

## LARVAL DEVELOPMENT AND SURVIVAL RATE OF GIANT CLAM *TRIDACNA SQUAMOSA* REARED UNDER LABORATORY CONDITIONS

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### ABSTRACT

Culture of *Tridacna squamosa* was initiated with a view to restock depleted populations of giant clam in Indonesia. Larvae of *T. squamosa* were obtained through induced spawning of the broodstocks by injection of serotonin. The pelagic larvae spent 16 days as trochophore, veliger, and pediveliger larvae before metamorphosing into juveniles. Unfortunately, the survival rate of the larvae reared under laboratory condition was very low, probably because of poor water quality.

### INTRODUCTION

*Tridacna squamosa* used to be common in Karimunjawa Island, Indonesia. But, the population of this giant clam species is depleted because of over-exploitation by man. Through broodstock conditioning and induced spawning we wanted to produce *T. squamosa* which could be released in nature to replenish the stock.

The morphology of larvae of giant clam changes considerably as they develop. Each stage has its specific behaviour and physiological needs (Raven 1958; Jameson 1976) which implies much experimental work in order to avoid heavy mortality. Therefore, experiments on production of juveniles in the hatchery and by ocean nursery are continued in Karimunjawa Island.

This paper reports our first results on development of *T. squamosa* larvae and their survival under laboratory condition.

### MATERIALS AND METHODS

Broodstock of *Tridacna squamosa* was induced to spawn following injection of serotonin. After fertilisation, the developing larvae were kept in aquarium with 50% shading. The stocking density of was 10 larvae l<sup>-1</sup>. On day 2, the larvae were fed with *Chlorella* sp. to a concentration of 10<sup>3</sup>-10<sup>5</sup> cells ml<sup>-1</sup> for 2 days. Zooxanthellae from adult *T. squamosa* were introduced at day 4, 6, and 8 at the same concentration as *Chlorella*. The

zooxanthellae were collected by scraping a small piece of excised adult mantle tissue to release them into suspension and then rinsed through a 25 µm mesh.

The observations of larval development started after fertilisation. Samples of the larvae in every stage were measured with a micrometer eyepiece. Shell length was measured along the longest axis and expressed as mean ± standard deviation.

The survival rate (SR) of the larvae was calculated for every stage as follows:  $SR = N_t/N_0 \cdot 100\%$ , where  $N_t$  = number of individuals at time  $t$ , and  $N_0$  = number of individuals at  $t_0$ .

### RESULTS AND DISCUSSION

The developmental stages of *Tridacna squamosa* did not differ from those reported for other tridacnid species (Jameson 1976; Alcazar 1978). The development of *T. squamosa* larvae in the present study is compared to *T. crocea*, *T. maxima*, and *T. derasa* in Tab. 1. Growth in length for different stages of *T. squamosa* and their survival rate are shown in Tab. 2.

#### Larval development

The trochophore stage was reached in 22 hours. This is slower than for other species (Tab. 1). The average length of the trochophore was  $109.57 \pm 3.59$  µm. The trocho-

Table 1. The development of the *T. squamosa* larvae (the present study) and other species according to \*) Jameson 1976 and \*\*) Alcazar 1978.

Stage	Time (h)			
	<i>Tridacna squamosa</i>	<i>Tridacna crocea</i> *	<i>Tridacna maxima</i> *	<i>Tridacna derasa</i> **
Trochopore	22	15	16	17
Veliger	2	20	20	3.5
Veliger	6 (6 - 9)	-	6 (2 - 6)	-
Pediveliger	10 (10 - 11)	10 (9 - 17)	9 (8 - 19)	5
Metamorphosis	12 (12 - 15)	13 (12 - 18)	12 (11 - 20)	7
Juvenile	16	19	21	25

Table 2. Length of developing stages and survival rate of *Tridacna Squamosa*.

Stage	Time (days)	Number	Length ( $\mu\text{m}$ )	Number	Survival rate (%)
Trochopore	22 hours - 2	7	109.57 $\pm$ 3.59	9	61.75 $\pm$ 14.58
Veliger	3 - 5	10	137.85 $\pm$ 9.14	10	53.20 $\pm$ 11.69
Veliger	6 - 9	12	165.43 $\pm$ 11.01	18	7.10 $\pm$ 3.57
Pediveliger	10 - 11	8	181.50 $\pm$ 6.30	8	2.06 $\pm$ 0.25
Metamorphosis	12 - 15	14	212.93 $\pm$ 6.28	13	1.47 $\pm$ 0.33
Juvenile	16	5	218.00 $\pm$ 4.12	5	1.09 $\pm$ 0.06
Juvenile	18	5	228.00 $\pm$ 4.12	5	0.87 $\pm$ 0.01
Juvenile	20	3	240.00 $\pm$ 5.00	5	0.69 $\pm$ 0.12
Juvenile	24	5	263.00 $\pm$ 5.15	5	0.34 $\pm$ 0.06
Juvenile	27	3	271.67 $\pm$ 7.64	5	0.17 $\pm$ 0.07
Juvenile	31	5	284.00 $\pm$ 2.92	5	0.04 $\pm$ 0.007

phore swam with a spiral movement propelled by the beat of cilia. At day 2, the larvae had a length of  $137.85 \pm 9.14 \mu\text{m}$ .

At day 3, the trochophore changed into the veliger stage, and prodissoconch I and II developed (days 3 and 4), characterised by the D-shape of the shell (Bayne 1976). Jameson (1976) named this stage the D-type veliger. They swam actively; four apical flagella present at the velum, foot and shell starting to develop. The intestine developed and the straight-hinge veliger fed actively on *Chlorella* sp. The green colour could clearly be seen in the digestive system. Food was kept in motion by cilia in the stomach. At all times the digestive gland was either dark brown or golden (Jameson 1976). Straight-hinge veligers, as described by LaBarbera (1974, 1975), have their own characteristic arrangement of retractor muscles in which *T. squamosa* is identical to *T. crocea*.

At day 6, they measured  $165.43 \pm 11.01 \mu\text{m}$ . By day 10, pediveligers developed. The shell began to lose its definite straight-hinge appearance because of the slight growth of

the umbo and the foot began to probe out. Cilia were present at the tip of the foot to the byssus gland. The digestive system developed. Pediveligers alternately swam about and crawled on the bottom. Crawling was accomplished by attaching the tip of the foot to the substratum and then pulling the rest of the body forward. The foot was also used for testing and orienting the clam relative to the substratum. The average size of a pediveliger was  $181.50 \pm 6.30 \mu\text{m}$ .

#### *Settlement, metamorphosis and juvenile development*

Settlement and metamorphosis are gradual processes. The appearance of an active foot signifies that the time for settlement is near and that metamorphosis is beginning. Pediveliger, with a functional velum and foot, alternately swam about and crawled on the bottom. After a period of alternately swimming, crawling, and making temporary attachment to the substratum, the pediveliger became increasingly sedentary in its habits. The velum gradually degenerated, the clam stuck to the substratum while func-

Tabel 3. Comparisons of lengths of developing stages of *Tridacna squamosa*, *Tridacna crocea*, and *Tridacna maxima* according to the present study, \* ) Dwiono 1994, and \*\* ) Jameson 1976.

Stage	Length ( $\mu\text{m}$ )		
	<i>Tridacna squamosa</i> *	<i>Tridacna crocea</i> **	<i>Tridacna maxima</i> **
Trochopore	104.50 $\pm$ 24.4	-	-
Veliger	137.70 $\pm$ 13.4	155.00 $\pm$ 4.10	168.00 $\pm$ 4.50
Pediveliger	145.60 $\pm$ 15.5	173.00 $\pm$ 2.70	192.10 $\pm$ 9.10
Metamorphosis	151.20 $\pm$ 15.0	168.00 $\pm$ 2.70	195.00 $\pm$ 8.70
Juvenile	200.40 $\pm$ 12.6	182.00 $\pm$ 5.70	203.00 $\pm$ 2.30

tional gills developed. The metamorphosis started at day 12 at a length of  $212.93 \pm 6.28 \mu\text{m}$ . The foot and velum disappeared and labial palps were present and active. At day 13, the juveniles achieved a length of  $284.00 \pm 2.92 \mu\text{m}$ . About 50 percent of the clams from the culture had completed metamorphosis by day 15.

Juveniles (day 16) demonstrated definite thigmotaxic reactions, and after metamorphosis they continued to crawl until they found a suitable place to attach the byssus, preferably the corners of the tank. Apparently, according to Jameson (1976), maximum protection governs the choice of site of permanent settlement. At day 16, the average length of juveniles was  $218.00 \pm 4.12 \mu\text{m}$ . Zooxanthellae were present in stomach at day 16, and in the mantle of juveniles at day 27. Actually, the clams acquire zooxanthellae soon after metamorphosis (Fitt *et al.* 1984).

Length measurements of developing stages of three species of Tridacnidae are compared in Tab. 3. After the pediveliger stage, the size of *T. squamosa* (Tab. 2) was bigger than found in other species (Tab. 3). It seemed that the addition of zooxanthellae supplied nutrients for the clam.

#### Survival rate

Generally, the survival rate of *T. squamosa* was very low. Although, in the beginning of the study, over 60 % of the trochophores survived, the transition between veliger stages (from day 2-3 to D-type veliger day 6) resulted in high mortality (over 90 %, Tab. 2). In comparison, Fitt *et al.* (1984) concluded

that high mortality occurred at the transition from pediveliger to juvenile. The difference is most likely due to changes in the mode of acquiring nutrition. Braley (1992) suggested that the zooxanthellae suspension should be fed to larvae on day 3 post-fertilisation, and then again on days 5, 7, and 9. It can be observed whether settled larvae, or early juveniles, take up the zooxanthellae because of the golden-brown spheres in the gut or developing mantle. Zooxanthellae were fed as recommended but a low survival rate ( $7.10 \pm 3.57 \%$ ) still occurred in this study.

After the pediveliger, survival rate decreased from 2.06 % down to 1.47 % at the metamorphosis stage. The survival rates of juveniles at day 16, 18, 20, 24, and 27 were 1.09, 0.87, 0.69, 0.34, and 0.17 % respectively. At the end of the observation (day 31) there were only 130 juveniles left out of 297,000 trochophores (0.04 %).

#### ACKNOWLEDGEMENTS

We are grateful to DANIDA/TMMP for funds which made the present study possible.

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