of *B. superba*
A (control)	0 % in diet weight
B	0.05 % in diet weight
C	0.5 % in diet weight
D	5 % in diet weight

Table 3-2. The ingredients of the giant freshwater prawn diet formulation.

Ingredients	Dry weight (g 100 g-1 of diet)			
	0% BS	0.05% BS	0.5% BS	5% BS
Fish meal	30	30	30	30
Soybean	40	40	40	40
Wheat flour	11	11	11	11
Rice oil	2	2	2	2
Palm oil	1	1	1	1
Vitamin mixture[a]	1	1	1	1
Mineral mixture[b]	1	1	1	1
Wheat gluten	6	6	6	6
Shrimp head meal	2	2	2	2
Lecithin[c]	0.5	0.5	0.5	0.5
Cholesterol[d]	0.5	0.5	0.5	0.5
Whple wheat	5	4.95	4.5	0
Butea superba (BS)	0	0.05	0.5	5
Total	100	100	100	100

[a]Vitamin mixture 1,000 g contains: vitamin A 10,000,000 IU, vitamin D_3 1,000,000 IU, vitamin E 10,00 mg, vitamin K_3 1,000 mg, vitamin B_1 500 mg, vitamin B_2 5,000 mg, vitamin B_6 1,500 mg, vitamin C 10,000 mg, folate 1,000 mg and D – L - methionine 16,038 mg

[b]Mineral mixture 1,000 g contains: calcium 147 g, phosphorus 147 g, iron 2,010 mg, copper 3,621 mg, zinc 6,424 mg, manganese 10,062 mg, cobolt 105 mg, iodine 1,000 mg, selenium 60 mg and binder 682,718 mg

[c]Soy lecithin, feed grade

[d]Ninety five percent cholesterol, laboratory grade, Sigma

4. Experimental rearing unit

The rearing system in this experiment was situated in the farm, Nongsur, Pathum Thani. Twenty rectangular net cages were placed in the same pond. The blue nylon net

cages are obtained in the grow-out system. Twenty cages with 1.8 x 2 x 1.5 m^3 of rearing area were established in the rearing unit.

5. Experimental animals

The broodstock of postlarvae was obtained form the nursery of the progeny of thirty fertilized female prawns in Marine Biotechnology Research Unit, Chulalongkorn University. All prawns were derived from a single hatching cycle, and were then nursed in a intensive primary nursery from late of July to the mid of September. The postlarvae from the same hatching batch were transported to the demonstration farm of Marine Biotechnology Research Unit (MBRU), Nongsur, Pathum Thani. The amounts of 2,000 postlarvae were randomly selected into the cultured experiment. One hundred postlarvae (average weight 0.01 g) are randomly stocked per cage. The detail of the density of prawns per rearing unit has been described by Menasveta (1999).

6. Feeding strategy

The experimental feeding regime of the prawns in each treatment, pelleted diet was fed 3 times a day at 6.00, 12.00 and 18.00 entirely culture period. The prawns were fed by percentage of body weight based on a feeding schedule modified from Menasveta (1999). They were fed at an amount of 10% and 7% body weight a day in the first month and second month, and decreased the amount of feed to be 5% body weight a day in the third month, and 4% and 3%body weight a day in the fourth month and the fifth month. Feeding rates were adjusted weekly based on weight and assumed survival as described

by Menasveta (1999). The prawns were fed diet containing different concentrations of *B. superba* following the same feed regime.

7. Water quality analysis

The water was analyzed every 2 weeks during the experimental period. The temperature and pH were measured by the temperature and pH meter. The ammonia, nitrate, nitrite and alkalinity were determined by test kits (Prima Tech Co., Ltd).

8. Data collection

8.1 Weight and length measurement

Every four weeks, twenty prawns of each cage were randomized sampling for determining the weight and length for recording the growth rate. The prawns were absorbed the body surface water by the hand towel prior to individually weigh. The body lengths of each prawn were measured with a ruler. The body length was defined as the distance from the posterior margin of the orbit to the posterior margin of the telson. After individual measurement of weight and length of these prawns, they were returned to their cages immediately. At the end of the experiment, every prawn was harvested; they were measured weight, body length, claw length and the extension of the first segment of the abdomen. The measurement of the claw length was categorized into the length of propodus, carpus and the fusion of merus and ichium, as shown in **Figure 3-1**. The extension of the first abdominal segment of individuals was measured by a caliper.

The growth rate of the experimental prawns were determine according to weight basis and length basis, calculated by

$$\text{Growth rate base on weight (g day}^{-1}) = \frac{W_2 - W_1}{t_2 - t_1}$$

Where

W_1 = Weight (g) at time, t_1

W_2 = Weight (g) at time, t_2

$t_2 - t_1$ = the duration of the growth period (days)

$$\text{Growth rate base on length (mm day}^{-1}) = \frac{L_2 - L_1}{t_2 - t_1}$$

Where

L_1 = Length (mm) at time, t_1

L_2 = Length (mm) at time, t_2

$t_2 - t_1$ = the duration of the growth period (days)

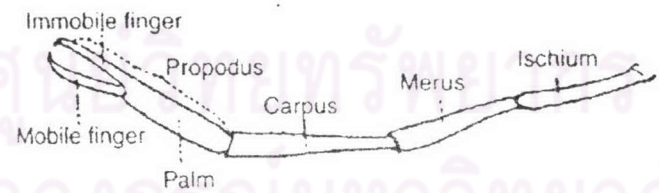
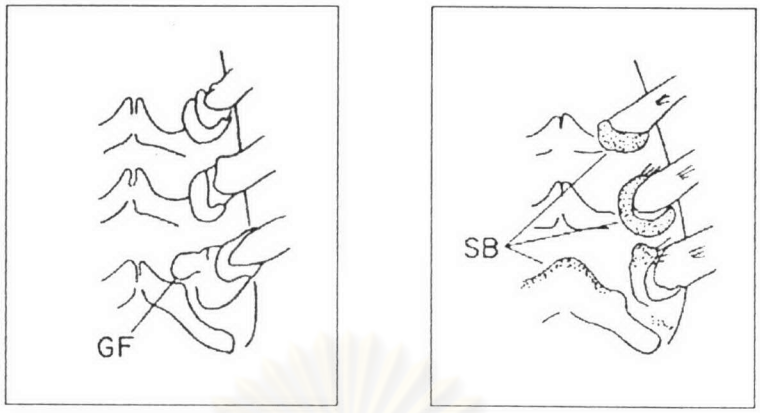


Figure 3-1. The structure of mature cheliped of *Macrobrachium rosenbergii*.



(a) (b)

Figure 3-2. Sexual dimorphism of the ventral surface of sexually mature male and female *Macrobrachium rosenbergii* (a) The sexually mature male gonopores. (b) The sexually mature female ventral surface. GF is gonopore and SB is setal buds.

8.2 Sex classification and survival rate

At the the carry out period, the prawns in each cage were classified into male and female. The prawns were classified according to the presence of male gonopores. They are situated at the base of the coxae of the fifth pereiopods while they are absence in female, as shown in **Figure 3-2**.

The amounts of the prawns in each cage at the end of the experiment were hand-counted and recorded for the survival rate.

8.3 Ovigerous female observation

At the end of the experiment, the female prawns in each cage were observed the egg in the abdominal segments. The amounts of ovigerous female in each cage were recorded individually.

8.4. Histological studies

At the end of the culture experiment, one male and one female prawn in each cage were sacrificed to examine the reproductive system. The total part of the cephathorax of prawns was removed to fix in Davidson's fixative (see appendix B) in an adequate supply a minimum of approximately 10X their volume of fixative. Inject fixative (0.1 to 10 ml depending on size of prawn), via needle and syringe (needle gauge dependant upon prawn size). The site of injection was laterally in the hepatopancreas proper, in the region anterior to the hepatopancreas. The prawns allowed remaining in the fixative at room temperature for 24 to 72 hr. Following proper fixation, the prawns were transferred to 50% ethyl alcohol to store for an indefinite period. The tissues were dehydrated in a series of alcohol and embedded in paraffin (mp 56-58 °C). The 7μm sections were prepared, strain with Harris's haematoxylin and counterstained in eosin (Bell and Lightner, 1988). The sections were viewed under light microscope.

9. Statistical analysis

The data of weight, body length and claw length were analyzed by one way ANOVA. Compare the different of each treatment by Duncan multiple's range test. Chi-square analysis was used to test the homogeneity of sex ratio for determining the distribution of male and female in the samples.