CHAPTER 4

DISCUSSIONS

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

1. Induction of spawning

There are several methods of induced spawning i.e. natural spawning, dessication, thermal shock and UV irradiated sea water. Each method has its advantages and disadvantages which should be taken into account. Hahn (1989) gave a very good review on the methods for induced spawning in abalone. He concluded that dessication and thermal shock have limited success in induced spawning and should not be used alone due to their unreliability. He further suggested that UV irradiated sea water and hydrogen peroxide are very reliable and induced spawning in almost 100% of ripe animals. Ebert and Houk (1984) also summarized that UV irradiated sea water is one of the most significant contributions to abalone cultivation but some of Haliotid species respond both UV irradiated sea water and hydrogen peroxide so they prefer UV irradiated sea water techniques because it requires no chemical addition. Therefore, the UV irradiated sea water techniques was chosen in this experiment. This technique allows a better control of fertilization and also avoids problems originated from sperm competition as well as abnormality of larvae due to spawning of immature.

During acclimation, photoperiod and temperature control were not used as important factors for broodstock acclimation because this experiment was done in summer season in Thailand, therefore, daytime and night (6.00 a.m. to 6.00 p.m.) was separated clearly and temperature between daytime and night was not difference.

Tropical abalones may require time for release gametes more than abalones in temperate zone and subtropical zone. In this experiment, time that broodstock required to released gametes was 3 hours 20 minutes after initiated induced spawning at amount of UV irradiation 800 mWh/l. Uki and Kikuchi (1984) reported that temperate abalones, *H. discus hanai* which collected from the wild, the average time to spawning was 1 hours 27 minutes in male and 1 hours 36 minutes in female. When compared with *H. diversicolor supertexta*, a subtropical abalone. They also requires a little longer times than *H. ovina* (within 4 to 6 hours to release the gametes) (Chen, 1989).

It should be noted that this technique can not be used year-round because fully mature broodstock (stage 3,4) from the wild is not available throughout the year. Broodstock which obtained from the wild are fully mature in 2 peaks in a year from June to July and from November to January (Jarayabhand, 1995),thus, broodstock conditioning should be the further studied in order to solve this problem.

2. Embryonic development and larval development

Compared with temperate Haliotids, egg diameter of *H. ovina* is clearly smaller such as *H. discus* (230 microns), *H. sieboldii* (280 microns) and *H. gigantea*

(270 microns) etc. (Ino, 1952 cited from Hahn, 1989), however, the mean egg diameter obtained in this study is slightly larger than the egg diameter of the same species reported by Naidenko (1992). Time required for embryonic development and larval development in *H. ovina* is shorter than temperate abalones such as *H. discus hannai* which spend 99 hours from fertilization to completion of larval development (Seki and Kan-no, 1977). However, it is in the same range as in subtropical species like *H. diversicolor supertexta* (Chen, 1989) and other tropical species like *H. asinina* (Singhagraiwan and Sasaki, 1991 a ; Singhagraiwan and Doi, 1993). In addition, both embryonic and larval developmental rates obtained in this study were clearly faster than that reported by Naidenko (1992) in the same species. This could be explained by a lower incubation temperature (23-25°C) used in the observation by Naidenko compared to one in this experiment. Temperature will increase the metabolism in the eggs, therefore, in higher temperature developmental rate is faster, Viana (1995). First respiratory pores of *H. ovina* occur in 20 to 24 days after fertilization which is faster than 28 days reported in other local species , *H. asinina* (Singhagraiwan, 1991). Both embryonic and larval developmental stages showed no major difference from those reported in other species either temperate or tropical abalone (Hahn, 1989 ; Singhagriwan and Doi, 1993).

The critical procedures in cultivating abalone are egg washing and decanting by nitex screen. This procedure must be avoided due to a very fragile egg membrane of *H. ovina*. Likewise, the bottom part of larval hatching and rearing tank (in this experiment used the same tank) containing shed egg membranes and undeveloped eggs which may serve as contaminant causing high larval motality rate. So a better larval rearing apparatus should be design for hatchery-produced

abalones. Study on the optimal sperm density and temperature for larval rearing in *H. ovina* are also necessary to avoid the problems of polyspermy and high mortality caused from temperature. Furthermore, a method to induce larvae to metamorphosis and settle should be studied in order to reduced time for development.

3. Early juvenile growth rate.

Early growth of three months-old juvenile, *H. ovina* is very close to growth rates of the other local abalone species in Thailand, *H. asinina* (Singhagraiwan and Sasaki, 1991 b ; Singhagraiwan and Doi, 1993), but generally grow faster than sub-tropical species, *H. diversicolor supertexta* reported by Chen (1989). Average size in shell length of *H. diversicolor supertexta* were 3 to 5 mm. while average size in shell length of *H. ovina* (three months-old) in this study were 11.0 to 22.90 mm. When compared with temperate abalone species (*H. discus hannai*), growth rate during 30 days after fertilization of *H. ovina* was lower than but during 90 days after fertilization growth rate of *H. ovina* were higher (Uki *et. al*, 1981). However, the study on the early growth rate of these juvenile to market size should be carried out.

The larval mortality rate in this experiment is very high. Amount of larvae which complete larval development and ready to settle were about 100,000 individuals but within 3 months after fertilization amount of early juvenile drastically reduced to 99. The major problem may arisen from uncleaned bottom of larval rearing and juvenile tank. Certain phytoplankton and zooplankton species can also pass through 5 microns filter. Thus, bacteria, copepod, nematode and ciliated protozoa can contaminated on benthic diatom plates. Another problem has arisen if

settlement occured was from variable abalone grazing rates according to size, grazing intensity relative to abalone density, succession of diatom population and the change in food preference from diatom to algae. In this study, mixed species of diatom was used. From these reason there may caused infection and competition condition may occurred in the culture system. Application of antibiotics, diatom maintenance on diatom plates and water quality improvement in the culture system should be studied in the future.

Weaning stage of juveniles, *H. ovina* started at 3 months after fertilization. They migrated from diatom plates to bottom of the tank. It was necessary to provided macroalgae for them. In this study *Enteromorpha intestinalis* and *Gracilaria changii* were used. The juveniles accepted these macroalgal diets to a certain extent. This finding is similar to other subtropical abalone species, *H. diversicolor supertexta* as by Chen (1989). However the tropical abalone, *H. asinina* accepted only *G. salicornia* (Singhagraiwan, 1991). Further investigation are required on this area.

Experiment 2. Effect of Different Macroalgal Diets on Growth of Juveniles Abalone, *Haliotis ovina* (Gmelin, 1791)

The result in experiment I indicated that the rate of increase in the growth rate in shell length of juveniles when fed with *Gracilaria changii* (GC) was higher than those fed with *Enteromorpha intestinalis* (EI) and those with mixed macroalgae. The trend from this experiment was not conclusive because of some toxic substance has leached into the tanks. Thus, care must be taken during the run of the experimental unit to prevent this problem and can be detrimental to the experimental results. However, there is a trend that juveniles of 4 to 5 months olds fed with *G. changii* give a higher growth rate than those fed with *E. intestimalis*.

The objective of the experiment II was to confirmed the former result in experiment I. For over 2 years, seed production were tried several times without any sucess. Many problems have caused poor production of juveniles. Broodstock which collected from the wild did not reach maturity all year-round. Jarayabhand, 1995 reported that abalones, *H. ovina*, have 2 peaks of maturity in a year from June to July and from November to January. Outside these periods, broodstock did not spawn when UV irradiated sea water technique was used. Moreover, the broodstock which collected from the wild during the peaks of maturity were not fully mature. Sometimes the broodstock did not release gametes synchronizly. Broodstock conditioning has been tried but fail due to the unadequate supply of macroalgae all year round for the broodstock. Therefore, wild juveniles were chosen in this experiment. In this case, if they have enough time to acclimated in the hatchery, the result from this study can be used to demonstrated the growth rate and food preference when fed with different macroalgal diets of hatchery-produced juveniles.

In this experiment *G. changii* and *E. intestinalis* were chosen to feed juveniles as they have been used in the experiment carried out in other tropical abalone, *H. asinina* (Singhagraiwan, 1991) and subtropical abalone (Chen, 1989). The imported macroalgae from Japan, *Euchema* sp., is another potential source if the seaweed culture can be maintained all year round as in case of the Prachubkirikan Fisheries Station. This species showed an acceptable growth rate throughout the year (Nakranard, personal communication).

Prior to the measurement of shell length, shell width and body weight, juveniles shell should be brushed because of fouling organisms might interfered in the measurement. The result from experiment II indicated that *G. changii* gave the better growth than *E intestinalis* and *Euchema* sp. which clearly expressed in the specific growth rate. In treatment 2 when fed with *Euchema* sp., high mortality rate occured. The remaining juveniles in this treatment had the poor growth rate. Therefore, it can be concluded that *Euchema* sp. is not suitable as a food for juvenile abalone of this species.

At the beginning of this experiment, average of initial size of juveniles fed with *G. changii* seem to be higher than others but there were no significant different in sizes. In this experiment, inital shell length and initial weight have had effect on growth of juveniles. Correlation between these parameter showed that small size juveniles have the higher rate of increase in shell length and specific growth rate than those large size juveniles. But in this experiment, daily increase in shell length and specific growth rate of juveniles fed with *G. changii* were higher than juveniles fed with *E. intestinalis*. Thus, juveniles when fed with *G. changii* resulted in the better growth rate. During 60 to 90 days specific growth rate of juvenile were declined in treatment fed with *E. intestinalis* and *G. changii* or

slightly increased in treatment fed with *Euchema* sp. These may resulted from one or the combination of these following reasons : it was the natural growth pattern i.e. compensatory growth and the effects of environmental factors on culture system. It has been reported that abalone are easily stress by handling and light (Hahn, 1989), suggesting stress was the cause of different growth performances over time (Viana *et al.*, 1995).

However, the results obtained from this experiment are very close to study on subtropical abalone, *H. diversicolor supertexta* reported by Chen (1989) that juveniles fed with *Gracilaria.* sp. produced the best growth followed by those fed with *Ulva* sp. and *Enteromorpha* sp. respectively. *H. iris* prefers *Gracilaria* sp. and the growth produced by *Gracilaia* sp. was twice as high that produced by *Macrocrysis pyrifera.* (Tong, 1983). Singhagraiwan (1991) found that juveniles of tropical abalone, *H. asinina* prefers *G. salicornia* and does not accepted *E. intestinalis.* Food preferences of *H. ovina* are different from abalone in temperate zone as summarized by Hahn (1989). Temperate zone abalones usually prefer brown algae especially those in order Laminariales.

Considering the nutritional value of macroalgaes used in this study and body composition of juveniles, it may conclude that macroalgae which has the high protein content also produce the high protein content in body composition in juveniles. Food conversion efficientcy (FCE) and feeding rate are necessary to study for supported this results. It should be carried out in the future.

Nutritional value of macroalgae in the same species may differ depending on the sources of macroalgae and season they are collected (Viana, personal

communication) but nutritional value obtained in this study excepted *Euchema* sp. is similar to reported by Singhagraiwan (1991)

As regards to the texture of the macroalgae, this might be one of the causes for the differences in the growth rate between three kinds of macroalgae in this experiment, *Euchema* sp. is hard in texture and this can reduced the feeding efficiency of juveniles. While the texture of *G. changii* and. *E. intestinalis* are rather soft. In general, when fed *E. intestinalis* to experimental unit, the algae always dispersed in the tanks. So shelter must be put on this macroalgae to prevent it from flaoting on the bottom of the tank which would reduced the feeding efficiency of juveniles. *E. interternalis* has the strong smell it may serve as the good attractant to juveniles to grazed upon. *G. changii*, can also dispersed on the bottom of the experimental unit so juveniles could easily grazed upon it. From this reason juveniles which fed with *G. changii* may have the better growth rate than others.

With respect to the overall results which have already been disscussed, it may conclude that *G. changii* in this experiment shown better growth while *E. intestinalis* can used as food for juveniles but with lower growth rate. *Euchema* sp. is not suitable for used as food for juveniles. However, in hatchery condition, mass abalone cultivation need large supply of fresh macroalgae. But *G. changii* resource in Thailand is limited. The growing and harvesting period is also limited from February to May. Furthermore, *G. changii* is economically important, particularly for agar which is used as food. Previous reports have shown that single species macroalgae offered in the laboratory do not to appear to provide a nutritionally balanced diet (Day and Fleming, 1992; Viana *et* al.,1993a). In nature,

abalones feed on various speciess of macro- and microalgae which together presumably supply balanced diets (Viana, 1995). Thus, development of appropriated artificial diets for abalones should be the next step in oder to solve the problems of mass abalone cultivation in hatchery.