AN EXPERIMENT ON HATCHERY SEED PRODUCTION OF THE SCALLOP *CHLAMYS SENATORIA* GMELIN

Jintana Nugranad & Kanchanee Promjinda
Prachuap Khiri Khan Coastal Aquaculture Development Center, Klong Wan, Prachuap Khiri Khan 77000, Thailand

ABSTRACT

Breeding of the scallop *Chlamys senatoria* Gmelin was performed successfully in the Prachuap Khiri Khan Mollusc Hatchery. Spawning was induced by injection with 0.3-0.5 ml saline solution with 2 mM serotonin (5-Hydroxytryptamine creatinine sulphate) into either adductor muscle or gonad, or by sea water manipulation combined with air exposure methods. Fertilised eggs developed into the D-shaped larval stage within 18 hours. The larvae took 8-9 days to reach the pediveliger stage. Settlement and metamorphosis began after 10-11 days at a size between 220-240 μm shell length. The survival from fertilisation to the D-shaped larval stage ranged from 2.5 to 66.9 %. Survival from D-shaped larvae to pediveligers was 0-38 %, and from pediveligers to one month old spat with 0.5-1.0 mm shell length about 5-10 %. Spat grew to 1-4 mm shell length at the age of 2 months and reached the size of 7-18 mm as young juveniles at 3 months of age, with approximately 40-50 % and 80-90 % survival, respectively.

INTRODUCTION

The scallop *Chlamys senatoria* Gmelin, is one of the pectinid bivalves found in Thai waters, both in the Gulf of Thailand and on the Andaman Coast. They are collected and utilised as food by local fishermen, while diving for other valuable shells, and known for delicacy like other scallop species.

In Thailand, scallop culture has yet not been established. The whole production of scallops is from natural harvest of *Amusium pleuronectes*, the Asian moon scallop. This species, although being the most commercial one, is not feasible for aquaculture since they show very active swimming behaviour, causing difficulties in enclosure culture. To develop scallop culture in Thailand, it is necessary to find other suitable species. Among the scallops present in Thai waters, the local species *C. senatoria* is interesting. The characteristics of *C. senatoria* are very similar to the species *C. nobilis* in Japan. Preliminary attempts to grow the scallops collected in nature in Prachuap Khiri Khan Bay have also been promising. Therefore, the Prachuap Khiri Khan Coastal Aquaculture Development Center has included this species to be studied under the research project on scallop culture.

C. senatoria is normally found inhabiting areas off coral reef flats, using the byssus to affix itself onto the substrate. However, if being disturbed they can free themselves by releasing the byssus and swim away. The species is dioecious. During gonadal maturation sexes can be easily distinguished by difference in gonad colours, which is creamy white in the male and yellow-orange in the female. The gonad is conspicuous, lying in a semi-circle around the anterior and ventral part of the adductor muscle. Based on samples from Bang Sapan, Prachuap Khiri Khan, it was found that this species possesses mature gonad almost all year round. The aim of this study was to collect information on larval development and growth, as well as to define the hatchery techniques to be used with the scallop *C. senatoria*.

MATERIALS & METHODS

Broodstock

Adult *C. senatoria* (Fig. 1) were collected from natural habitats in the sea, and placed

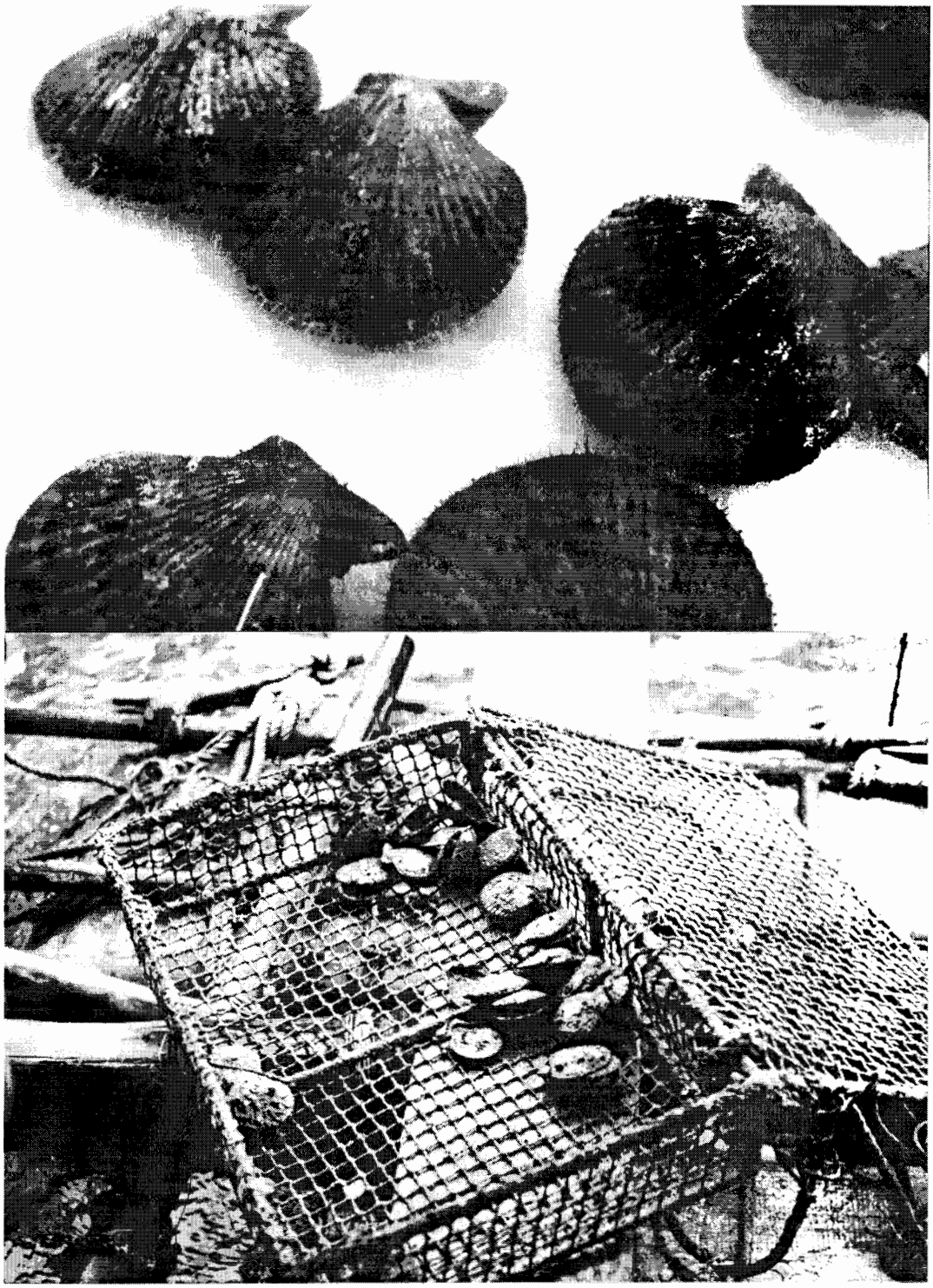

Figure 1. The scallop *Chlamys senatoria* Gmelin and broodstock culture tray.

in metal-framed nylon net cages, 40 x 60 x 15 cm, hanging from bamboo rafts in Prachuap Khiri Khan Bay for gonad maturation. Gonad condition was easily determined by looking into the body cavity while the scallop opened the valves. The scallops with plump or full gonads, were considered mature and were selected for breeding. They were cleaned to remove any fouling organisms and sediments, and transferred for spawning induction in the hatchery.

Spawning induction

The methods applied for spawning induction were thermal stimulation, short-period desiccation or air exposure combined with sea water manipulation, and serotonin injection. The thermal stimulation method was performed using heated sea water at 32-34 °C and ambient temperature sea water at 27-29 °C, flowing through the spawning tray alternating with a period of 0.5-1 h each, two or three times until spawning occurred. The short-period desiccation or air exposure method was done by leaving the scallops out of sea water for about 5-10 minutes, then placing them back into the spawning tray provided with running sea water. Water in the spawning tray was drained off from time to time after every 1-2 h of flowing water until spawning occurred.

The serotonin injection method utilises a neurotransmitter reagent, serotonin (5-Hydroxytryptamine creatinine sulphate, Sigma Co.). The solution was prepared at a concentration of 2 mM serotonin in filtered sea water (Gibbons & Castagna 1984). The dosage of 0.3-0.5 ml solution was injected into either adductor muscle or the gonad of the scallop. As it has been observed that males usually respond to this induction almost immediately while females take longer time to respond, the females were given injection prior to the males. The injected scallops were placed individually in glass beakers containing filtered sea water for spawning.

Larval rearing

Spawned eggs and sperm were collected separately, and then mixed later for fertilisation. About 100-200 ml of sperm suspension, depending on density, was used for 20 l of egg suspension. Fertilised eggs were suspended at the density of 5-10 eggs ml^{-1} in 500-1,000 l cylindrical fibreglass tanks filled with 1 μm filtered and UV sterilised sea water provided with gentle aeration. On the following day, when the D-shaped larval stage completely developed, the water in the rearing tanks was totally changed using nylon screen sieves to retain the larvae. Unicellular algae, *i.e.*, *Isochrysis galbana, Chaetoceros calcitrans,* and *Tetraselmis* sp. were used for feeding the larvae at the concentration of 10,000-25,000 cells ml^{-1} at a time. Changing of water thereafter was performed every other day.

Juvenile rearing

When the pediveliger stage developed and the larvae were about to reach settlement age, differentiated by eye-spot and foot development, settling substrates such as nylon filaments, nylon rope, plastic shade-cloth (Fig. 2) were provided in the rearing tanks. Newly settled juveniles were held in nursery rearing tanks provided with running filtered sea water and phytoplankton for feeding in the re-circulating system. Juveniles attaining 3-5 mm shell length were transferred to the open sea nursery in Prachuap Khiri Khan Bay, where they were held in nylon mesh bags hanging from the raft or long-lines. Growth, survival, and development of the hatchery-produced larvae and juveniles were determined.

RESULTS

The adult scallops held in Prachuap Khiri Khan Bay developed mature gonads almost through the year. The mature scallops used in spawning induction had shell lengths (anterior-posterior axis) of 5.6-8.0 cm with a

mean of 6.5 cm, shell height (dorso-ventral axis) of 6.0-8.2 cm with a mean of 6.8 cm, and a total weight of 28.6-68.8 g with a mean of 43.8 g.

Spawning induction

Spawning of the scallop *C. senatoria* was successfully induced by either serotonin injection or combination of air exposure and sea water manipulation. The thermal stimulation by alternating between the temperatures 32-34 °C and 27-29 °C was unable to induce spawning in the scallops in this study. This method even weakened the induced animals resulting in 50 % mortality after spawning induction. Results of spawning induction of the scallop by these three methods are shown in Tab. 1.

Table 1. Results of spawning induction in *Chlamys senatoria* at Prachuap Khiri Khan hatchery. (*) signifies 3 trials inducing both males and females; and 1 trial inducing only females (See details in Tab. 2).

Method	No. of trials	No. of scallops induced	Number of trials spawned		Remarks
			Egg	Sperm	
Thermal	1	100	-	-	50 % died on the following day
Air exposure	8	35-450	5	5	average 1.1 % died
Serotonin	4*	20-32	4	3	average 15.21 % died

Serotonin injection into either adductor muscle or gonad was found to be very effective in inducing spawning of the scallops, with no obvious difference between the ar-

Figure 2. Some collecting materials provided for spat settlement: the nylon filaments or artificial weed (originally used for collection of fish eggs), and nylon rope formed to make a filamentous lattice.

Table 2. Results of spawning induction in *Chlamys senatoria* with serotonin injection.

Trial	No. of Males		No. of Females		% Spawning	
No.	Injected	Spawned	Injected	Spawned	Males	Females
1	7	6	13	9	85.7	69.2
2	3	3	29	27	100	93.1
3	-	-	28	25	-	89.3
4	2	2	14	7	100	50.0
Total	12	11	84	68	91.7	80.9

Table 3. Results of larval rearing in *Chlamys senatoria*. Ex = Air exposure & water manipulation method; Sr = Serotonin injection; Spn = Spontaneous spawning; n.d. = not determined.

Batch No.	Method	No. of females spawned	No. of Eggs	Hatched (%)	Survival to settlement	Remarks (rearing period)
1	Ex	n.d	10,080,000	17.7	-	13 days
2	Spn	n.d.	4,640,000	n.d.	< 1 %	30 days
3	Ex	n.d.	>5,000,000	n.d.	-	9 days
4	Spn	n.d.	19,820,000	14.1	-	13 days
5	Ex		7,240,000	2.9	-	1 day
6	Ex		75,222,000	4.9	< 1 %	23 days
7	Spn	n.d.	5,600,000	22.1	< 1 %	25 days
8	Ex	n.d.	16,120,000	66.9	-	21 days
9	Sr	9	3,620,000	26.3	< 1 %	13 days
10	Sr	27	33,274,000	n.d.	-	1 day
11	Spn	23	7,080,000	2.5	-	11 days
12	Spn	1	1,200,000	47.8	-	9 days
13	Sr	25	40,608,000	50.7	38.17 %	Successfully rearing to juveniles
14	Sr	7	Very few	< 1 %	-	Discarded

eas of injection. The males started spawning within 0.5-3 minutes after injection, while females stayed for 2-30 minutes before beginning to spawn. There were no differences between the doses of 0.3, 0.4, or 0.5 ml solution per individual with respect to spawning induction. Total percentage of response was 91.7 % for males, and 81 % for females. Tab. 2 shows the results of induced spawning by serotonin injection.

The combination of air exposure, leaving the scallops out of water for 5-10 minutes, and sea water manipulation was effective in inducing spawning of the ripe scallops. Spawning occurred within 5-30 minutes after filling new sea water in the inducing tank. Besides spawning induction, the adult scallops just brought from the sea into the hatchery also produced spontaneous spawning without any direct induction, but mostly after the cleaning procedure.

Larval rearing

From 9 successful spawning inductions (Tab. 1) and 5 spontaneous spawning events, a total of 14 larval batches were obtained, though the latest batch was discarded because of very low hatching rate (Tab. 3). Of 13 batches of viable larvae being reared, most suffered from high mortality, low

Table 4. Larval rearing of the scallop *Chlamys senatoria*.

Age (days)	Stage	Size (µm)	Density (larvae ml^{-1})	Food conc. (cells ml^{-1})
1-2	D-shaped veliger	100-120	5-10	10,000-12,000
3-7	Veliger	130-190	3-5	12,000-20,000
8-11	Pediveliger	220-240	1-3	15,000-20,000
10-15	Settlement	-	-	20,000-25,000
30	Spat	500-1,000	-	> 30,000

growth, and failure to develop through metamorphosis. Five batches reached pediveliger stage. Only one batch passed metamorphosis through settlement and grew up to juvenile stage to be cultured in the open sea successfully with the survival of 38.2 % from D-shaped to pediveliger stage.

Salinity of the sea water in the larval rearing tanks ranged from 33 and 35 ‰, and temperature from 27-29 °C. The rearing density of veliger larvae was 3-5 larvae ml^{-1}. Unicellular algae used for feeding the larvae were *Isochrysis galbana* and *Chaetoceros calcitrans* with the concentration of 10,000-25,000 cells ml^{-1}. After settlement, *Tetraselmis* sp. was added as food with increasing concentration to reach spat requirement. The scallop spat would settle onto every substrate provided in the rearing tanks with no significant preference. Details of larval rearing is shown in Tab. 4.

Development of the larvae and juveniles

Fertilised eggs of *C. senatoria* developed into the D-shaped veliger larval stage within 18 hours after fertilisation. One day old veligers had a shell length of 100-105 µm, and 70-80 µm shell height (Fig. 3A). Healthy larvae, indicated by active swimming and good feeding, as seen by a brownish colour of algae in the stomach, exhibited a growth rate of around 10 µm per day and reached a size of 130-150 µm in 4-5 days (Fig. 3B). The pediveliger stage was reached in 8-9 days, by which the foot and eye-spots were well developed (Fig. 3C). They began settlement by producing byssus to affix themselves onto the substrate at the age of about 10-11 days (shell length of 220-240 µm, shell height of 170-190 µm). Next they metamorphosed, the velum disappeared, the gills developed, and the spat shells were formed (Fig. 3D). Shell growth expanded both anteriorly and posteriorly until a straight line appeared at the hinge, with a byssal groove on the anterior of the right valve. This straight hinge characteristic was seen at a size of about 500 µm (Fig. 3E).

The statocysts were seen in one month old spat with a size of about 1 mm shell length, appearing at the edge of the mantle tissue among mantle tentacles which developed a little earlier (Fig. 3F). Shell growth increased more in the dorso-ventral axis than the anterior-posterior axis, and the radial ribs were formed on the shells (Fig. 3G). Shells of the young juveniles appeared in a variety of colours such as yellowish orange, bright orange, brownish, and dark brown (Fig. 3H) from an age of about 2 months, or from the size of 3-4 mm.

Growth and survival rates

In the 13 rearing batches (Tab. 3), the success of development from fertilised eggs into D-shaped larvae ranged between 2.5-66.9 %, with the average of 30.4 %. Survival rate from D-shaped larvae to pediveligers was estimated at 0-38 %. Pediveligers developed through metamorphosis to one month old spat of 0.5-1 mm shell length with a success rate of about 5-10 %.

Table 5. Development and survival of the hatchery-reared scallop *Chlamys senatoria*.

Age	Stage	Size	Survival %
17-18 hours	D-shaped veliger	100-105 µm	2.5-66.9
4-5 days	Veliger	145-150 µm	70-80
8-10 days	Pediveliger	210-230 µm	0-38
10-11 days	Settlement	230-240 µm	-
1 month	Spat	0.5-1 mm	5-10
2 months	Young juvenile	1-4 mm	40-50
3 months	Juvenile	7-18 mm	80-90

Spat grew up to 1-4 mm shell length, with the mean size of 3.0 mm at an age of 2 months, with approximately 40-50 % survival. At this stage they were transferred into the nursery in the sea. They reached the size of 7-18 mm as young juveniles at 3 months of age, with approximately 80-90 % survival.

Development and survival of the hatchery-produced larvae and juvenile scallops is summarised in Tab. 5. Growth of the larvae and juveniles is shown in Fig. 4.

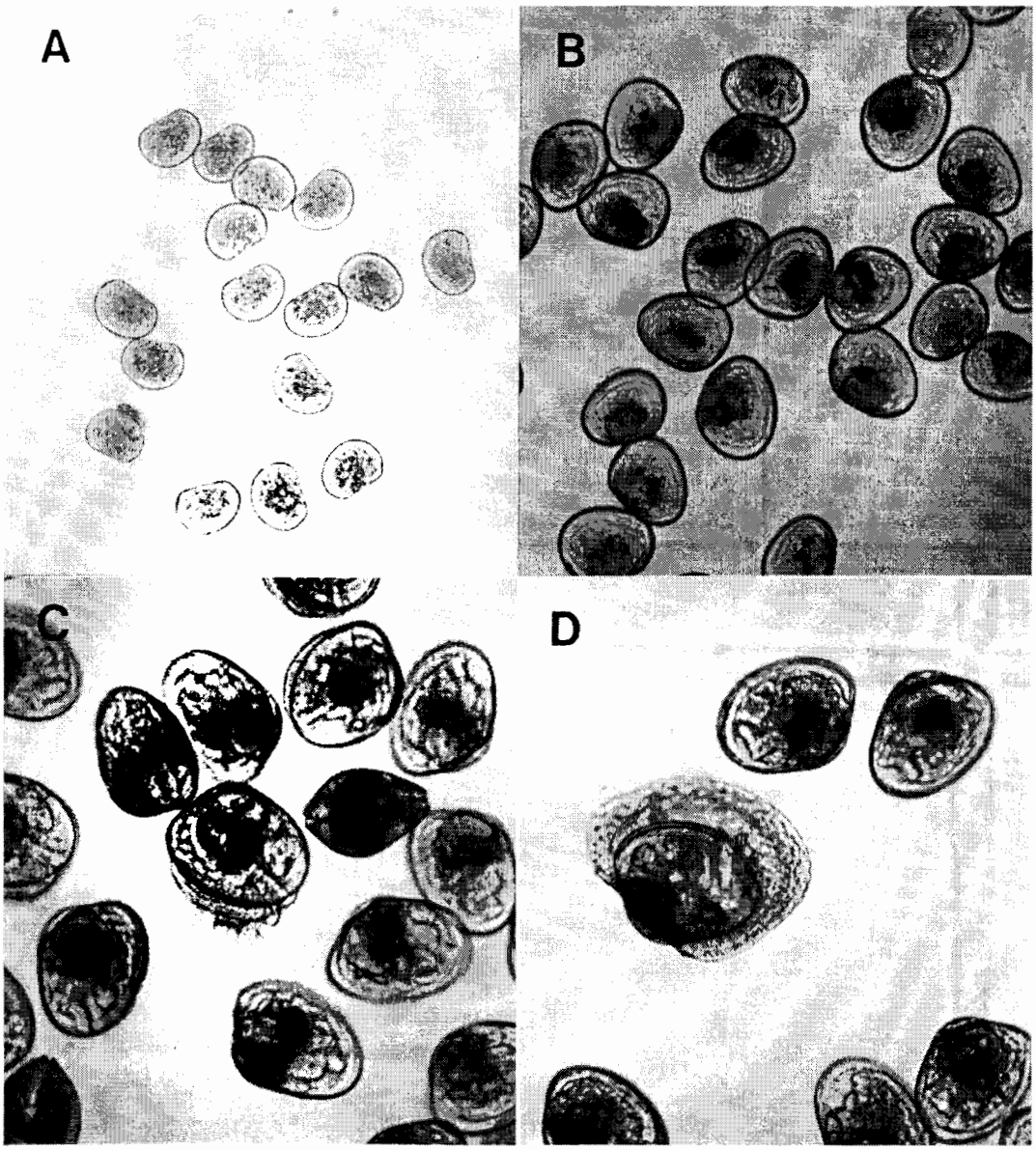

Figure 3. Development of the hatchery-reared larvae and young juvenile scallop *Chlamys senatoria*; (A) D-shaped larvae, 100-105 µm shell length, 20 hours after fertilisation; (B) 4-5 days old veligers, 130-150 µm shell length; (C) Pediveligers, 11 days old, 220-240 µm; (D) Metamorphosed spat, 15 days old.

DISCUSSION

Serotonin injection is a very effective method in breeding of the scallop *Chlamys senatoria*. The serotonin has been successfully used with many bivalve species, including scallops (Matsutani & Nomura 1982; Gibbons & Castagna 1984). As this reagent is a strong inducer, releasing of immature gametes may occur. Thus, it should be used only with mature broodstock in order to yield healthy larvae. As gonad maturity of the scallops can be determined properly, spawning induction

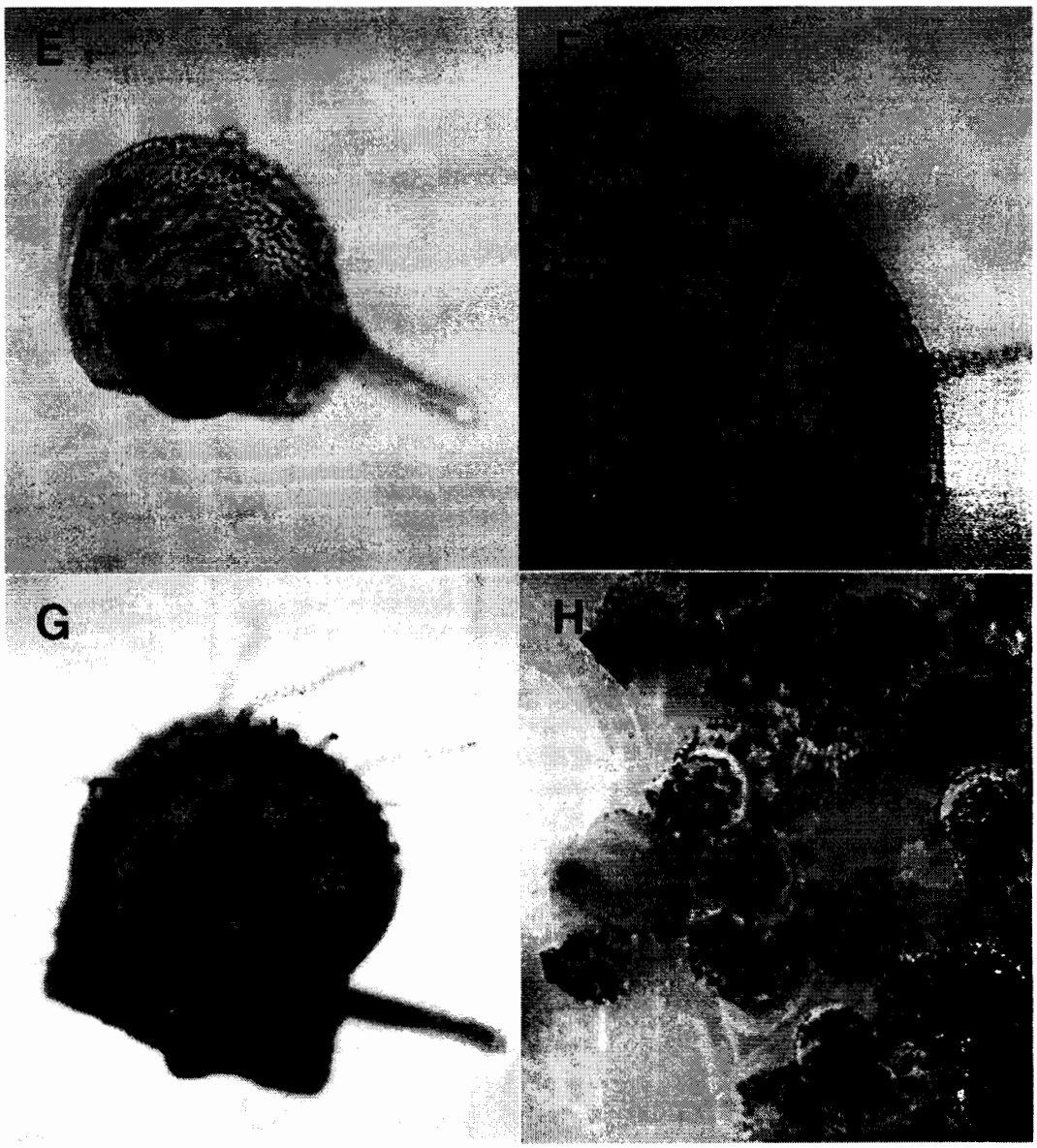

Figure 4. Development of the hatchery-reared larvae and young juvenile scallops *Chlamys senatoria*; (E) Straight hinge spat, 19 days old, 600 μm shell length; (F) 30 days old spat with tentacles and statocysts at the mantle margin; (G) 35 days old spat, with radial ribs appeared on the shell; (H) 3 months old juveniles, 7-18 mm shell length.

with serotonin injection is desirable.

Serotonin was reported to be applicable for spawning induction of other scallops especially in the dioecious species *Patinopecten yessoensis* (Matsutani & Nomura 1982) but without much success in the hermaphroditic species *Pecten albicans* (Tanaka & Murakoshi 1985), *Pecten ziczac* (Velez *et al.* 1990), and the Asian moon scallop *Amusium pleuronectes* (own observation), in which we were able to induce only sperm release.

Injection of serotonin to induce spawning of the scallops can be applied to either gonad or adductor muscle. However, giving injection into the adductor muscle seems to be much easier while injection into the gonad may cause injury to some delicate internal organs and may lead to weakening or mortality after induction.

Spawning induction by thermal stimulation is found to be effective and is widely used with many bivalve species including scallops (Bourne *et al.* 1989). In this study it was tried only once. Therefore, the failure might have resulted from immature broodstock or due to inappropriate period of induction.

Broodstocks of the scallops held in Prachuap Khiri Khan Bay developed mature gonads almost all year round. The environmental conditions in the Bay can serve the requirements for scallop growth and gonad maturation, thus scallop culture could be developed in this area. This species was found sexually mature at a size as small as 4 cm shell length. The reproductive biology of this species should be studied further.

Hatchery seed production of the scallop *C. senatoria* is one promising way of scallop culture development in Thailand. The hatchery-produced juveniles could survive and grow well in coastal waters. However, techniques for larval and juvenile rearing should be further improved to obtain better survival rate and production.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of Prachuap Khiri Khan Mollusc Hatchery for their contributions in the hard hatchery work, especially Om-duan Meejui who helped taking care of larval and juvenile rearing, and Waraporn Saleetid who managed phytoplankton culture for feeding the scallops. Thanks are also due to Mr. Songchai Sahavacharin, the Director of Prachuap Khiri Khan Coastal Aquaculture Development Center, for his advice and encouragement. And last, but not least, we would never forget to thank the Deputy Director of the Department of Fisheries, Mr. Chanintorn Srithongsuk, who encouraged our work on scallop culture.

REFERENCES

Bourne, N., C.A. Hodgson & J.N.C. Whyte. 1989. A manual for scallop culture in British Columbia. - Canadian Technical Report on Fishery and Aquatic Sciences (1694): 230 pp.

Gibbons, M.C. & M. Castagna. 1984. Serotonin as an inducer of spawning in six bivalve species. - Aquaculture **40**: 189-191.

Matsutani, T. & T. Nomura. 1982. Induction of spawning by serotonin in the scallop, *Patinopecten yessoensis* (Jay). - Marine Biological Letters **3**: 353-358.

Tanaka, Y. & M. Murakoshi. 1985. Spawning induction of the hermaphroditic scallop, *Pecten albicans*, by injection with serotonin. - Bulletin of the National Research Institute of Aquaculture **7**: 9-12. (In Japanese, with English abstract)

Velez, A., E. Alifa & O. Azuaje. 1990. Induction of spawning by temperature and serotonin in the hermaphroditic tropical scallop, *Pecten ziczac*. - Aquaculture **84**: 307-313.

ISSN 0858-3633