INDUCED SPAWNING, SEED PRODUCTION, AND JUVENILE GROWTH OF THE DONKEY'S EAR ABALONE *HALIOTIS ASININA* LINNÉ, 1758

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ABSTRACT

A total of 180 hatchery-raised abalone *Haliotis asinina* Linne were conditioned to photo-periods of 12 h light and 12 h darkness. Reversal of day and night periods made abalones change spawning behaviour and release gametes during day time. Spawning started after 7-10 days and continued from February to August 1996 without further treatment. The abalones produced a total of 40 million fertilised eggs. Survival of individual stages varied considerably. Calculated from the previous stage, survival was 60 % to veliger stage, 48 % to creeping larval stage, 10-20 % to shell length of 2 mm (1 month), 40 % to shell length of 5 mm (2 months), and 60 % to shell length of 10 mm (3 months). The monthly hatchery production was 39,497 juveniles with a shell length of 10 mm. Juveniles were reared in plastic cages suspended in the sea or kept in the raceway of the hatchery. The latter showed higher growth rate but lower survival rate. However, no statistical difference was found between the treatments.

INTRODUCTION

Three species of abalone, *Haliotis ovina* Gmelin, 1791, *H. varia* Linnaeus, 1758 and *H. asinina* Linnaeus, 1758 occur in Thai waters (Nateewathana & Hylleberg 1986; Tookvinas *et al.* 1986; and Nateewathana & Bussarawit 1988). The donkey's ear *H. asinina*, seems to have the best potential for commercial culture because of a higher mass of soft body. Its maximum shell length is 10 cm (Singhagraiwan & Doi 1993). The interest in abalone culture began in Thailand in 1988 when the Department of Fisheries succeeded in abalone culture for the first time at The Eastern Marine Fisheries Development Center. Since then the Prachuap Coastal Aquaculture Development Center has improved some techniques in order to achieve year-round seed supply. Abalone has a potential for commercial exploitation. The price is high and the market extensive, particularly in China, Japan, and Korea (Training Manual 1990) In this paper, we show results of induced spawning by artificial photo-period, rearing of larvae and juveniles, and we include data on growth of juveniles cultured in different environments.

MATERIALS AND METHODS

Conditions for induced spawning and larval development

Hatchery-raised abalone, 3 years old, were cultured in a raceway tank (capacity 11 tons) at the mollusc hatchery of Prachuap Khiri Khan Coastal Aquaculture Development Center. Broodstock abalones ranged in shell length from 7 to 10 cm. Abalones were fed intensively on red seaweed *Gracilaria* sp. Gonadal condition was determined every week by methods of Singhagraiwan & Doi (1992). When the gonad reached ripeness stage it could easily be defined by the naked eye. At the edge of the shell muscle, the colours of female and male were green and creamy respectively.

Ninety abalones were transferred to a 300 l fibreglass tank at a male to female ratio of 1:5. Strong aeration was provided to ensure sufficient supply of oxygen. Abalones were fed on fresh seaweeds, *Gracilaria* sp. Three bent 30 by 30 cm PVC plates were placed at the bottom of each tank of the adults. Filtered sea water was renewed at a rate of 10 litre min^{-1}, or according to the biomass of

abalone and amount of oxygen consumed (Training manual 1990). Photo-period spawning was induced according to the technique of Kikuchi & Uki (1974a). Tanks were covered with black cloths in daytime (AM 6.00 to PM 18.00). During night time cloth was removed and lights turned on (PM 18.00 to AM 6.00).

Fertilised eggs were collected 15 min after spawning. They were siphoned to a 45/50 µm sieve and washed with filtered sea water (0.1 µm) to remove excess sperm, debris, *etc.*, and then kept in 10 litre plastic bins filled with filtered and UV treated sea water. The progress of fertilisation was determined under compound microscope (haemacytometer). Fertilised eggs were stocked at a density of 5-10 eggs ml^{-1}. Rearing containers were kept at ambient temperature (27- 29 °C). Sea water was changed daily, growth and survival of larvae were observed, and mortality was estimated from the quantity of empty shells recovered on screening sieves after sea water changes. Mild aeration was provided and antibiotics (10 ppm neomycin and streptomycin, ratio 1:1) was added to the rearing tank throughout the 3 days of veliger stage in order to control bacterial infection.

Post larval, juvenile rearing and seed production

Transparent polyethylene sheets were grouped into bundles of 10-15 sheets arranged at 3 cm intervals. Three to four sets of culture sheets were immersed 4 to 5 days in a 300 l diatom culture tank. Next, film-forming pennate diatom (unidentified) were added to the cultures.

After larvae entered the creeping stage they were transferred to culture tanks provided with diatoms growing on sheets or container surfaces. Approximately 100,000 larvae were stocked in the 300 litre diatom culture tank provided with 0.1 micron filtered sea water, flow-through at 6 litre min^{-1}.

Settled abalones were fed on *Nitzschia* sp. and *Navicula* sp., as required, during the first 3 weeks of culture. After abalones reached 5 mm shell length, they were transferred to 1,000 litre rectangular fibreglass tanks with a continuous flow of sand-filtered sea water. At this time, abalones were fed on pieces of *Gracilaria fisheri* and *Acanthophora* sp. Supplementary feeding on microalgae continued until the young abalones were fully capable of consuming the macroalgae. Growth, survival rates and seed production were determined at critical stages.

On-growing of juveniles

Thirty abalone seeds, 18.23 to 18.34 mm shell length, were put in cylindrical plastic net cages (12 cm high, 10 cm in diameter, mesh size 0.5 cm). Each cage was provided with a bent PVC plate as substratum and shelter. Growth was compared in cages suspended from raft (Prachuap Khiri Khan Bay) and cages kept in raceway (3 replications of each treatment). Raft cages were hung at 1 m depth. They were cleaned every week and shells were measured every month.

Raceway cages were put in a 11 tons outdoor tank provided with sand filtered sea water at 12 litre min^{-1}. The water was constantly agitated by low-speed paddle wheels (electrical motor) to keep water conditions uniform throughout the tank. Both treatments were given fresh red sea weed *Gracilaria fisheri* (10 % of total wet weight of abalones). The experiments lasted 6 months. Differences between mean growth and survival rates were tested for significance using Students *t*-test.

RESULTS AND DISCUSSION

Spawning

Males and females began to spawn at 7 days after conditioning (AM 11:45 to PM 12:30) and around 10 to 15 % of the abalones in each tank released their gametes in the same period throughout this study. (Fig. 1) Males and females spawned almost at the same time. Duration of spawning was around 45 min. Repeated spawning was not

observed. The artificial photo-period is harmless and inexpensive. Furthermore, the release of gametes can be triggered to occur at any desired time of day. It is not fully understood why the artificial photo-period has this effect on spawning of the abalone. But, the method works all year round and stimulates spawning of *Haliotis* out of season.

Early development, larval rearing and seed production

During culture it is very important to provide sufficient feed to the abalones. Gonadal maturation does not occur unless the snails are fed on macroalgae. Singhagraiwan & Doi (1993) used *Gracilaria salicornia*, *etc*.

In this study the hatching rate was 46 to 72 % depending on quality of egg or overabundance of spermatozoa. Lack of spermatozoa caused low fertilisation rate. A density of

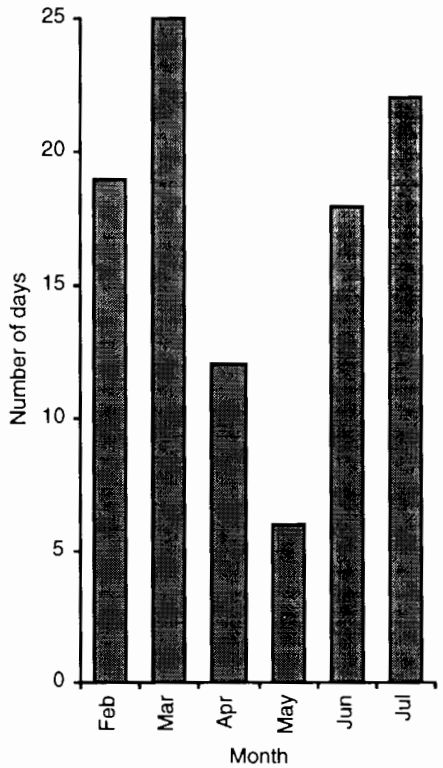

Figure 1. Number of times abalones released gametes during conditioned artificial photo-period.

200,000 sperms ml^{-1} gave good fertilisation and decreased problems with excess sperm. Excess sperm can result in abnormal abalone in the early stage of development (Kikuchi & Uki 1974b).

Eggs were spherical, dark green in colour and had a clear jelly around the nucleus (Fig. 4). The size of unfertilised egg was 0.150 mm. Egg cleavage was total, unequal, and of the spiral type. After 10 min the first polar body was seen, and the second polar body after 15 min. After 20 min the first division took place in the meridional plane (Fig. 5). After 30 min the second cleavage produced four equal blastomeres by a meridional division perpendicular to the first. After 60 min the third division took place, yielding four micromeres at the animal pole and four macromeres at the vegetative pole. The embryo passed through morula and blastula stages and reached gastrula stage in 80-120 min. After 5 h the embryo reached the trochophore stage, with a girdle of cilia allowing rotation within the egg (Fig. 6). After 6 h from fertilisation, hatching took place. The size of newly hatched larvae was 0.145 x 0.170 mm. After 9 h most larvae had developed transparent larval shells, reached the veliger stage, and formed the velum (Fig. 7). After 24 h veliger torsion of 90° was completed, and operculum, foot, muscles, eyes, mantle and cephalic tentacles developed. At that time the larvae measured 0.150 x 0.180 mm. After 28 h most of the veligers metamorphosed to creeping larvae, the foot began to grow, and the velum disappeared. At this stage the larvae should be transferred to the setting tank. Most larvae settled on the plates within three days and shifted from planktonic to benthic mode of life. Abalones measured approximately 2.5 mm after 34 days (Fig. 8). They continued to grow on the diatom culture sheets until 5 mm length, or 2 months age. After 3 months, they reached about 10 mm in shell length (Fig. 9) and could feed on macro-algae together with benthic microalgae.

Approximately 40 millions of mature eggs

were collected during February-August 1995. They hatched to 24,000,000 trochophore larvae (hatching rate 60 %). About 11,520,000 survived to the creeping stage at day 2 (survival rate 48 %) but about 1,152,000 survived to 2 mm shell length fed on diatom cultured sheets after 60 days (survival rate 10 %). The survival rates at 5 and 10 mm shell length were 60 and 80 %, respectively. As a final result the Mollusc Hatchery produced 468,000 seeds of 5 mm shell length and 276,480 seeds of 10 mm shell length. The average production was around 39,497 seeds of 10 mm shell length

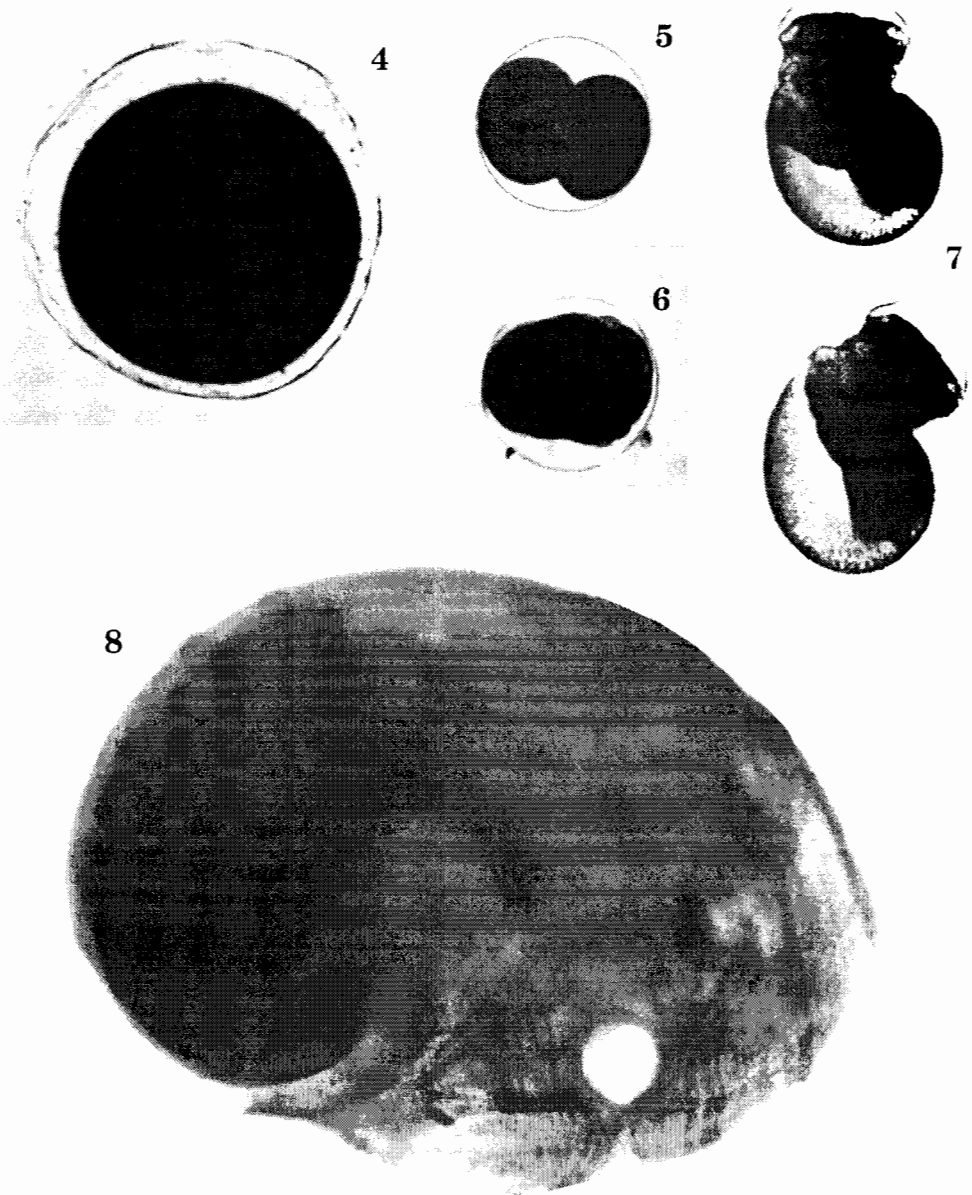

Figure 4-8. (4). Fertilised egg - 5 min. (5). 2-cell stage - 30 min. (6). Rotating trochophore within egg membrane - 5 h. (7). Late veliger stage with transparent shell and velum - 18 h. (8). Juvenile 2.5 mm with 1st respiratory pore - 34 days.

Table 1. Time required and size for embryonic and larval development of *Haliotis asinina*.

Developmental stage	Time required	Size (μm)
Unfertilised egg	0 min	150
First polar body	10 min	150
Second polar body	15 min	150
First cleavage	20 min	160
Second clevage	30 min	160
Third-sixth cleavage	60 min	160
Rotating trochophore	5.15 h	170
Hatching	6.35 h	170
Early veliger	9 h	180
Late veliger	24 h	180
Early creeping larvae	28 h	220
Creeping larvae	2-3 days	245
Juvenile (1-3 respiratory pores)	30-40 days	2500

per month. In several cases, mortality rates were high during the first 60 days due to poor egg quality, contamination by microorganisms, water quality, and quality and quantity of feed on the diatom sheets. Several interrelated environmental factors affect the growth rate such as the level and type of nutrients available, as well as the range of environmental conditions. Some of these factors include feed, water temperature and stocking density. Water in the rearing tank should be filtered until juveniles reach 5 mm shell length. Occasionally the mortality rate may be high due to the overgrowth of food organisms. A technical system to control the growth of diatom and filamentous algae should be established.

Juvenile rearing

The experiment was conducted from day 45 to the sixth months of age. The results are summarized in Tab. 2. Better growth rates were observed in the raceway group where 18.23 to 32.78 mm shell length and 1.39 to

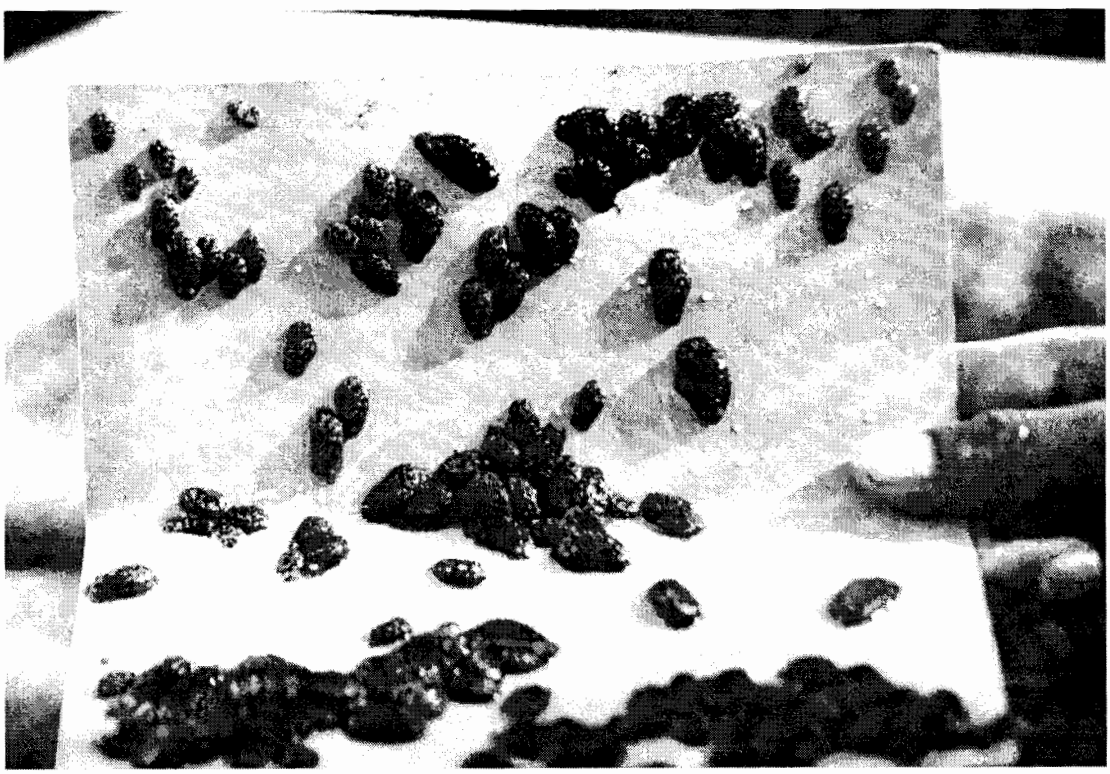

Figure 9. Juvenile 10 mm shell length - 3 months.