Shell morphology and culture of *Tridacna squamosa* larvae (Bivalvia: Tridacnidae).

Norainy Mohd. Husin, Zulfigar Yasin & Aileen Shau-Hwai Tan

Husin, N.M., Z. Yasin & A. S.-H. Tan. 2001. Shell morphology and culture of *Tridacna squamosa* larvae (Bivalvia: Tridacnidae). - Phuket Marine Biological Center Special Publication 25(1): 169-172.

The shell morphology of *Tridacna squamosa* larvae is described and illustrated based on SEM pictures. The increase in total shell length and shell width was more distinct compared to the length of hinge teeth. Prodissoconch 1 and prodissoconch 2 could clearly be differentiated. Other important morphological characteristics are discussed.

Norainy Mohd. Husin, Zulfigar Yasin & Aileen Tan Shau-Hwai. Centre for Marine and Coastal Studies, University Science of Malaysia, 11800 Penang, Malaysia.
E-mail Zulfigar Yasin: zulfigar@usm.my

INTRODUCTION

In Indo-Pacific tridacnid bivalves face potential extinction (Gwyther & Munro 1981; Fitt & Trench 1981). *Tridacna gigas* and *Tridacna derasa* are the most exploited species (Hardy & Hardy 1969; Heslinga *et al.* 1984).

In Malaysia, distribution of giant clams has been studied by on several occasions (Tan 1993, 1997; Tan & Zulfigar 1997). Adib *et al.* (1993) worked in Pulau Redang, Pulau Kapas and Pulau Perhentian in East Coast Penisular Malaysia where only *Tridacna maxima* and *T. crocea* were common while *T. squamosa* and *T. derasa* were endangered. *Tridacna gigas* was not recorded from the sites. In Pulau Tioman, *T. maxima* were the most abundant species followed by *T. squamosa* and *T. crocea* (Tan *et al.* 1998). Information on larvae is very limited compared to studies on distribution adult giant clams. We have selected *T. squamosa* for larval studies because it is quite endangered and a good candidate for aquaculture (Tan *et al.* 1998).

MATERIALS AND METHODS

Adult *T. squamosa* were collected at Pulau Besar at a depth of 8 m. They were transported in sea water to the nearby island Pulau Pemanggil, which is part of the Johore archipelago situated at the southern Peninsular Malaysia (Figure 1).

The adult clams were acclimatized 24 hrs on the reef flat in front of the Pemanggil Island. The clams were cleaned and put into aerated 200-l fiberglass tanks with 5 μm filtered sea water. Induced spawning followed Gibbon & Castagna (1984) using 2 ml 2.0 mM serotonin injected through the inhalant siphon in the late evening. Sperms and eggs were collected in separate containers. Only a small part of the sperms were used to fertilize the eggs. Concentration of 10 ppm streptomycin was mixed into each tank to avoid bacterial growth. Water was changed 24 hours after fertilization and every alternate day thereafter. Streptomycin was added every time water was changed and the tanks were cleaned.

Larvae were reared in natural light from 7 am to 7 pm and under fluorescent light from 7 pm to 7 am. The density of larvae was 3/ml as recommended by Fitt *et al.* (1984). Salinity was 36 ‰ and temperature ranged from 27-29 °C.

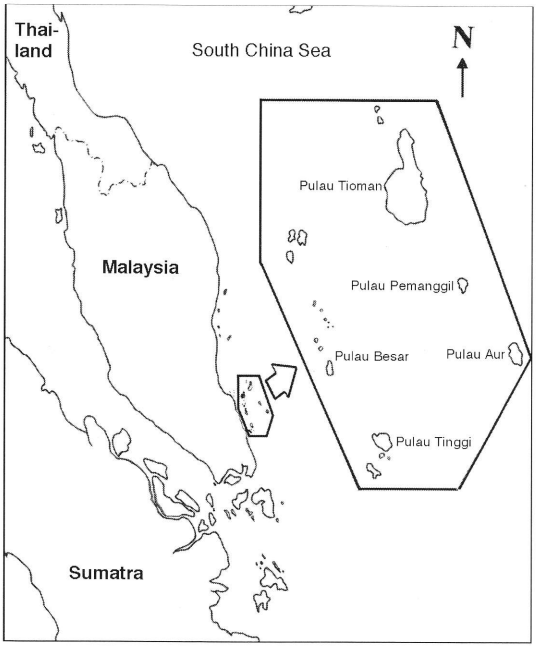

Figure 1. Research area: Pulau Besar and Pulau Pemanggil, southern Malaysia.

After 24 hours the digestion system had developed and larvae were fed phytoplankton *Chaetoceros calcitrans*. Initially 100 ml phytoplankton (5 x 10^3 cells/ml) was given. From third day onwards 300 ml phytoplankton was given daily at the same concentration. During each water change, all larvae were collected in 2-l containers. Larvae were counted(5 replications) using a zigzag counter under light microscope, and 20 larvae were removed for study of morphology. The larvae were fixed in Carriker's fixative (1% formalin, 0.05% $NaHCO_3$ and 10% sugar in sea water). The ligament was dissolved and the valves separated in 0.05% sodium hypochlorite solution (Clorox). Larval shells were washed with distilled water and prepared for scanning electron microscope (SEM) according to LaBarbera (1975). The shells were vacuum coated with gold using Sputter Coater Polaron SC-5 15. SEM observations and photographs were done using a Leica Cambridge S-360 scanning electron microscope.

RESULTS

Larval culture and growth. - No mortality was observed in injected clams. Five to ten minutes after injection of serotonin, muscle convulsions were observed. Ten minutes after injection, sperms were released through the exhalent siphon. The release of sperms was milky, which made the water cloudy. The release of sperms continue for 10 min. Eggs were released after the release of sperms had stopped 20 min after the injection. Intervals from one release of gametes to the next were getting longer and stopped after 40 to 50 min. Each clam released 12-46 million eggs. The diameter of *Tridacna squamosa* eggs ranged from 85 to 105 μm (mean 94 ± 5.2 μm) Larval samples were taken from day-1 to day-5. The larvae were competent at day-6. Figure 2 shows growth of the larvae. Increase in total length from day-1 to day-5 was more

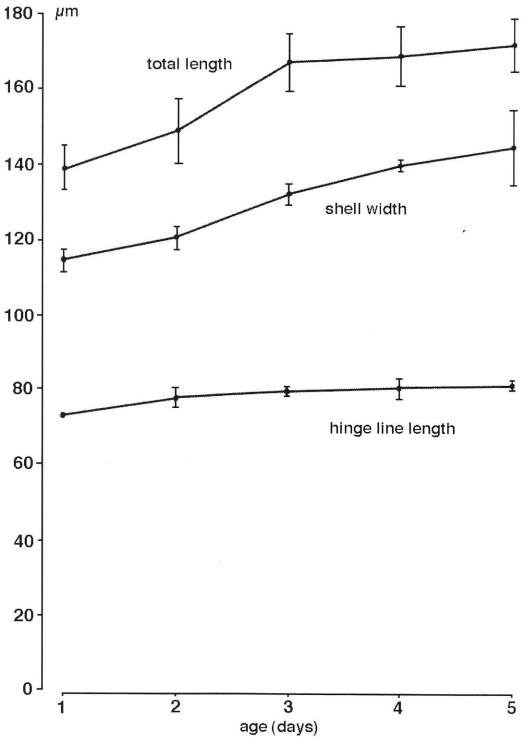

Figure 2. Total length, width, and hinge line of *Tridacna squamosa* larvae from day-1 to day-5.

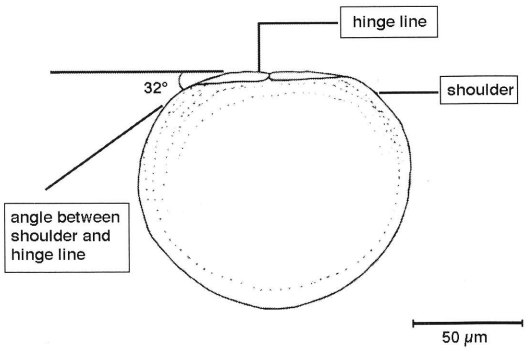

Figure 3. One day old larva of *Tridacna squamosa* showing a wide angle between the shoulder and hinge line.

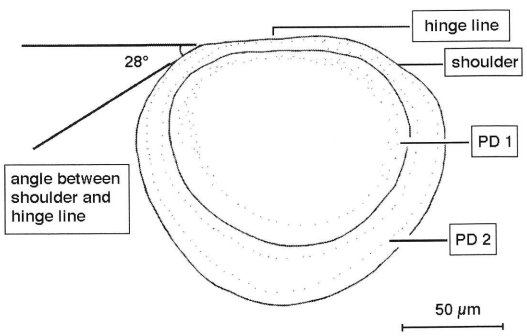

Figure 4. Four days old larva of *Tridacna squamosa* showing a narrow angle between the shoulder and hinge line. PD1 is clearly distinguished from PD 2.

pronounced than increase in shell width and length of hinge line. The hinge line did not show distinct increment from day 1 to day 5.

Larval morphology. - The right and left valves are equal and the shell shape broadly rounded. D-hinge larvae, also known as prodissoconch 1 (PD 1), developed 24 hrs after fertilization. The shoulder became less steep because it grew towards the hinge line and the angle between shoulder and hinge line became smaller. (Figs. 3 and 4). SEM studies showed that 4 days old larvae had a PD 1 with smooth surface compared to PD 2. Growth lines were very distinct on PD 2 compared to PD 1. Otherwise, no obvious difference could be seen from day-1 to day-5 (Fig. 4). The provinculum area is almost the same for larvae of different age. Hinge teeth line is straight with a small dent in the middle and many big and small dentitions. The width of these dentitions is approximately 1 µm. The dentitions on day-1 larvae are smaller compared to dentitions of day-3 larvae. These dentitions are rectangular and at a right angle to the hinge line, or slightly slanted.

DISCUSSION

Yuhana (1999) found sperm release 1.5 minutes after injection of 2 ml 2 mM serotonin. Sperm release continued for 13 minutes and egg release for 42 minutes. In our study the timing was slightly different; the sperm was released after 5-10 minutes. Braley (1985) found that *T. squamosa* started to release sperm 3 min after injection but no eggs were released

Our studies on shell morphology of *T. squamosa* larvae agrees with LaBarbera (1975). PD 1 was seen on day-2. The very large size of PD 1 and relatively small size at time of settling indicates that this species normally has a very short free swimming existence (LaBarbera, 1975).

ACKNOWLEDGMENTS

We would like to thank University Science of Malaysia Reef Research Group for their support and dedication. We especially appreciate the help and cooperation from Pak Cik Mazian's family and University Science Malaysia SEM team. This study was supported by the Johore Government.

REFERENCES

Adib,H., A.Pauzi & Nordin, 1993. Pembiakan aruhan kimia *Tridacna squamosa* (Bivalvia: Tridacnidae) di Taman Laut Pulau Redang, Terengganu, Malaysia. MFRDMD/ SEAFDEC Malaysia Department of Fisheries, 14 p.

Braley,R.D. 1992. (ed). The giant clam: hatchery and nursery culture manual. - ACIAR Monograph No. 15, 144 p.

Hardy.J.T. & S.A.Hardy, 1969. Ecology of Tridacna in Palau. - Pac. Sci., 23(4): 467-472.

Heslinga,G.A., F.E.Perron & O.Orak, 1984. Mass culture of giant clams (F. Tridacnidae) in Palau. - Aquaculture, 39: 197-215.

Fitt,W.K. & R.K.Trench, 1981. Spawning, development and acquisition of zooxanthellae by *Tridacna squamosa* (Mollusca: Bivalvia). - Biology Bulletin, 161: 213-235.

Fitt,W.K., C.R.Fisher & R.K.Trench, 1984. Larval biology of tridacnid clams. - Aquaculture, 39: 181-195.

Gibbon,M.C. & M.Castagna, 1984. Serotonin as an inducer of spawning in six bivalve species. - Aquaculture, 39: 34.

Gwyther,J. & J.L.Munro, 1981. Spawning induction and rearing of larvae of tridacnid clam (Bivalvia: Tridacnidae). - Aquaculture, 24: 197-215.

LaBarbera,M. 1975. Larval and post-larval development of the giant clams *Tridacna maxima* and *Tridacna squamosa* (Bivalvia: Tridacnidae). - Malacologia, 15(1): 69-79.

Tan,S.H. 1993. Aspek-aspek biologi dan perkembangan larva tiram tropika *Crassostrea belcheri* (Sowerby). Dissertation for Masters, School of Biological Sciences, University Science of Malaysia.

Tan,S.H. & Y. Zulfigar, 1998. The reproduction cycle of *Tridacna squamosa* and *Tridacna maxima* in Rengis Island (Tioman Island), Malaysia. - Phuket Marine Biological Center Special Publication, 18(1): 107-112.

Tan,S.H., Y.Zulfigar, S.Ibrahirm & Y.A.Aziz, 1998. Status of giant clams in Pulau Tioman, Malaysia. - Malayan Nature Journal 52(3 & 4): 205-216.

Yuhana,U.L.A. 1999. Perkembangan awal kerang gergasi, *Tridacna squamosa* dan *Hippopus hippopus*. Final year dissertation for Bachelor Degree, School of Biological Sciences, University Science of Malaysia.