HATCHERY SEED PRODUCTION OF THE FLUTED GIANT CLAM (*TRIDACNA SQUAMOSA* LAMARCK, 1819) AND OCEAN NURSERY OF THE JUVENILES FOR RESTOCKING IN KOH TAO, THAILAND.

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ABSTRACT

Five batches of hatchery-produced juvenile fluted giant clam *Tridacna squamosa* were raised in Prachuap Khiri Khan Mollusc Hatchery. The largest batch encompassed more than 150,000 juveniles, 1-2 cm length. Spawning was either spontaneous or induced after a short-period of desiccation combined with sea water change. Zooxanthellae were collected from excretion of adult clams and supplied into rearing tanks from day 4 or 5 by when the veligers were transferred to outdoor culture tanks exposed to sunlight. Pediveligers established symbiosis from day 8, and completed metamorphosis at the age of two weeks. Thereafter, juveniles were reared in a concrete raceway with flowing sea water. Growth of the juveniles varied from batch to batch, as well as from tank to tank, mainly depending on management. High mortality occurred during metamorphosis, and subsequently in small juveniles prior to the size of 5 mm shell length due to overgrowth with benthic diatoms and algae in the rearing tanks. Herbivorous fish *Siganus* spp. were found effective to reduce this problem. Ocean nursery trials were conducted in coral reef area in Koh Tao in the Gulf of Thailand, using plastic net cages lined on sea floor with coral rubbles as substrate to raise the juveniles from 1.5 cm shell length. Juvenile transportation, nursery methods, results, and problems are discussed.

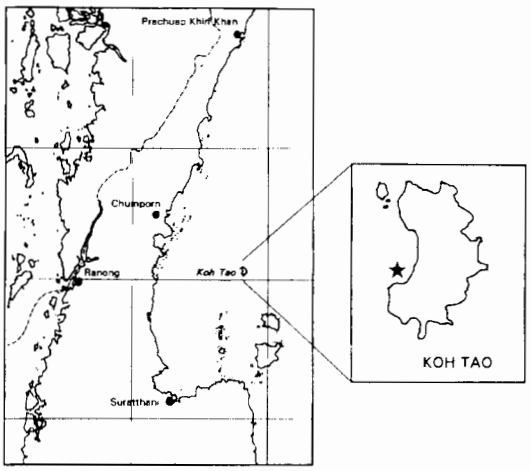
INTRODUCTION

The fluted giant clam *Tridacna squamosa* Lamarck, 1819 is one of the largest bivalves presently existing in Thai waters. Four other species of the tridacnids have been reported from the Gulf of Thailand and/or the Andaman Sea: *Tridacna crocea, T. maxima, T. gigas,* and *Hippopus hippopus* (Lucas 1988). The natural stocks of giant clams have been declining, and presently only the first three mentioned species are found existing. *T. squamosa* is the most vulnerable species. It has been overexploited till near extinction in most of its geographical range.

Giant clams possess interesting biological characteristics. They are simultaneous hermaphrodite, capable of producing both male and female gametes at the same time in each individual. Natural release of sperms and eggs of each clam usually occur in separate events to restrain inbreeding. However, self-fertilisation can also yield viable larvae (Murakoshi & Hirata 1993). Another unique feature of giant clams is the symbiotic relationship with zooxanthellae living in their mantle tissues (Svane 1996). These peculiarities have made giant clams differ from other bivalve molluscs in terms of hatchery culture methodology.

While the culture technique for giant clams has developed progressively around the Indo-Pacific during the last decade, work on giant clam culture in Thailand has just started a few years ago. The Department of Fisheries, Thailand, started a giant clam research project attempting to produce juveniles from hatchery to be used primarily for restocking the depleted reefs. One of the sites designated for restocking and conservation is Koh Tao, a small island located about 70 km east off Chumporn in the Gulf of Thailand, Suratthani Province (Fig. 1). The island is considered as one of the best marine tourist places in the Gulf, with clear and clean water excellent for snorkelling and

Figure 1. Location of Koh Tao, the Gulf of Thailand. Ocean nursery marked with an ★.



diving activities. It used to have abundant giant clam stocks, but overexploitation has brought the natural population of *T. squamosa* close to extinction. This study was aimed at producing giant clam seed from the hatchery to be used for restocking purposes, as well as studying the appropriate ocean nursery technique to mass culture juveniles to a size suitable for restocking on reef areas.

MATERIALS AND METHODS

Hatchery Culture

Prachuap Khiri Khan Mollusc Hatchery is located in an area where sea water is highly turbid. Several steps of water treatment are necessary to prepare sea water to be used in the hatchery and nursery, as shown by the diagram in Fig. 2.

Adult clams were collected during 1990-93 by diving in Prachuap Khiri Khan and Chumporn, the Gulf of Thailand. Broodstock was maintained in an outdoor, round-cornered, concrete raceway, 2 m width, 10 m length, 0.75 m depth, about 15 tons capacity, provided with running sand-filtered sea water at a flow rate of 8-10 litre per minute, and an electric-driven paddle wheel for water circulation. The raceway was covered with plastic shade-cloth for 50 % sunlight reduction. Herbivorous fish and gastropods were used for controlling growth of benthic diatoms and algae in the raceway. Sediments, faeces, and debris were siphoned out daily. A total of 69 clams, 20-38 cm shell length, were used for breeding.

Spawning was induced by short-period desiccation combined with sea water change in order not to stress the clams too much. It is a gentle method, saving the broodstock. The clams were taken out of the holding tank, sea water drained, and the tank cleaned. The clams were cleaned and fouling organisms removed from their shells. Clams were out of water for about 15-30 minutes, then put back into the tank, refilled with fresh filtered sea water. If spawning did not occur after about 1 hour, the water was drained, leaving the clams desiccated for another 15-30 minutes, then refilled. When spawning occurred, sperms and eggs were collected separately and mixed later for fertilisation.

Fertilised eggs were suspended in 500-1,000 litre fibreglass rearing tanks filled with 1 µm filtered & UV sterilised sea water, at a density of 5-20 eggs ml^{-1}. Gentle aeration was provided. Cultures were maintained in sea water, 33-35 ‰ S, range of temperature 27-29 °C.

When the D-shaped veliger larval stage was accomplished, sea water was changed, us-

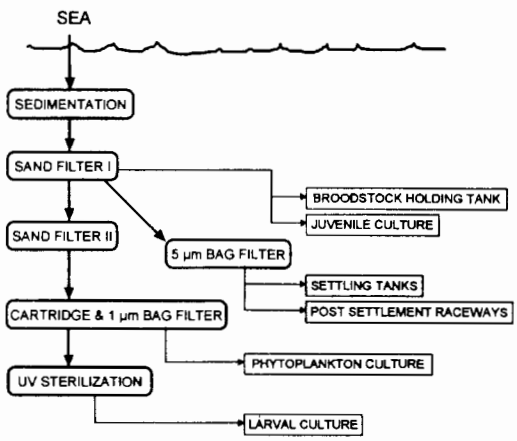


Figure 2. Seawater system applied at Prachuap Khiri Khan Mollusc Hatchery.

ing a siphon hose and nylon screen sieves to retain the larvae. The rearing tanks were cleaned and replenished with fresh filtered and UV sterilised sea water. The veligers were reared at a density of 5-20 larvae ml^{-1}, feeding with *Isochrysis galbana* and *Chaetoceros calcitrans*, 1-2 x 10^4 cells ml^{-1}. Further sea water change was performed every second day, with 100 % replenishment. Faeces of adult clams constituted the source of zooxanthellae. Faeces was collected, sieved (Fig. 3), and put in the rearing tanks on day 4 or 5. By that time the veligers were transferred to 50 % shaded, outdoor tanks. Settlement occurred in either 1,000 litre rectangular fibreglass tanks or 3-15 tons concrete raceways filled with 5 µm filtered sea water. No particular substrate was provided for settlement.

After settlement, a flow-through system was applied to the rearing tanks, using filtered sea water with a flow rate of about 8-10 litres per minute. Juvenile rabbitfish (*Siganus* spp.) were collected from the sea and used to control growth of benthic diatoms and filamentous algae in the rearing tanks. Juvenile clams smaller than 1.5 cm shell length, reared in concrete raceways were left attaching directly on the cement surface without special substrate. Square cement plates, about 30 x 30 cm, were provided as substrates for juveniles reared in fibreglass tanks. Perforated plastic trays lined with a layer of coral rubbles were used for holding the juveniles of the sizes larger than 1.5 cm shell length.

Ocean Nursery

Ocean nursery trials were performed in a coral reef area at the south west side of Koh Tao (Fig. 1), in front of the Fisheries Patrol station. The area had coarse sand and coral rubble substrata among patchy reefs in 3-5 m depth of water (Fig. 4).

Juveniles, 1.5-4.5 cm shell length, were dislodged from the substratum. Their byssus was cut with a sharp knife one day prior to transportation. They were transported in Styrofoam boxes using cotton cloth moistened with sea water for lining and covering. The juveniles were put between moistened cloth, arranged into several layers containing 100-200 juveniles per layer and 5-10 layers per box. Small packs of ice were

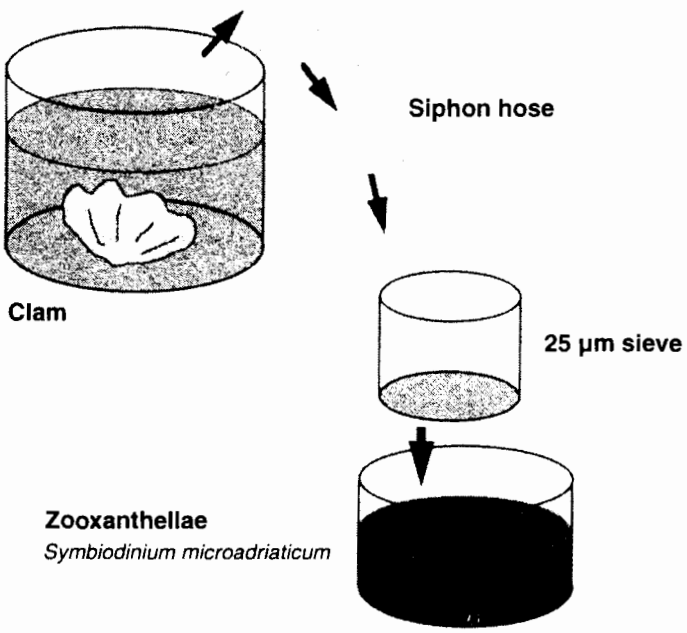


Figure 3. Method for collecting symbiotic dinoflagellate zooxanthellae from adult clams.

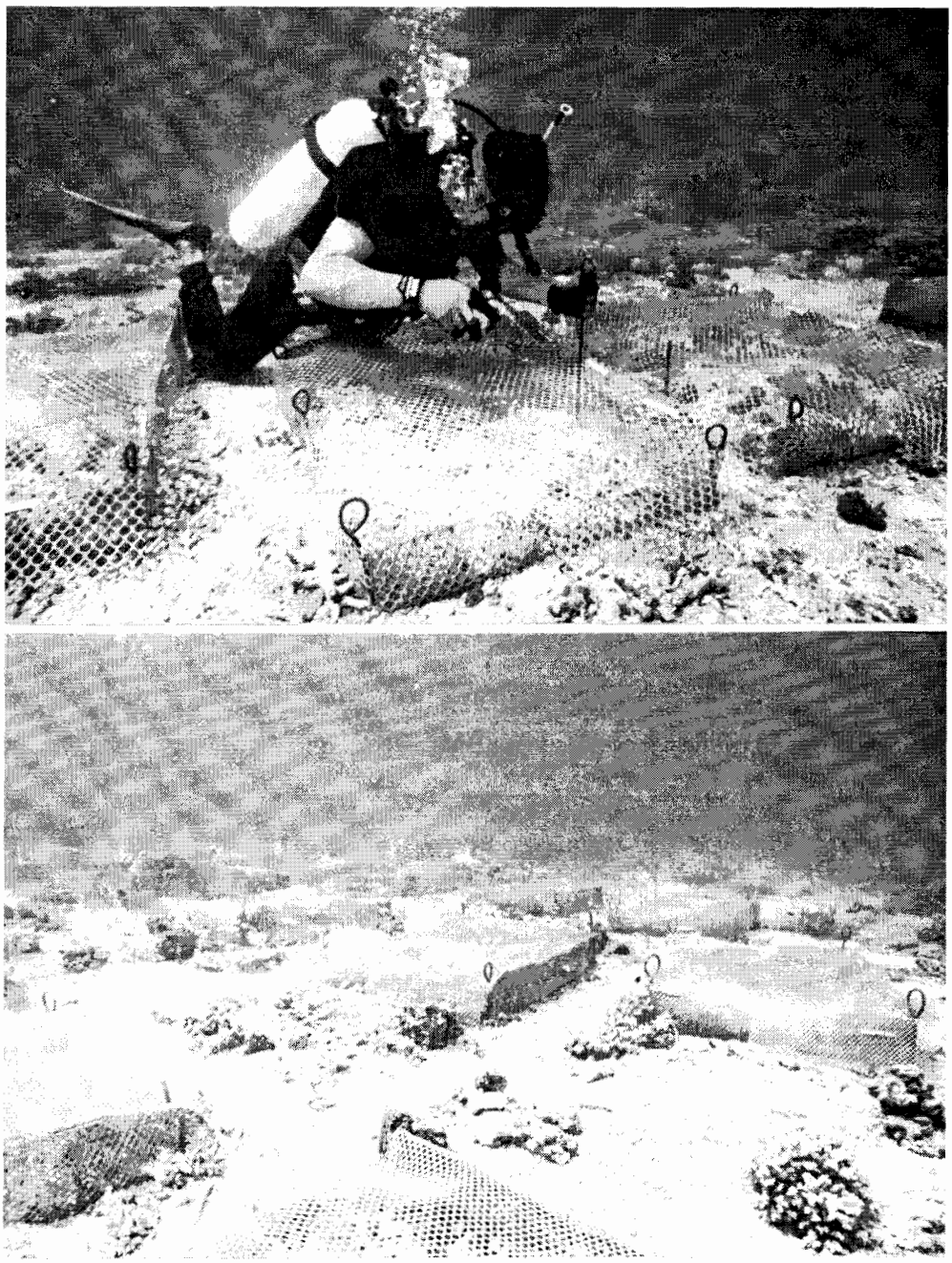


Figure 4. Ocean nursery cages in Koh Tao, the Gulf of Thailand.

Table 1 Development and survival of the hatchery-cultured giant clam *Tridacna squamosa*.

Age	Stage & Size	% Survival
-	Fertilised eggs (100 -105 μm)	-
26-28 hours	D-shaped veligers (150 ± 2.72 μm)	71.2 %
Day 6 - 10	Pediveligers (190 - 210 μm)	64.3 % from D-shaped
Day 8 - 14	Metamorphosis & Symbiosis	~ 35 - 70 %
2.5 months	Juveniles (0.5 - 4.3 mm)	16.3 % from pediveliger or 6.9 % from D-shaped

put in the box to prevent temperature from increasing.

Nursery cages were made of plastic netlon, 1-2.5 cm mesh size, formed into box-shape with cover, 70 cm width, 100 cm length, 15 cm depth. The cages were fixed onto the sea floor with metal pins, and lined with a layer of coral rubble as substratum for juvenile attachment. This type of cage was selected in consideration of simplicity of fixing, easiness to remove after termination of the nursery period, and minimal disturbance of the underwater scenery. Clams were stocked in the cages at densities of 200-300 juveniles m^{-2}. The cages were inspected by SCUBA divers once a month and survival and growth recorded.

RESULTS

Spawning

In the outdoor raceway clams spawned 5 times during the period from April 1994 to April 1996. But, the clams only responded to spawning induction one time in August 1993, when they began spawning within 2-3 hours after induction. The other four events were considered as spontaneous spawning because the clams spawned later than 1 day after induction.

Development, growth & survival of the larvae and juveniles

Fertilised eggs developed into the D-shaped veliger stage after 26-28 hrs in 33-35 ‰ S, 27-29 °C. Survival was 71.2 % on the average. The second day, veligers began feeding on unicellular algae reflected in brownish colour of the stomach. Experiments with feeding and non-feeding showed that veligers fed with *Isochrysis* and *Chaetoceros* showed better growth than the unfed ones. The larvae developed into pediveligers on day 6-7. Settlement and metamorphosis was reached by day 8-10, and completed within the age of two weeks. Symbiosis was accomplished shortly after. Development of larvae and juveniles of *T. squamosa* is summarised in Tab. 1. Growth of the hatchery-cultured juveniles is shown in Fig. 5.

High mortality occurred during metamorphosis, and subsequently in the small juveniles prior to the size of 5 mm shell length. During this outdoor nursery period, the juveniles suffered from overgrowing of benthic diatoms and filamentous algae in the rearing tanks. The rabbitfish, *Siganus* spp., was found to be effective in reducing this problem, as their feeding habit was harmless for the juvenile clams while the grazing gastropods such as *Trochus, Tectus,* and *Turbo* spp. caused damage to the small juveniles. Growth of the juveniles varied from batch to batch, as well as from tank to tank, mainly depending on management.

Seed Production

Five batches of hatchery-produced giant clam juveniles were successfully cultured (see Fig. 6). The production of the 3-months-old seeds (0.4 to 1 cm size) varied from hundred to a few hundred thousands per batch. Production and growth of the juveniles in each batch are shown in Tab. 2.

Ocean Nursery

A total of 40,000 juveniles, 1.5-4.5 cm length, were transferred to the ocean nursery on 5 occasions from March to June 1996 (Tab. 3). Results until September 1996 showed that juveniles could adapt well to the natural environment in the nursery area. The transplanted juveniles showed no mortality during the first 24 hours in nursery cages, with one exception in June 1996 when 20,000 individuals were transplanted. They had approximately 10 % mortality.

Most of the transplanted juveniles displayed increments of about 0.5-1 cm per month during the first three months. Survival of the juveniles depended mainly upon the condition of the nursery cages. Juveniles attained more than 90 % survival in the well fixed cages, while there was 100 % mortality in some cages that were flipped upside down by storm or wave action. High mortality also occurred in the damaged or opened cages, where predators could get inside. Mortality caused by predators was easily recognised by broken shells of the clams. Overall survival of the transplanted juveniles until September 1996 was estimated at 50 %.

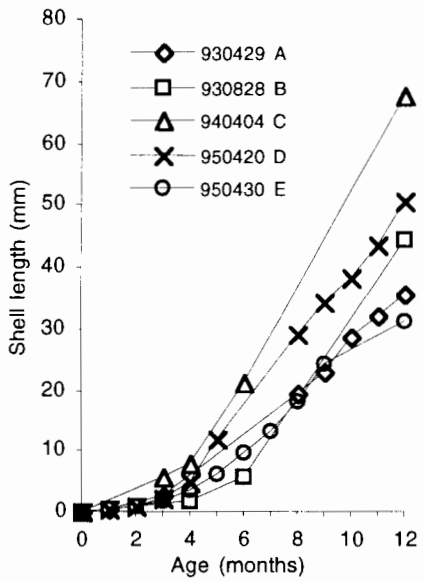


Figure 5. Growth (mm length) of the hatchery-reared juvenile giant clam *Tridacna squamosa*.

Table 2. Hatchery seed production of the giant clam *Tridacna squamosa* and growth of the juveniles in different culture batches. *: Growth was estimated by shell length increments from the age of 3 months to 1 year.

Batch No.	No. of juv. 3 months	Mean size of 1 year old (mm)	Growth * (mm/month)
930429 A	< 100	35.5 ± 4.3	3.8
930828 B	~ 140,000	44.5 ± 8.4	4.4
940404 C	< 30,000	67.8 ± 7.8	7.0
950420 D	~ 300,000	50.4 ± 7.4	5.0
950430 E	< 20,000	31.2 ± 4.2	2.9

Table 3. Juveniles transferred into ocean nursery at Koh Tao. Shell length in cm; Mortality as % after 24 h.

Month	No. of juveniles	Age (months)	Shell length	Mortality
March 1996	2,000	11	1.5-4.5	0
April 1996	5,000	12	2.5-4.5	< 0.1
May 1996 (I)	3,000	12	3.0-4.5	0
May 1996 (II)	10,000	13	2.5-5.0	< 0.1
June 1996	20,000	14	2.5-5.0	9.5

DISCUSSION

The clams of the present study produced viable gametes yielding several batches of the offspring. A broodstock of *Tridacna squamosa* can be maintained in captivity for many years. This advantage should be implemented in further planning of selective breeding programmes to produce seeds for restocking.

Growth of the hatchery-cultured juvenile clams was observed to display great variation, not only among different batches but also within the same batch. This variation may be due to the quality of the offspring itself or depended on culture techniques such as stocking or rearing densities and management.

The present ocean nursery technique has been successful when employed in the study area, but there is room for improvements.

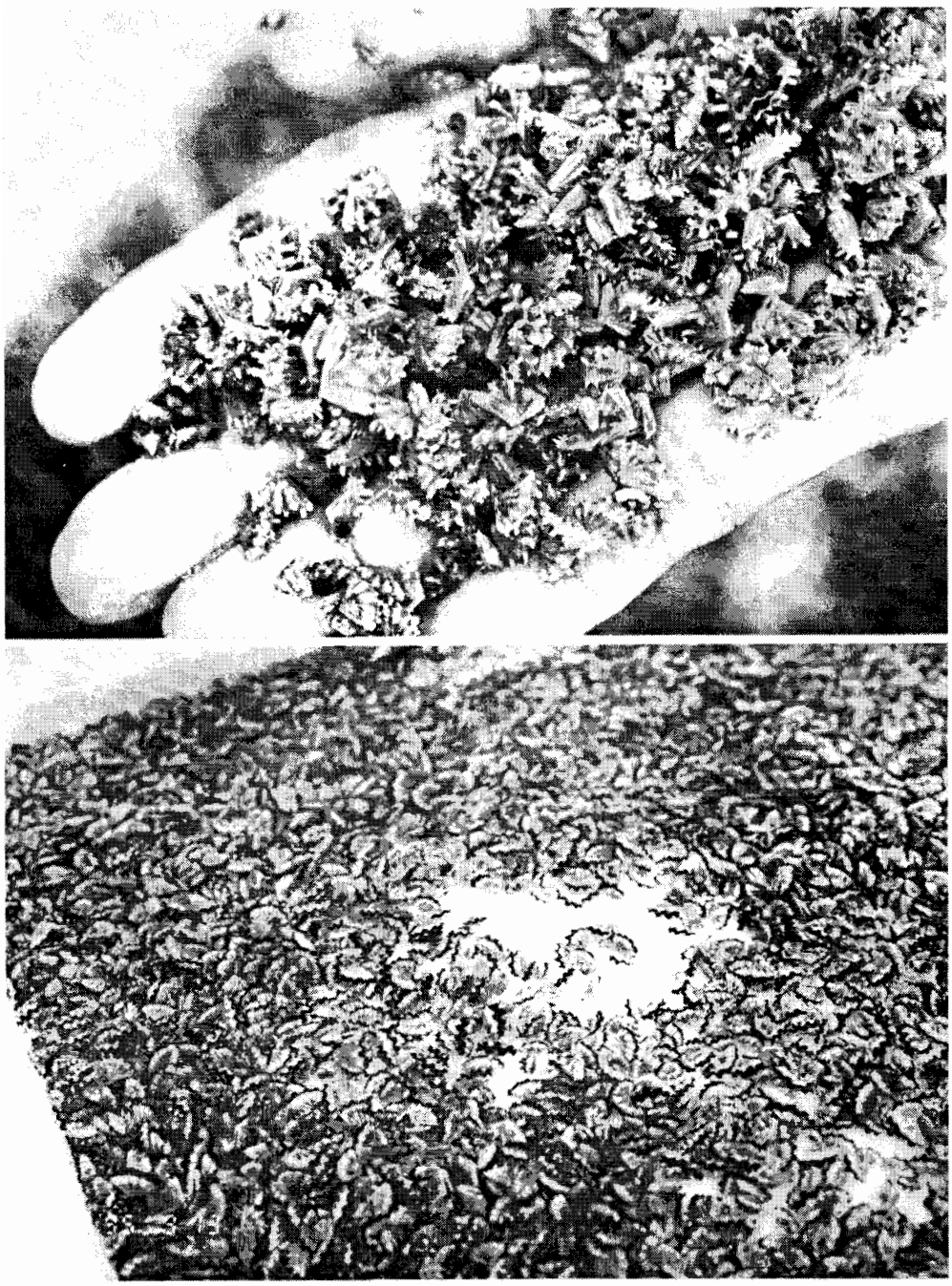


Figure 6. *Tridacna squamosa* produced in hatchery. Juveniles 4 months old.

Growth of juveniles remains to be studied in nature, and the most suitable size for restocking should be identified.

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