Redescription of Larval Development in Cultured Pearl Oyster *Pinctada maxima*

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Abstract

Larval development plays a major role in efficient pearl culture. The cultured larvae will be later used for recipient and donor oysters in cultured pearl production. Larval developmental stage of silver lip pearl oyster Pinctada maxima has been reported by several studies. Those studies used female and male oyster parents directly taken from natural habitat.This study aimed to redescribe larval development of P. maxima from commercial pearl oyster culture farm in Indonesia. Larval development of this species whose parents are originated from selected groups in the pearl culture farm has not been reported yet, thus it is necessary to be described. This species undergoes specific larval developmental stage. The larvae were observed under microscope, and then the average shell length (SL) and shell height (SH) were measured. D-shaped veliger larva (77.4±0.3 µm SL; 65.4±1.1 µm SH) appeared 20 h after fertilization. Tenday-old larva (156.2±2.8 µm SL; 149.5±5.6 µm SH) had developed umbo region so it was called umbonal larva. Umbonal larva then developed further into plantigrade larva (411.3±9.8 µm SL; 380.5±6.9 µm SH) in 25 days after fertilization. Developmental stage and larval sizein P. maxima is similar with those observed in P. fucata and P. margaritifera.

Keywords: *growth, larva, plantigrade, shell, umbo, veliger*

Introduction

Pinctada maxima is the biggest pearl oyster species in genus *Pinctada* (Wada and Temkin, 2008). *P. maxima* is naturally distributed in region around northern Australia and Southeast Asia, including Indonesia. This species produces the most lustrous and expensive pearls ever produced by pearl oyster.The pearl is large with various colors; i.e.white, yellow, greenish and golden. Indonesia has been *P. Maxima* cultured pearl producing country with the biggest volume reaching almost 4 tons and contributes as much as 40% to the total pearl production (Southgate *et al*., 2008).

Pearl oyster has dispersed larval phase in its life cycle. Fertilized egg needs 12 until 24 days to fully develop (Rose and Baker, 1994). Larval developmental of *P. Margaritifera* has been reported by Alagarswami *et al*. (1989) and Doroudi and Southgate (2003), while Alagarswami *et al*. (1983) and CMFRI (1991) reported of *P. fucata*. Rose and Baker (1994) described larval developmental stage of *P. maxima* in western Australia. Larval development of *P. maxima* in Indonesia has also been previously reported by Winanto (2009) and Hamzah *et al*. (2017). Those studies used female and male oyster parents directly taken from natural habitat which were induced to spawn in the laboratory. Larval development of this species whose parents are originated from selected groups in the pearl culture farm has not been reported yet, thus it is necessary to be described. Besides, larva is the initial step in producing pearl oyster broodstock which then becomes one of the factors determining success in pearl culture industry (Southgate, 2008). This research aimed to redescribe larval developmental stage of *P. maxima* from commercial pearl oyster culture farm in Indonesia.

Materials and Methods

Larvae collection was done in pearl oyster culture farm in Kupang, East Nusa Tenggara, Indonesia in 2016. *P. maxima* larvae with different age after fertilization were taken separatedly from 1 ml water inside the tanks. The age of the larvae were 20 h, four-day-old, ten-day-old, 17-day-old, and 25-day-old after fertilization. They were stored in

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different bottle samples and were centrifuged. Groups of larvae gathered in the bottom of the bottles were preserved with 600 µl RNAlater.

Observation and measurement of larval developmental

P. maxima larvae of 20 hour-old, 4 day-old, 10 day-old, 17 day-old, and 25day-old after fertilization were observed under microscope. The average shell length (anteroposterior measure; APM) and shell height (dorsoventral measure; DVM) were measured on every developmental age.

Results and Discussion

P. maxima larva at 20 h after fertilization was in D shape or also called as straight-hinged larva (Figure 1A.) with size of 77.4±0.3 µm shell length (SL) and 65.4±1.1 µm shell height (SH). This larva at this phase had possesed ciliated organ called velum to support movement and capture food particles. Larva with velum is called veliger with transparent shell. Embryonic shell (*prodissoconch I)* growth started at D-shaped larva and continued to grow in 4-day-old veliger larva (Figure 1B.). Four-day-old veliger larva had embryonic shell measured 84.5± 1.1µm SL and 79.7±0.06µm SH. Velum was seen to be more developed in larva with the age of 10 days after fertilization (Figure 1C.). Umbo region started to appear on day 10 so that the larva was in umbonal larval stage. Umbonal larva changed the shell shape from D-shaped into clam-shaped with equal right and left valve. The averagesize of umbonal larva was 156.2±2.8 µm SL and 149.5±5.6µm SH. Adult shell growth was observed in 17-day-old larva (Figure 1D.) with larger shell size measuring 234.9±3.1 µm SL and 213.8±4.4 µm SH. The shell shape of this larva began to change and represent adult shell of pearl oyster.Twenty five-day-old larva called plantigrade showed typical shell morphology of adult pearl oyster (Figure 1E.) and reached 411.3±9.8µm SL and 380.5±6.9µm SH. Plantigrade larva had developing gill filaments to replace the function of velum. Larval developmental stages is summarized in Table 1.

Developmental stage and size of *P. maxima* larva in this study was similar to the one reported by Rose and Baker (1994), Winanto (2009), and Hamzah *et al*. (2017). At 20 hours D-shaped larva formed, umbonal larva in 10 until 12 days and plantigrade larva appeared in 25 days after fertilization. Two other species of genus *Pinctada*, *P. margaritifera* (Alagarswami *et al*., 1989; Doroudi and Southgate, 2003) and *P. fucata* (Alagarswami *et al*., 1983; CMFRI, 1991) also have similar developmental stage reached at the same period with *P. maxima*.The size of *P. maxima* plantigrade larva in this study tended to be bigger compared to the report of same species by Rose and Baker (1994), Winanto (2009) as well as Hamzah *et al*. (2017), *P. margaritifera* (Alagarswami *et al*., 1989; Doroudi and Southgate, 2003) and *P. fucata* (Alagarswami *et al.,* 1983; CMFRI 1991). Developmental period and larval size of *P. fucata*, *P. margaritifera*, and *P. maxima*at each stage is similar, as stated by Alagarswami *et al*. (1989). D-shaped and umbonal larva of *P. maxima* in this study appeared at nearly the same time with same stages larva of *Pteria penguin*, 18-22 hours and 12 days respectively (Wassnig and Southgate, 2012).

Various factors are known to influence development of pearl oyster larva, including water temperature, availability of appropriate substrate for attachment, and food availability (CMFRI, 1991; Saucedo and Southgate, 2008; Winanto, 2009; Hamzah *et al*., 2017). Microalgae is common feed for larvae of fish, crustaceans, echinoderms, and molluscs (Hemaiswarya *et al*., 2011), including pearl oyster. Microalgae provides energy and specific nutrition for tissue synthesis required by developing pearl oyster larvae. Species from genus *Isochrysis*, *Pavlova*, and *Chaetoceros* are common microalgae used for bivalve larvae feed in hatcheries (Hemaiswarya *et al*., 2011). Mixture of at least two microalgae species are given as feed for pearl oyster larvae (Southgate, 2008). Similar type of microalgae feed might be the reason of similarity in larval developmental period and size from three pearl oyster species, *P. fucata*, *P. margaritifera*, and *P. maxima*.

Economic value improvement is one of breeding objectives. Growth rate is important factor to achieve the breeding objectives in aquaculture. Growth rate is usually measured by body length (Gjedrem, 1983). Parental line for breeding used in pearl oyster culture farm is carefully chosen based on beneficial traitswhich appear on the pearls. Certain larval stage duration might differ between selected pearl oysters in pearl oyster culture farm and individuals in natural habitat, even though the developmental period and larval size are similar. Further verification is necessary to be performed in order to confirm any longer or shorter larval stage durations.

Table 1. Timing of larval developmental stages of *Pinctada maxima* and the mean shell length and shell height (SL and SH, in µm) at each stage.

Stage	Timing	SL	SH
D-shaped	20 h	77.4 ± 0.3	65.4 ± 1.1
Early umbo	4 days	84.5 ± 1.1	79.7 ± 0.06
Umbo	10 days	156.2 ± 2.8	149.5 ± 5.6
Plantigrade	25 days	411.3 ± 9.8	380.5 ± 6.9

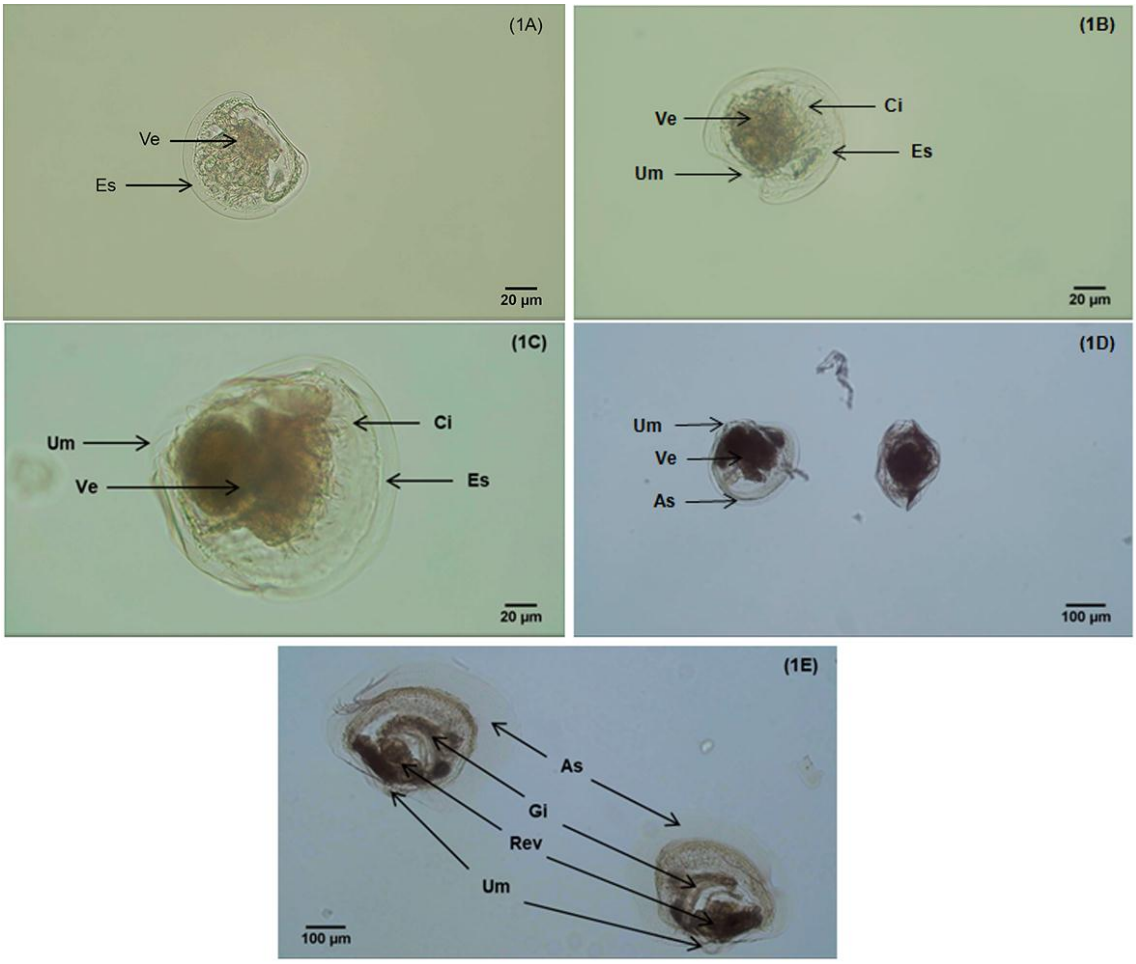

Figure 1. Larval developmental stage of pearl oyster *Pinctada maxima*: (A) D-shaped larva at 20 hours after fertilization; (B) 4-day-old larva showing growth of embryonic shell (*prodissoconch I*) and velum; (C) 10-day-old umbonal larva with distinct umbo region; (D) 17-day-old larva with initial development of adult shell; (E) plantigrade larva aged 25 day-old showing gill filaments with thin and transparent adult shell (*dissoconch*). Abbreviations: Ve, velum; Es, embryonic shell; Ci, cilia; Um, umbo; As, adult shell; Gi, gill filaments; Rev, reduced velum.

Conclusion

D-shaped veliger larva of cultured *P. maxima* appeared at 20 h after fertilization. Umbonal larva formed at the age of 10 days which then further developed into plantigrade larva at day 25 after fertilization. Developmental period and larval size of cultured *P. maxima* in this study is similar to *P. maxima* from natural habitat and also to *P. fucata* and *P. margaritifera*. Noticable difference obtained from this study is shell size of 25 day-old plantigrade larva which tends to be bigger compared to previous reports from other parts of the world.

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