

## CHAPTER 1

### INTRODUCTION



Abalone is one of the economically marine gastropods. Its good flavor and taste make abalones in high demand in the world market. Abalones, in nature, are found attached to the rocks and boulders in shallow coastal areas and are easily available for divers who collect on them from the rocks. They have slow growth rate and with such efficient methods to capture can lead overfishing. Due to depletion of its natural stock from overfishing and long production time to attain large size, several studies on abalones cultivation have been conducted since late 1800's in Japan and early 1900's in United States (Mottet, 1978). These studies were aimed to ensure an adequate supply of abalones production for the world consumption.

In 1993, FAO Fisheries Statistics estimated that the world production of abalone was approximately 9,272 metric tons. However, this number was expected to be far under estimated as there was production from unreported farms from many countries. Abalones are mainly produce in Japan , Australia , New Zealand , Mexico, United States, Korea and South Africa. Abalones are very popular among consumers in many countries especially in Asia such as Japan, mainland China, Hong Kong and Singapore. The market price is quite high. The annual production of abalones from various countries are shown in Table 1. Abalone products can be sold as live abalones, frozen whole abalone meat , canned abalones and dried abalones ( market size over 70 mm.) and cocktail abalones ( market size 40-70 mm.) (Fallu, 1991).

Table 1. Annual production (metric ton) of abalones from various countries  
(FAO, 1995)

| Countries    | Species              | 1990  | 1991  | 1992  | 1993  |
|--------------|----------------------|-------|-------|-------|-------|
| Japan        | <i>H. gigantea</i>   | 3,353 | 3,066 | 2,496 | 2,353 |
| South Africa | <i>H. midae</i>      | 624   | 573   | 738   | 561   |
| Australia    | <i>H. rubra</i>      | 347   | 326   | 285   | 327   |
| Mexico       | <i>Haliotis</i> spp. | 3,655 | 2,849 | 3,132 | 2,180 |
| N. Z.*       | <i>Haliotis</i> spp. | 1,228 | 1,294 | 1,481 | 1,099 |
| U.S.A.       | <i>Haliotis</i> spp  | 265   | 117   | 252   | 209   |
| Korea Rep.   | <i>Haliotis</i> spp  | 344   | 376   | 320   | 361   |
| Oman         | <i>Haliotis</i> spp  | 116   | 49    | 42    | 34    |
| Phillippines | <i>Haliotis</i> spp  | 61    | 63    | 73    | 122   |
| Solomon Is.  | <i>Haliotis</i> spp  | 28    | 25    | 29    | 30    |

\* = New Zealand

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Abalones are found along the coast of most temperate and tropical regions except for South America and the eastern part of North America. They can be found along the west coast of North America (Baja California to Alaska) ; along the eastern and western of Asia (Korea , Japan , China, Taiwan, Indonesia, Malaysia, Thailand , Cambodia , India and Sri Lanka); The Pacific Ocean islands as far as Tumulus , Australia , New Zealand ; Africa (Egypt , Mozambique , Madacasca , Cape of Good Hope , Gold Coast ) and Europe (France , Spain , Italy, Yugoslavia and Greece) (Hahn, 1989). Table 2. shows a list of some commercially important abalone species.

Thailand has at least 3 species of tropical abalones. They are *Haliotis asinina*, *H. platana*, *H. varia* and *H. ovina* as reported by Tantanasiriwong (1978) Later, it was found that they may be only 3 species instead. This probably may be due to mixing up *H. platana* with *H. varia* , Nateewattana and Hylleberg (1986) and Nateewattana and Busarawit (1988). Apart from *H. asinina*, *H. ovina* is one of those species that has potential for commercial cultivation (Nateewattana and Busarawit, 1988). Because of its meat texture, morphology and taste that are very close to *H. diversicolor supertexta* (Jarayabhand, 1995), cultivation of this species may lead to the possibility of cocktail size abalones production in Thailand. However, aquaculturists lack the knowledge on basic biology, ecology, nutrition, genetics and cultivation techniques of *H. ovina*. Basic biological studies of this species and appropriated techniques are essential for commercial abalone productions in Thailand.

Table 2 List of some commercially important abalone species. (summarized from Hahn, 1989)

| scientific name                   | average shell length (mm.) | common name                        |
|-----------------------------------|----------------------------|------------------------------------|
| <b>North America</b>              |                            |                                    |
| <i>Haliotis rufescens</i>         | > 275                      | red abalone                        |
| <i>H. fulgens</i>                 | 125-200                    | green, southern green or blue      |
| <i>H. corrugata</i>               | 150-175                    | pink or corrugated abalone         |
| <i>H. sorenseni</i>               | 125-200                    | white or sorensen abalone          |
| <i>H. asimilis</i>                | < 100                      | threaded abalone                   |
| <i>H. cracherodii</i>             | 75-125                     | black abalone                      |
| <i>H. kamtschatkana</i>           | 100                        | pinto abalone                      |
| <b>Japan and Korea</b>            |                            |                                    |
| <i>H. discus hannai</i>           | 180-200                    | ezo awabi                          |
| <i>H. discus</i>                  | 200                        | kuro awabi, oni or onigai          |
| <i>H. diversicolor supertexta</i> | 50                         | tokubushi                          |
| <i>H. gigantea</i>                | 250                        | makada                             |
| <i>H. siebodii</i>                | 170                        | megai                              |
| <i>H. asinina</i>                 | 70-100                     | mimigai                            |
| <b>Australia</b>                  |                            |                                    |
| <i>H. rubra</i>                   | 120-140                    | black-lip or red-ear shell abalone |
| <i>H. laevigata</i>               | 130-140                    | green-lip abalone                  |
| <i>H. roei</i>                    | 70-80                      | roe's abalone                      |
| <b>New Zealand</b>                |                            |                                    |
| <i>H. iris</i>                    | 170                        | paua or black abalone              |



## Objective

The main objectives of this study are as follow :

1. To study embryonic development , larval development and early growth of hatchery-produced abalone, *Haliotis ovina*.
2. To study the effects of different macroalgal diets on abalone growth under hatchery operation.
3. To study and develop some techniques on abalone cultivation in Thailand.

## Expected results

An outcome of this study will provide some basic biological information on abalone, *H. ovina*. It is also expected that, this knowledge can be used in developing techniques for commercial abalone production in Thailand.

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## LITERATURE REVIEW

### Taxonomy

The taxonomy of abalone, *Haliotis ovina*, is as follows : (modified from Nateewattana and Hylleberg, 1986 and Hahn, 1989)

Kingdom : Animalia

Phylum : Mollusca

Class : Gastropoda

Subclass : Prosobranchia

Order : Archeogastropoda

Suborder : Zygobranchia

Superfamily : Pleurotomariacea

Family : Haliotidae

Genus : *Haliotis*

Scientific name : *Haliotis ovina* Gmelin, 1791

Common name : Sheep Ear Shell

### General morphology

Shell ovate and solid, with radiating ribs which runcate in a concentric row of nodes around the center apex, perforation tubular and elevated. Between 3 - 5 open respiratory pores. Generally olive-green in color, ornament with white or creamy-yellow radiating streaks (Nateewattana and Hylleberg, 1986).

## Distribution

Members of Haliotidae are globally distributed in modern ocean, especially are found in the tropical western Pacific, Australia, Japan, South Africa and along the coast of northeastern Pacific margin. They occur from the intertidal to approximately the depth of 400 m.

The genus *Haliotis* has been divided into 15 subgenera. The characters on which these taxa based on are the ratio of shell to body size, the shell sculpture, epipodial structures and the biology of tremata. (Linberg, 1992).

In Thailand, Nateewattana and Hylleberg (1986) and Nateewatana and Busarawit (1988) reported that Thailand has at least 3 species of abalone which are classified as tropical species (Figure 1). They are

1. *Haliotis asinina* Linnacus, 1758
2. *H. ovina* Gmelin, 1791
3. *H. varia* Linnacus, 1758

These species can be found both in the Gulf of Thailand and Andaman Sea coastlines (Fuze, 1981, Robert *et al.*, 1982 and Purchon and Purchon, 1981). The first survey on the abundance and distribution of *Haliotis* spp. along the Andaman Sea coast of Thailand was carried out in 1985-1986 (Nateewattana and Busarawit, 1988) A survey on Thai abalone around Phuket Island and feasibility study of abalone culture in Thailand was reported by Nateewatana and Hylleberg



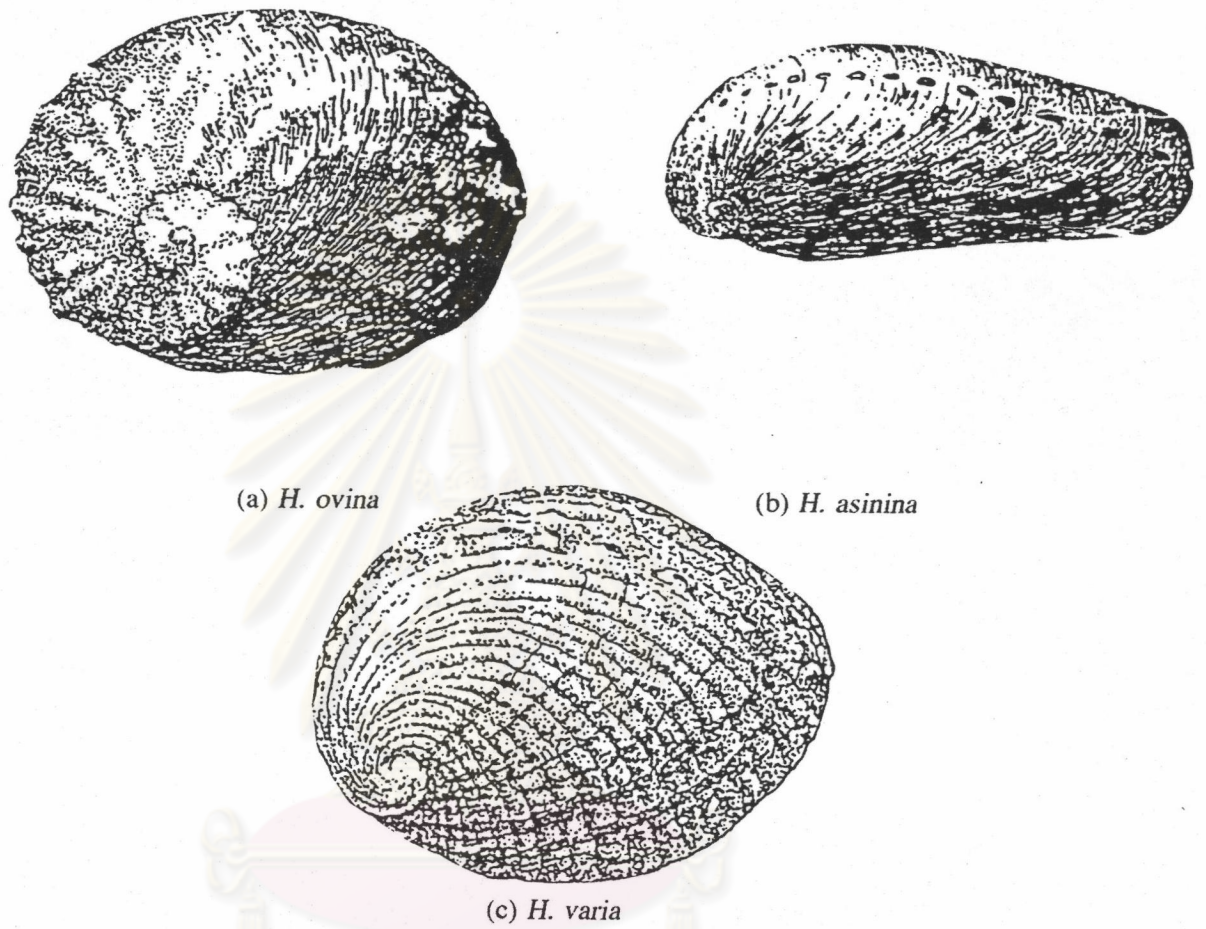


Figure 1. General morphology of abalone in Thailand

(a) *Haliotis ovina* (b) *H. asinina* (c) *H. varia*

(from Jarayabhand et. al, 1992)

(1986). The survey on species and distribution of *Haliotis* spp. in Surat Thani, Nakorn Sri Thammarat, and Songkla in the Gulf of Thailand are reported in the same year (Tookvinas *et al.*., 1986)

Kakhai and Pechpradub (1992) studied on the broodstock and collection of abalone in coral reef and rocky shore areas in Chonburi province. They found two species of abalones collected at the depth of 2.0-8.0 meters, *H. asinina* of shell length from 37.77 - 83.08 mm and *H. ovina* of shell length from 35.29 - 64.19 mm.

Jarayabhand *et al.*, (1992) studied the distribution of abalone around Khang Kao Island and revealed that *H. ovina* was the dominant abalone species. It can be found in a reasonable number in the rocky shore around Khang Kao Island at the depth of 3-5 meters. These animals are found attached underneath the rocks and some coral head (*Porites* sp.), in the same area where sea urchin (*Diadema serosum*) and some gastropods such as top shell (*Trochus* sp.) were found. They are also attached firmly on rocks of exposed side of the island where strong water current created well oxygenated sea water. Shell length of specimens collected from study sites were in the range of 20-74 mm. Size distribution and sex ratio of abalone showed no significant difference among stations.

### **Reproductive biology**

Jarayabhand *et. al* (1992) studied the reproductive biology of *H. ovina*. They reported that *H. ovina* are dioecious (sexes are separated) with external fertilization as found in other species. So far, there is no evidence of hermaphrodite



in this species. During spawning season, males can easily be distinguished from female by simple external observation on the color of reproductive tissues lined over the surface of conical appendage (Figure 2).

The conical appendage can be seen by folding back the mantle edge at the rear part of a live specimen. The colors of testes are ranged from creamy white to orange where the colors of ovary are ranged from dark green to black. There are two peaks of spawning seasons, June and from November until January. The criteria for assigning the gonad developmental stage are shown in Table 3.

### **Abalone culture**

The major problem in abalone culture is to produced abalone seed production. There is the need for determining the specific conditions for spawning. There were also some difficulties in devising the equipment for rearing juvenile abalone (Seki ,1980). The system of abalone seed production are shown in Figure 3.

#### **A. Broodstock Conditioning**

The term "broodstock conditioning" means the induction of gonad maturation and spawning ability in adult abalones which are not necessary in the spawning season. Conditioning is an extensive term which include several biological processes, i.e. gametogenesis , synthesis of hormones controlling gametogenesis and spawning (male and female) vitellogenesis and synthesis of hormones controlling vitellogenesis. In addition to the gonad maturation and spawning ability, organisms must be in proper physiological state (good nutrition,



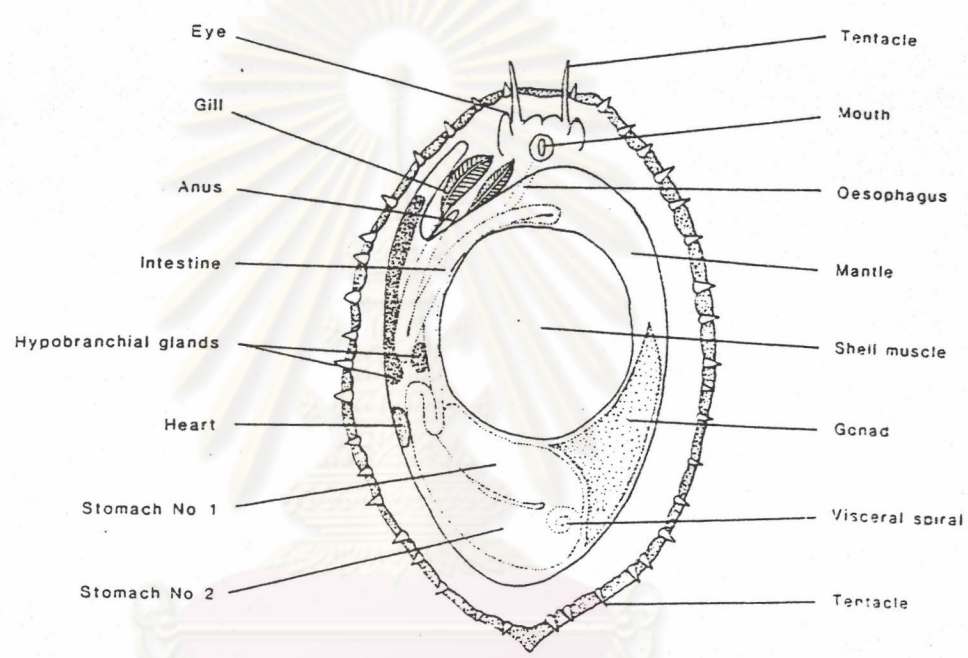


Figure 2. Internal organ of abalone (From Fallu,1992)

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Table 3. Gonad developmental stage for *Haliotis ovina* (Gmelin, 1791).  
(summarized from Jarayabhand *et. al*, 1991)

| Stage number | Description   |
|--------------|---|
| 0            | Sex can not be determine  |
| 1            | Sex can be determine and reproductive tissue cover about 10-25% of conical appendage      |
| 2            | Sex can be determine and reproductive tissue cover about 20-50% of the conical appendage  |
| 3            | Sex can be determine and reproductive tissue cover about 50-75% of the conical appendage  |
| 4            | Sex can be determine and reproductive tissue cover more than 75% of the conical appendage |

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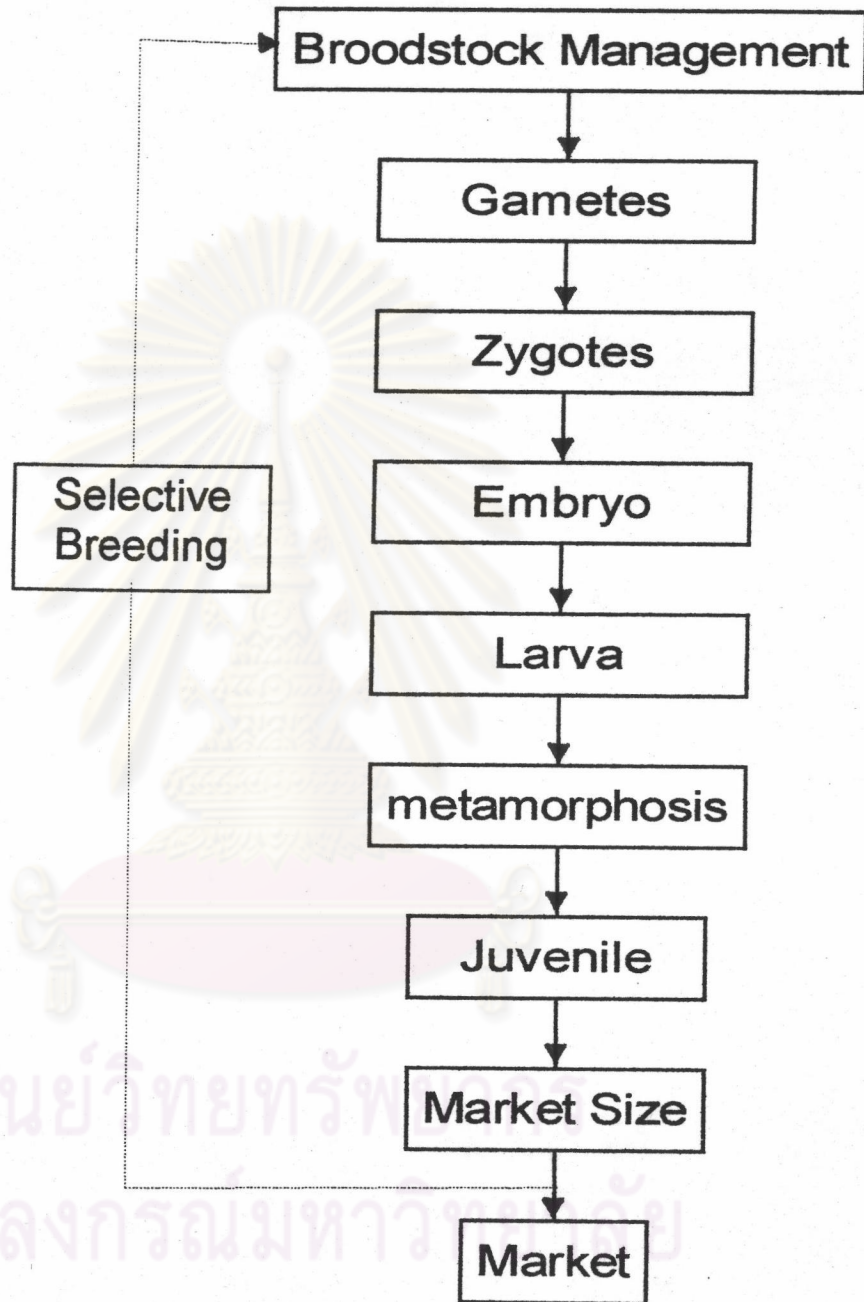


Figure 3. A general diagram of complete life cycle aquaculture which can also be applied to abalone culture in Thailand. (From Jarayabhand *et al*, 1991)



water quality, photoperiod and water temperature). The main reasons why broodstock conditioning should be promoted because firstly, broodstock conditioning can provide year round supply of fully mature males and females abalone for efficient culture of juvenile abalones. Secondly, it provides opportunity for researchers to investigate the reproductive biology of abalone (Jarayabhand *et al.*, 1992)

Broodstock conditioning in some commercial abalone species :

a. *H. discus hannai*

Uki and Kikuchi (1984) reported that gonad maturation of *H. discus hannai* was proportional to the effective accumulative temperature (EAT) which can be calculated from the following formula :

$$Y_n = \sum_{i=1}^n (t_i - \theta)$$

Where  $Y = \text{EAT}$  in degree-days ,  $t_i =$  temperature to which are expose daily ( $^{\circ}\text{C}$ ) and  $\theta =$  the biological zero for gonadal growth. They concluded that mature adult abalone could be obtained throughout the year by the temperature regulation based on EAT. The exogenous factor controlling gonad maturation is known for this species and gonad maturation can be quantified. Hahn (1989) also mentioned that once abalone reached fully mature stages , even slight fluctuation in water temperature in the conditioning tanks could induce spawning.

b. *H. discus*

This species also show a relationship between EAT and gonad maturation. But it has less response to artificial conditioning than *H. discus hannai* and requires 2-3 times the EAT to attain the level of gonad maturation. In addition within the same species, population which inhabit warm water tends to require a higher EAT than population inhabit cold water.

c. *H. gigantea*

There is no relationship between gonad maturation and water temperature in this species. Degree of gonad maturation depended on the length of cultivation rather than the water temperature at which the animal were raised (Hahn, 1989).

d. *H. rufescens*

The gonad maturation of this species was controlled by the food level. There was no direct correlation between gonad condition and exogenous physical factors such as water temperature and photoperiod (Hahn, 1989).

e. *H. fulgens*, *H. corrugata* and *H. cracherodii*

*H. fulgens* could be conditioning during winter, when the natural population were spent, by raising them in sea water between 20 to 24 °C. Accelerated gametogenesis , gonad maturation and spawning of California species (*H. fulgens* , *H.corrugata* and *H. cracherodii*) require *ad libitum* feeding at elevated



temperatures (1 to 2 months at 18 to 28 °C depending upon species, water quality and water exchange rate) (Hooker and Morse,1985 ; Morse,1984 cited by Hahn,1989)

In Thailand Jarayabhand *et al.*(1992) concluded that the degree of gonad maturation of *H. ovina* is the function of temperature (EAT). It will be possible to obtain fully mature of *H.ovina* if appropriate broodstock conditioning are applied.

#### B. Artificial spawning

Artificial spawning is usually an initial step to attempting abalone culture. The control of spawning which resulted in control over production of larvae, is an essential step for the success of the aquaculture operation. Induction of artificial spawning, allows the production of abalone larvae over a longer period than the natural spontaneous condition. However, induction of spontaneous spawning is not yet completely understood. Several endogenous and exogenous factors have been proposed as controlling factors (Jarayabhand *et al.*, 1992). Hahn (1989) reviewed that spawning might be induced by a sudden change in water temperature either increase or decrease in the temperature, air exposure during low tide, photoperiod , lunar cycle, release of gametes from other individuals in the population or some combinations of these factors.

Several methods of spawning induction in abalone have been tried over the year, some are successful and other are of limited use. These techniques are reviewed as follows :



#### a. Gamete Stripping

Kishinoue (1893) was the first person who attempt to take viable gametes from abalone broodstock. After fertilization, some of zygotes are developed to the 2 - 4 cell stage before dying. Hahn (1989) summarized that this method was usually not successful. Even if the eggs are mature, they are unfertilized when taken directly from the gonad before undergoing the final maturation step that normally occur immediately before spawning.

#### b. Desiccation

This technique is simply conducted by taking abalone out from the water about 1 hour. Spawning usually begins as soon as they are returned into the water at the former temperature level. Hahn (1989) suggested that this method was unreliable and has no biological significance because these animals are subtidal and would never experience this stimulus in nature. The desiccation method is also inefficient because it caused the release a large quantities of immature gametes.

#### c. Thermal shock

The first person who reported the use of thermal shock in order to induce spawning in abalone was Ino (1952), (cited by Hahn ,1989). The method consisted of placing ripe males and females together in the same tank and raising the 3-6 °C above ambient . Care must be taken not to raise water close to water temperature or above the lethal level. Moreover, it was important to avoid too much difference in water temperature between ambient and spawning water especially

during cold months. The other technique was to place animals directly into warm sea water and then gradually decrease water temperature to ambient. This method also causes releasing of immature gametes as in the former method. In addition, it is also difficult to assure the simultaneous release of eggs and sperm.

#### d. Ultraviolet Irradiated Sea water

Kikuchi and Uki (1974a) found by accident that sea water when irradiated with ultraviolet light effectively induced the spawning of *H. discus hannai*. Subsequent detailed experiments Kikuchi and Uki (1974b) were conducted on the relationship between amount of ultraviolet irradiation and time between induction and spawning. Irradiated sea water with ultraviolet light of 2537  $\text{\AA}$  wavelength at an intensity of 800 milliwatt hours per liters induced spawning in about three hours at the rate of 90% or better. No harmful effects were determined on the spawning adults or the obtained larvae and juveniles. The technique has been used successfully with other Haliotids as well as scallops (Uki and Kikuchi, 1974). Landau (1992) explained the mechanism of how ultraviolet irradiated sea water induces spawning in abalone. This was because of UV energy caused the conversion of some water molecules to the hydroperoxy free radical ( $\text{HO}_2$ ) and the peroxy diradical ( $\text{O}_2$ ), which were also the product of  $\text{H}_2\text{O}_2$  in water. These radical oxidizing agents are thought to stimulate enzymes (by acting as donors of charged oxygens) responsible for the synthesis of specific prostagaldins that control spawning. This method was used to induce most of the commercial abalones i.e. *H. discus hannai*, *H. fulgens*, *H. cracherodii*, *H. gigantea* and *H. diversicolor supertexta* (summarized from Hahn, 1989).



Uki (1989) studied on the time required for abalones to release gametes when applying this techniques. He reported that abalone required at least one hour in prior to release gametes. The time require for gamete release can be determined by the following formula :

$$Y = [c/(x+a)]^{-1} + b$$

Where Y = time require for gamete release (h)

x = amount of UV irradiation (mWh/l)

a,b,c = constant; for male = 231.3, 1.064 and 1422.4 respectively

female = 278.5, 1.350 and 1978.7 respectively

constant b for male and female are one hour four minutes and one hour twenty-one minutes respectively.

#### e. Hydrogen Peroxide

The hydrogen peroxide method causes spawning induction in abalone through the same mechanism as using of the UV irradiated sea water. Morse *et al.* (1977) reported that abalone are induced to spawn by sea water contains  $H_2O_2$  or prostaglandin (PG). The effect of  $H_2O_2$ -added sea water on spawning inducement are completely lost by adding aspirin, known as PG biosynthesis inhibitor, and thus PG plays an important role in the abalone spawning mechanism.

Landau (1992) described the steps of this method by placing broodstocks in a container of sea water adjusted pH at 9.1. Concentration of hydrogen peroxide varied depending on abalone species. After about 2.5 hours, they were transferred back into clean sea water where gametes were released within an



hour. Pena (1986) studied the induction of artificial spawning in *H. coccinea canariensis*. The best spawning rate were obtained when concentration of hydrogen peroxide were at 2,4 and 8 millimole for male and 4 millimole for female. Hahn (1989) reported that artificial spawning of *H. iris* in New Zealand was performed by using hydrogen peroxide (by adding 40 milliliter of 6% hydrogen peroxide solution (or approximately 7 millimole).

Hahn(1989) explored the different artificial spawning induction methods in abalone. He concluded that desiccation and thermal shock have limited success. These methods should not be use alone due to their unreliability. The other methods (UV irradiated sea water and hydrogen peroxide) are very reliable and result in almost 100% of ripe individuals to spawn.

### C. Fertilization

Fertilization of the gametes is another critical step in the hatchery seed production. The techniques used should guarantee rapid fertilization of short duration , high fertilization rate (approximately 100 %) with excellent survival, and low abnormality or polyspremy (Hahn, 1989)

Kikuchi and Uki (1974b) examined the fertilization rate of *H. discus hannai*'s gametes by preserving them under different temperatures. They found that the fertilization rates of the gametes kept at 14 °C and 17 °C remained above 90% for three hours after spawning. Erbert and Hamilton (1983) examined the fertilization rate of *H. refescens*. They reported that the fertilization rate of the ova fertilized with the sperm collected immediately after ejaculation was higher than

that of the ova fertilized with the sperm sample at the same time of the ova, and thus assumed that the fertility of sperm declines faster than that of ova.

Kikuchi and Uki (1974c) proposed the relationship between the sperm density and fertilization rate of *H. discus hannai* at the water temperature of 17 °C by the following equation:

$$f = 1 - (1 - 0.0005)^d$$

Where f = fertilization rate

d = sperm density (spermatozoa/ml)

#### D. Embryonic Development, Larval Development and Settlement

##### a. Embryonic Development and Larval Development

Embryonic development of Haliotid was described as early as 1899 when Boutan reported on the European species, *H. tuberculata*. This work was brief and offered little information on the late larval stage. Stephenson (1924) obtained trochophore of *H. tuberculata* but was unable to develop the larvae for more than 62 hours. Maruyama (1935) succeeded in obtaining fertilized eggs of the Japanese species *H. gigantea* (cited by Grant, 1981). Ino (1952) studied on detail the development of *H. discus hannai*. Shibui (1972) described in detail the normal development of the eggs and larval of *H. discus hannai* maintained under artificial condition. Seki and Kan-no (1977) made a careful observation of the early development of *H. discus hannai* at 20 °C from fertilization to veliger larvae.



Morphogenesis starts with the first cleavage and passes through the blastula, gastrula and trochophore stages to the veliger larva. Veliger morphogenesis completed after 99 hours with the formation of the fourth tubule on the cephalic tentacle. Leight(1974) found that larvae of *H. fulgens* could be cultured at 10 to 25 °C with the best development at the higher temperature. These larvae raised at 22 to 23 °C would settled after 4 days with good health. However, Beaudry (1982) conducted an investigation on the effect of water temperature on the larval development in *H. kamtchatkana*. His study was difficult to interpret. Larvae reared at 14 °C, the lowest temperature tested, had a highest number of normal larvae and survive from fertilization to trochophore larva. Larvae reared at 21 °C, showed sign of thermal stress and all larvae died. Although 14 °C produced the best development to early larval stage, the larvae reared at 16 °C had the most rapid growth after settlement while larvae reared at 18.5 °C had the highest overall survival rates from fertilization to 2 months old. The increase overall survival rate at higher temperature may be due to shorter settlement times, reducing the larval period when the highest mortality occur. Hahn (1989) summarized that development rate of abalone larvae is a function of optimum temperature range and time from fertilization to settlement . These factors are different among species of Haliotids (Hahn, 1989).

#### b. Larval Settlement

Planktonic larvae acquire creeping ability after the formation of the first epipodial tentacle. Morphogenesis continues until the fourth tubule forms on the cephalic tentacle. During this period, the larvae alternately creep and suspend while searching for a favorable settlement place (Uki, 1989). In their natural



environment, the larvae of abalone are reported to settle exclusively on coralline algae (Saito, 1981 ;Morse & Morse, 1984 ; Shephred and Turner, 1985 ; Clavier & Richard, 1986). Coralline algae provide good substrate for settlement of benthic larvae because concavities in the algae provide shelter for setting abalone larvae. Furthermore, post-settlement abalone may depend on the cuticle and epithelial contents grazed from coralline algae (Garland *et. al*, 1985). Hahn (1989) stated that in abalones cultivation, the quantity of larvae introduced into a tank depend on the water quality, mortality at settlement and the food source for the newly settled juveniles.

There were many studied on induced larvae settlement methods, first was the diatom and mucus trials from juvenile and adult abalone. Seki and Kan-no (1981) found that complete settlement of competent larvae was induced by a substratum covered with encrusting and diatoms and mucus secretions from grazing juvenile abalones. The second method was the induction of larval settlement with GABA, Morse *et al.* (1979) believed that abalone larvae, when they settled on crustose red algae, recognized “ $\gamma$ -aminobutyric acid (GABA)” that was covalently linked to a some protein or some other molecular substance, or recognized phycoerythrobilin which has a backbone similar to GABA at the center of tetrapyrrole derivative molecule. GABA is an amino acid, and a neurotransmitter in the human brain and other tissues of higher animals. However, there were contradictions in the literature on the effectiveness of these two methods. Arkashige *et al.* (1981) reported that GABA and other neurotransmitter caused death and abnormal behavior in *H. discus hannai* larvae. Seki and Kan-no (1981) showed that diatom and mucus trials from juvenile abalone induced selective settlement in *H. discus hannai* larvae. However, Morse *et al.* (1979) reported that

none of diatom species that were primary food eaten during juvenile rearing had any effect in promoting settlement, development and survival. They also reported that GABA caused normal settlement and metamorphosis. The results of these two studies appear to contradict to each other.

## E. Nutrition

### a. Diatom

Diatoms are the principle food source for newly settled juveniles. They are needed at sufficient levels until abalone are 7 to 8 millimeters in shell length, when the juveniles switch to feed on macroalgae. Most culture facilities rely on naturally occurring diatom species to supply food for juveniles (Ino, 1980 ; Norman-Boudreau, 1985 ; Morse and Hooker, 1985).

Diatoms are unicellular algae belonging to Phylum Crypsophyta, Division Bacillariophyta, Class Diatomaceae. Diatoms has siliceous cell walls that form two overlapping halves, and are golden brown from the presence of chlorophyll, carotene and xanthophyll pigments (Norman-Beudreau, 1985). Diatom growth in rearing tanks or microalgal culture tanks can be enhanced by supplying phosphate, nitrogen, silica, boron, vitamins and trace metals to the water (Uki and Kikuchi, 1979 ; Norman-Beudreau, 1985).

Norman-Beudreau *et. al* (1986) developed a technique to determine the diatom species eaten by newly settled juveniles. They found that all diatoms in the 2 day-old juvenile's digestive tract were of the Order Pennales and usually



smaller than 10  $\mu\text{m}$ . *Navicula* sp. and *Cocconeis* sp. were the most commonly found diatoms. The diatoms found in the gut were more uniform in size and species composition than those found on the rearing surface.

*Navicula* spp. and *Nitzschia* spp. are the usual diatom species given to juveniles for food. These diatoms occur naturally in the rearing tanks and produce good growth rates (Uki and Kikuchi, 1979; Ino, 1980 ; Ebert and Houk, 1984 ; Norman-Beudreau, 1985 ).

#### b. Macroalgae

Juveniles abalone with shell length about 10 millimeters begin to eat macroalgae with an amount range from 10 to 30% of their whole body weight (Norman-Beudreau *et. al* 1986). The high feeding rate of macroalgae is due to high water content and relative low protein content of fresh macroalgae.

Uki *et. al* (1981) tested the food value on 56 species of macroalgae found along the Pacific coast of northern Japan by measured growth rate (body weight and shell length) of *Haliotis discus hannai*, they found that brown algae in the order of Laminaria is the suitable food for this species.

Hahn(1989) summarized that abalones usually preferred brown algae, with some exceptions. Abalone species in California, depending on location and season, eat brown algae (*Macrocrysis* spp., *Nereocrysis* spp., *Egregea* spp., and *Eisenia* spp.), red algae (*Gigartina* spp., *Gelidium* spp., and *Plocamium* spp.), and green algae (*Ulva* spp.). New Zealand species (*H. iris* and *H. australis*) eat



*Macrocrysis pyrifera* when it is abundant but preferably red algae. The order of food preference for *H. iris* was *Gracilaria* sp., *Grossophora* sp., *M. pyrifera*, *Lessonia variegata*, *Champia* sp., *Uva lactuca*, and *Pterocladia* sp. *Gracilaria* sp. was the best food for juvenile and adult *H. iris* even though individuals would not normally eat this red algae in nature. The growth rate in *H. iris* fed by *Gracilaria* sp. was twice as high as the growth rate in the same species when fed by *M. pyrifera* (Tong, 1982).

There were two reports on the experiments on appropriated macroalgae diets for food of abalone in Thailand. These were aimed to find year-round sources of macroalgae to supported the local abalones. Singhagraiwan (1989) conducted the feeding experiments on juvenile abalone (*H. asinina*) with *Gracilaria salicornia*, *Enteromorpha intestinalis*, *Caulerpa macrophysa*. He reported that *Gracilaria salicornia* was the best macroalgae for growth of *H. asinina*. Jarayabhand et al.(1992) summarized from research and development on some aspects of abalone culture that *Enteromorpha intestinalis* is an appropriate food for adult of *H. ovina*. In Prachubkirikan Fisheries Station, Klongwan, Prachubkirikan province conducted a research on growth of *Euchema* sp. from Japan. This algal species has good growth rate all year-round in Thailand (Nagranard, 1995 personal communication). So considered from physical structure, *Euchema* sp. should be tested as another macroalgal food for abalone cultivation.

In this experiment, two potential local species of macroalgae in Thailand were also chosen. They are: 1) *Enteromorpha intestinalis*, the green algae with hollow tubular up to 12 cm high, attach by a discoid holdfast, basal part tapering, inflated and contorted above. Light green incolor. Growing on the rock or shells in upper intertidal areas. This species is common in backish water ponds. 2) *Gracilaria*

*changii*, the red algae with thallas 5-7 cm in high with many branches arising from small holdfast or from precurent axis with irregular branching. Growing on the pebble shells, rock fragments mangrove roots or fish cages in sandy muddy to muddy area of high turbidity. This species is rather common on the east coast of the Gulf of Thailand especially in Trat Province (Lewmanomont and Ogawa, 1995).



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