CHAPTER 2

MATERIALS AND METHODS

Experiment 1: Embryonic Development, Larval Development and Early Growth of Hatchery-Produced Abalone Seed, *Haliotis ovina* Gmelin, 1791.

The experiment was conducted at Angsila Marine Biological Research Station (AMBRS), Department of Marine Science, Chulalongkorn University, Chon Buri Province during March to June 1992.

1. Broodstock collection

Broodstocks of *H. ovina* were obtained from Khang Kao Island Chon Buri Province by SCUBA diving. These animals were brushed to get rid of the fouling organisms and maintained in 1x1.2x0.8 m^3 broodstock conditioning tanks for 5 days before the induction of spawning. These animals were fed *ad libitum* with fresh green algae, *Enteromorpha intestinalis*. The tanks were cleaned everyday and refilled with sea water (5 micron.) at ambient temperature and salinity.

2. Benthic diatom preparation

Acrylic plastic plate bundles (Figure 4.) were put into tank filled with 25 microns filtered sea water supplied with strong aeration. After 3-4 days benthic

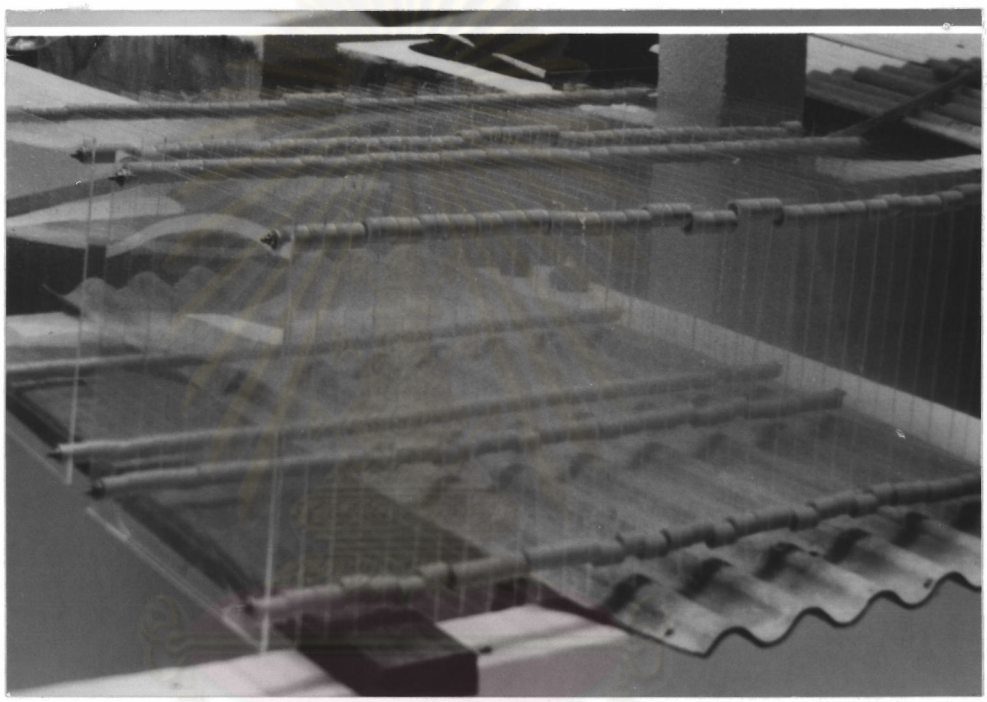

Figure 4. Acrylic plastic plates bundles used for diatom preparation

diatom film was appeared on acrylic plastic plate when they were ready for juvenile settlement.

3. Spawning induction

On the sixth day after conditioning, males and females abalones with high gonad index (stage 3 or more described by Jarayabhand *et al.*, 1991) were selected for induction of spawning. UV irradiated sea water techniques was chosen for this experiment. Males and females animals were put into two separated 20 l spawning aquariums continuously supplied with 1 μm filtered and UV irradiated sea water (Figure 5). An energy for spawning induction of 800 millwatt-liter/hour was used for both males and females. As soon as spawning begin, males and females were transferred into the separated 20 l aquariums for egg and sperm collection.

4. Fertilization

Insemination was initiated by adding an appropriated quantity of sperm into each egg tank. There was no attempt to check on sperm density during this experiment About 30 minutes after fertilization, this tank were sunk in 1 x3x0.8 m^3 half tank filled with 1 micron filtered sea water. The tank was covered with black plastic net to decrease light intensity and gently aeration was applied.

5. Larval rearing

When larvae were ready to settled, pre-prepared acrylic plastic plate bundles covered with thin films of mixed species of benthic diatoms were put into

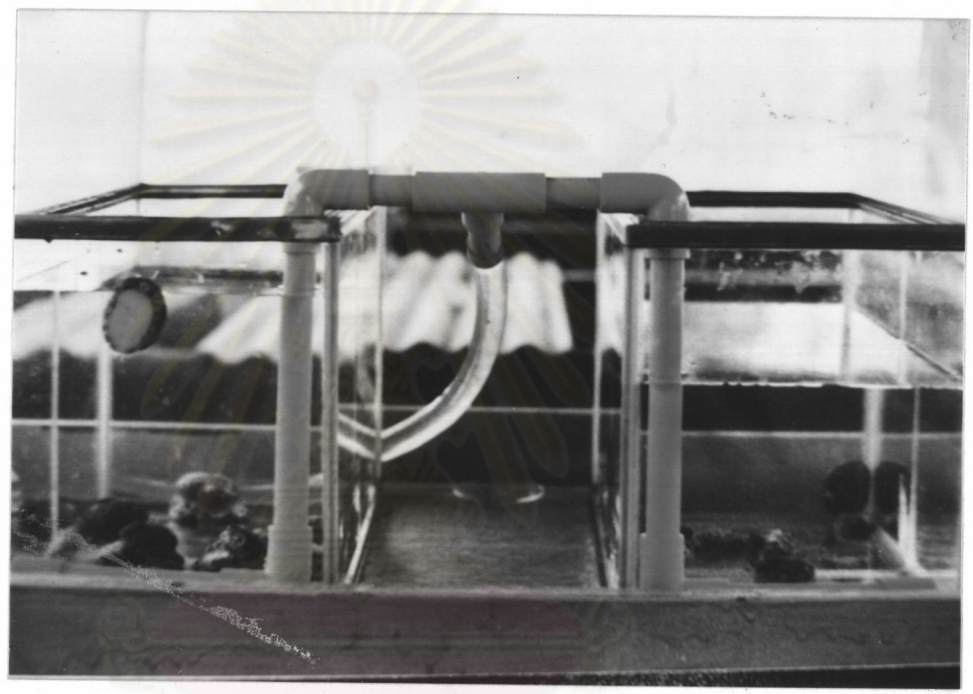

Figure 5. Spawning induction with UV irradiated seawater

larvae rearing tank. Aeration's was stopped for 2-3 hours, to allow creeping larvae to settle on those plates. To keep the tank clean, fresh 1 micron filtered UV treated sea water was applied continuously. Dead larvae mixed with debris were siphoned out from tank bottom during three days after fertilization. Six days after fertilization , the plate bundles were carefully hung in the same tank. The tank was cleaned everyday with continuous water supply. To maintained growth of benthic diatoms on the plates, the light intensity was controlled by covering the tank with black plastic net during the daytime as well as a supply of nutrient (urea or (CO $(NH_2)_2$) dropped into this tank which the optimum concentration of nitrogen about 4-8 ppm.

6. Weaning

Three months after fertilization, juveniles abalone were switched its diet from diatoms to macroalgae. It was necessary to prepared mixed macroalgae on the bottom of the tank as its diet. In this experiment juvenile abalones were fed with *Enteromorpha intestinalis* and *Gracilaria changii*.

7. Data collection

Embryonic development, larval development, newly settled abalone and three months old abalone were monitored. Photographs were taken by microscope equipped camera. Growth in shell length was measured at prominent stages by an ocularmeter , a vernier caliper. Whole body weight was measured to the nearest 0.01 g with an electronic balance.

Experiment 2: Effect of Different Macroalgal Diets on Growth of Juveniles Abalone, *Haliotis ovina*.

The experiment was conducted at Angsila Marine Biological Research Station, Department of Marine Science, Chulalongkorn University, Chon Buri Province during May to June 1992 and April to June 1995.

1. Juvenile preparation and collection

The pre-liminary experiment (experiment I during May-June 1992) was conducted by using 98 three months-old hatchery-produced juvenile abalones from experiment 1 and experiment II (during April to June 1995). Ninety juvenile abalones were obtained by SCUBA diving from Krok Island, Chon Buri Province were used. These animals were brushed to get rid of fouling organisms and transferred into the closed system 1x1.2x0.8 m^3 acclimation tank with aeration. Semi-circle 4 inch PVC pipe for shelter of juvenile and fed *ad libitum* with fresh algae, *Enteromorpha intestinalis, Euchema* sp. and *Gracilaria changii* (Figure 6 to Figure 8) for 15 days prior to the experiment. Water salinity was maintained at 32 ppt., temperature at 29-32 ^{o}C. The condition similar to natural condition of juveniles were collected

After preparation and acclimatization, juvenile abalones were tagged with cement glue. Shell length, shell width and weight were recorded, their average size were shown in Table 4 and Table 5

Figure 6. *Gracilaria changii* (GC)

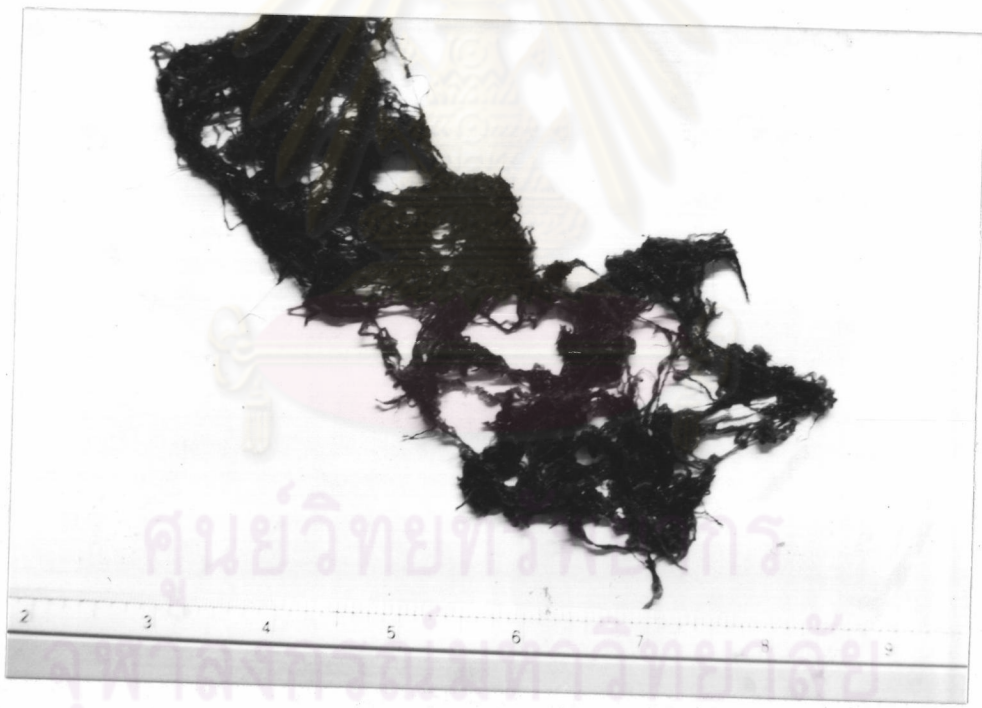

Figure 7. *Enteromorpha intestinalis*(EI)

Figure 6. *Euchema* sp (EU)

Table 4. Average sizes of juvenile abalone *H. ovina* (Gmelin, 1791) in experiments I.

Treatment	No.	Shell length (mm.)	Shell width (mm.)	Body weight (g.)
Gracilaria changii	32	17.03±2.451[a]	13.16±0.989[a]	0.55 ±0.246[a]
Enteromorpha intestinalis	33	16.30± 2.640[a]	12.76±1.160[a]	0.57 ±0.247[a]
Mixed	33	16.24± 2.500[a]	12.74±1.832[a]	0.51± 0.240[a]

Table 5. Average sizes of juvenile abalone *H. ovina* (Gmelin, 1791) in experiment II.

Treatment	No.	Shell length (mm.)	Shell width (mm.)	Body weight (g.)
Enteromorpha intestinalis	30	29.17±3.270[a]	23.06±2.215[a]	4.63±1.276[a]
Euchema sp.	30	29.51±3.946[a]	23.15±2.878[a]	4.70±1.564[a]
Gracilaria changii	30	30.14±3.701[a]	23.53 ±2491[a]	5.22±1.659[a]

a, b and c denoted significant different in average size ($p<0.05$)

2. Experimental design

A complete randomized design was used in this experiment which consisted of 3 treatments and 2 replications as follows :

Experiment I

Treatment 1 : Juveniles were fed with *Gracilaria changii* (GC)

Treatment 2 : Juveniles were fed with *Enteromorpha intestinalis* (EI)

Treatment 3 : Juveniles were fed combined with *Gracilaria changii* and *Enteromorpha intestinalis* (MI)

Experiment II

Treatment 1 : Juveniles were fed with *Enteromorpha intestinalis* (EI)

Treatment 2 : Juveniles were fed with *Euchema* sp. (EU)

Treatment 3 : Juveniles were fed with *Gracilaria changii* (GC)

Both experiments were conducted for 90 days after preparation and feeding acclimation for 30 days.

3. Culture system

Six units of circular resin tanks diameter 50 centimeters, 30 centimeters in height equipped with 4 overflow pores on top-side were used. Two semi circular 4 inch PVC pipe on bottom of tanks were used as shelters for juveniles. Closed water system were poerated by an underwater pump, sand filter tank, pressure controlling tanks, 0.5 inch PVC pipeline and valves. Culture system in this experiment is shown in Figure 9

4. Feeding

Juveniles in each treatment were fed *ad libitum* with fresh macroalgaes. Every three days the reminding macroalgaes were removed. Feces were siphoned everyday. In experiment I, the density of juveniles was 10-11 in each experimental unit and experiment II, the density of juveniles was 15 in each experimental unit.

5. Data collection

Shell length, shell width and weight were measured every 30 days. Specific growth rate and growth rate were obtained from the daily rate of shell increase specific growth rate (Viana *et. al*, 1995), equation is shown as follows

Specific growth rate (body weight) (%/day) = $100 \times \{\ln \text{final weight} - \ln \text{initial weight}\} * n^{-1}$

Daily rate of shell increase (μm/day) = $1000\ G_L * n^{-1}$

Figure 9. Culture system in this experiment

Where G_L = increase in shell length or shell width(mm.)

n = days

6. Proximate analysis

Proximate analysis of nutritional values in three species of macroalgaes in this experiment were done by using AOAC methods (AOAC,1980)

7. Statistical analysis

The statistical analysis used in this experiment were descriptive statistics, analysis of covariance and linear regression. SYSTAT, Sigma Stat and Sigma Plot programmed were used for all statistical analysis.