



Effects of artificial and natural materials ropes as substrates for settling and metamorphosis of pediveliger pearl oyster, *Pinctada maxima* (Jameson, 1901)

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Abstract. A study aimed at investigating the effects of four different materials ropes as substrates for settling and metamorphosis of tropical oyster, *Pinctada maxima*, pediveligers was conducted at laboratory scale. The number of pediveligers attached and metamorphosed on each substratum were counted at the termination of the experiment. Increment in shell length during settling and metamorphosis was also measured. Settling and metamorphosis were analyzed using One-Way ANOVA with substratum as the main effect. Two-Way ANOVA with time and substratum as the main effect on growth was also tested. The results showed that the settling and metamorphosis of pediveligers were affected by the substratum. The number of settled and metamorphosed pediveligers on polypropylene rope, natural ropes of *Arenga pinnata* and *Cocos nucifera* was significantly higher than on the bottom of Pyrex glasses. No significant difference between numbers of larvae settled on polypropylene and natural ropes of *A. pinnata* and *C. nucifera* appeared. A significant effect of time, but not of substratum, on growth occurred. Overall, settling and metamorphosis of *P. maxima* pediveligers larvae were strongly affected by artificial and natural ropes, while metamorphosis occurred haphazardly on the Pyrex glass. Growth occurred after settling and metamorphosis.

Key Words: pediveliger larvae, settlement, metamorphosis, substratum, pearl oyster.

Introduction. Pearl oysters, *Pinctada maxima* (Jameson, 1901), have been actively cultured and developed to be one of the main pearl oyster industry in North Sulawesi. Spat of this pearl oyster is mainly supplied from hatcheries or collected from the oyster bed using spat collectors. Nowadays the use of polypropylene and monofilament as substrate for spat collectors has been intensified. Yet the unused materials are being dumped into the sea and become wastes, affecting benthic organisms as it may be ingested, accumulated and possibly transferred to higher trophic levels. According to Chae & An (2017), plastic debris, including micro and nano plastics, can affect metabolism, fertility, and mortality of aquatic organisms.

To minimize the negative effects of plastic materials to the benthic ecosystem, it will be necessary to find alternative materials as spat collectors. Two types of natural fibers as organic materials obtained from sugar palm three, *Arenga pinnata* (Wurmb) Merr, and coconut tree, *Cocos nucifera* L. (Ferita et al 2015) can be considered to be such alternative materials. These materials are abundantly found in tropical areas and have been used for different purposes (Mogea et al 1991). Being organic materials, they are easily decomposed, and may be dumped to the sea bottom with no ill effects (Karliansyah et al 2010). The question is whether pearl oyster pediveliger larvae of *P. maxima* can be induced to settle and metamorphose on these materials?

Many researchers have shown that oyster larvae trigger on environmental cues, particularly on specific substrata when reaching the stage competent for settling and metamorphosis. For example, a significantly higher number of *Pinctada margaritifera* (Linnaeus, 1758) attached on shade meshes than other available substrates, while larvae of *Pinctada maculata* (Gould, 1850) were found more abundant on plastic sheeting than

other substrates (Friedman et al 1998). In the study of the Pacific oyster in Denmark water, a success attaching and recruits of *Crassostrea gigas* (Thunberg, 1793) on shelter tidal Limford occurred (Holm et al 2015). An abundant number of rocky oyster *Striostrea prismatica* (Gray, 1825) attaching on rocky natural substrata in the coastal eastern Pacific waters was recorded (Lodeiros et al 2017). In the study of eastern Virginia oyster, *Crassostrea virginica*, George et al (2015) reported that oyster larvae settled and recruited on various natural substrata too, which included concrete, porcelaine, limestone, rivers rock, and oyster shell. More examples, the larvae of *P. maxima* settled in significantly higher numbers on the combined PVC slat and rope collectors than on either nylon or PVC slat collectors (Taylor et al 1998). A higher number of larvae of *P. margaritifera* settled on garden shade spirals than on any other materials provided (Libini et al 2013). Furthermore, Somchai & Cedhagen (2012) reported that larvae of *Crassostrea belchery* (Sowerby) preferred substrates of their own species, such as shell periostracum protein and pheromones from their adults. The same behavior has been also reported for mytilid mussel larvae from South Australian waters (Ompi 2010). In the field, a high number of new recruits attached on their adult byssus threads and their adult shells were found (Ompi & Svane 2018). Another example, the new recruits of tropical box mussel, *Septifer bilocularis* (Linnaeus, 1758) was also high on their adults shells compare to others substrates (Ompi 1998). Interestingly up to now, information on using natural organic ropes from fibers of sugar palm and coconut as pearl oyster spat collectors is limited.

The purposes of this study were (1) to test whether the late larval stage, which is called pediveliger, of *P. maxima* settled and metamorphosed when exposed to various artificial and natural substrates such as polypropylene rope, ropes from natural fibers of *A. pinnata* and *C. nucifera*, and Pyrex glass (= no added substrate), (2) to test if there is any preferred substrate on which settling and metamorphosis would take place, and (3) to describe growth of pediveliger larvae after settling and metamorphosis had taken place.

This study provides valuable information for understanding the mechanism of the larval settling and metamorphosis of this species, particularly when natural substrates were provided, and it might be useful information of using more environmentally friendly materials as spat collectors in the future.

Material and Method

Settlement and metamorphosis. These experiments used larvae of *Pinctada maxima* which had been spawned and reared until 18 days, from 6 to 24 September 2015 at the hatchery of Pt Samudra in Lembe Strait, Bitung Town, North Sulawesi. Four types of substrate, namely (1) polypropylene rope, (2) natural fiber rope from sugar palm tree, *A. pinnata*, (3) coconut fiber rope from *C. nucifera* tree and (4) Pyrex glass, were soaked with seawater for one week before use. Three replicates for each type of substrate were performed. Each type of rope was cut into sections of 9.5 cm length and fit into an aluminum tube of 13 cm length. One end of the rope was fitted at the same level as one end of the aluminum tube. No rope was attached to the rest of 3.5 cm length. However, this end was bent into a curve shape. The curved end of the tube was wrapped around an aluminum cable lying horizontally across the top of a 500 ml Pyrex container. The substrates were hung vertically, and the other end was attached to the Pyrex glass bottom. The Pyrex glass container was filled with filtered sea water before adding the larvae. About 1 larva per mL was added into each of the Pyrex containers. All containers were placed into a box chamber system culture with circulating sea water system at 28-29°C. For control, three Pyrex glass containers were filled with filtered sea water and larvae but no substrates and placed into the same culture chamber. Larvae were fed every day with cultured diatom, *Chaetorus* sp.

The attached and metamorphosed larvae were observed after 96 hours at the termination of the experiment. Each rope was removed from the containers and observed under the microscope connected to a monitor and digital camera. The larvae on the glass

bottom were also siphoned out from the container and placed in a counting chamber and observed under the microscope to find any metamorphosed larvae. Larval metamorphosis can be identified when eyes and umbo have appeared, and at the same time, velum and cilia are lost and the gill begins to develop. A larva capable of undergoing this metamorphosis change successfully was identified as competent. Larvae with velum and cilia swimming close to the bottom were not metamorphosed. Here, the larvae were not counted.

Growth. Shell length of attached and metamorphosed larvae was also measured. Substratum was removed quickly and placed under the microscope, connected with a monitor to measure shell length. Three to five larvae were measured from each substratum. Substratum with attached larvae was brought back as soon as possible to the culture system after the measurement was finished.

Data analysis. Settling and metamorphosis of larvae were analyzed using One-Way ANOVA with types of substrate as the main factor. Shell length of attached and metamorphosed larvae was tested using Two-Way ANOVA with time and substrates as the main factors. In order to fulfill the assumptions of analysis variance, the data were tested for homogeneity of variance using the F_{\max} test (Fowler et al 1998). In cases where the ANOVA-test showed significant treatment effects, the means were compared using the SNK-test (Sokal & Rohlf 1981).

Results

Settling and metamorphosis. Larvae of *P. maxima* settled and metamorphosed on substrata of polypropylene rope, natural fiber of *A. pinnata* and *C. nucifera* rather than on Pyrex glass. Here, presence of eyes, foot, and umbo was observed when settlement took place. Pediveligers crawled on the substrata by using their foot. Sometimes pediveligers swam back again into water column, where velum and cilia extended from the shell valves. Metamorphosis occurred when velum and cilia were lost, hard shell valves produced, and juveniles were attached on the substrata as shown at Figure 1. When moved, the foot attached on substrata, dragging its body moved forward, until byssus was produced and attached permanently on suitable substrata. More byssus produced and attaching on substrata indicated a strong attachment on suitable substrata.

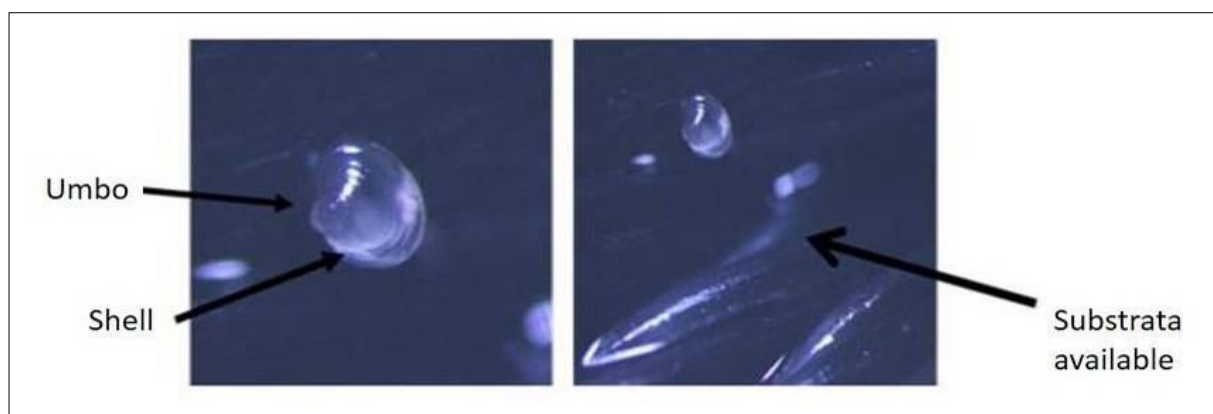


Figure 1. Pediveliger *P. maxima* attached on substrata available.

The number of settled and metamorphosed pediveligers were analyzed using One-Way ANOVA with substratum as the main effect. The results showed a significant effect of substratum on settling and metamorphosis (One-Way ANOVA, $df = 3$, $F = 112.272$, $p < 0.01$).

A significantly higher number of settled and metamorphosed pediveligers occurred on each of the added substrates than on Pyrex glass (PG) (SNK-test, $p < 0.05$) (Figure

2). There was no significant difference in number of settled and metamorphosed pediveligers between polypropylene rope (PE), sugar palm rope (IP), and coconut rope (IK) (SNK-test, $p > 0.05$) (Figure 2).

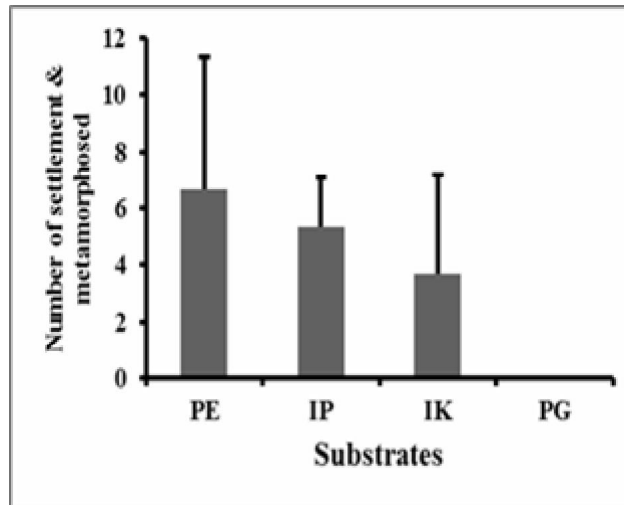


Figure 2. Mean number of settled and metamorphosed pediveligers of *P. maxima* attached on different substrates: PE = polypropylene rope, IP = sugar palm rope, IK = coconut rope, and PG = Pyrex glass (Bars = 95% confidence intervals).

Growth. A pattern of consistently slightly increase in shell length from day 1 to day 2 and further slowly increase until day 4 of pediveligers of *P. maxima* attaching on each type of substrate was observed (Figure 3).

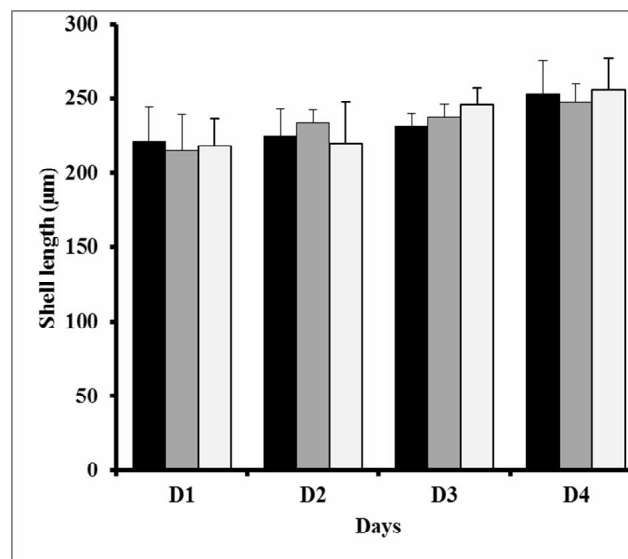


Figure 3. Mean shell length of pediveligers of *P. maxima* settled and metamorphosed on different substrata from day 1 until day 4. D1 = first day, D2 = second day, D3 = third day, and D4 = fourth day. Black bars = coconut fibers, grey bars = sugar palm fibers, and white bars = polypropylene ropes (Bars = are 95% confidence interval).

The increase in shell length of settled and metamorphosed pediveliger larvae as affected by substratum and time was analyzed using Two-Way ANOVA. The results showed that the shell length of settling and metamorphosis of pediveliger larvae were affected by time (2 - way ANOVA, $df = 2$, $F = 17.753$, $p < 0.05$), but not by substratum (2 - way ANOVA, $df = 2$, $F = 2.897$, $p > 0.05$). No effect of interaction on shell length increment between observed factors (substratum and time) was found (2-way ANOVA, $df = 6$, $F = 1.784$, $p > 0.05$).

Furthermore, since shell length was only affected by time, all of the data were combined and analyzed using One-Way ANOVA with time as the main effect. The result showed a significant effect of time on shell length increment (1 – way ANOVA, $df = 3$, $F = 13.712$, $p < 0.05$). Shell length of settled and metamorphosed pediveliger larvae on day 4 was significantly larger than larvae on days 1, 2, and 3 (Figure 4). However, no difference in shell length among settled and metamorphosed larvae on day 1 and day 2 ($p > 0.05$) as well as day 2 and 3 ($p > 0.05$) occurred (Figure 4).

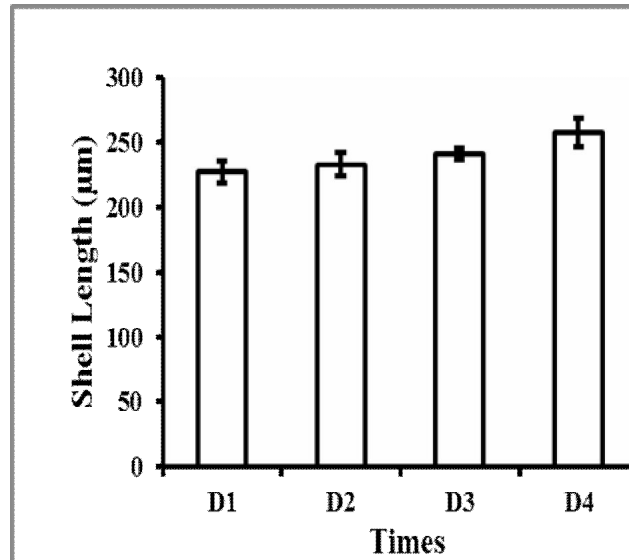


Figure 4. Mean shell length of settled and metamorphosed pediveligers of *P. maxima*. D1 = first day, D2 = second day, D3 = third day, and D4 = fourth day.

Discussion. Settlement for many of marine benthic larvae may be defined as the process of descending from the water column, searching for suitable substrates, and attaching on it, while metamorphosis is defined as a sequence of morphological and organ transformations preparing the larvae for benthic life (Svane & Young 1989; Ompi 2010; Young et al 2002; Carl et al 2012).

In the present study, *P. maxima* settled and metamorphosed clearly on polypropylene rope, natural ropes of sugar palm and coconut tree. This might imply that polypropylene and natural ropes of sugar palm, *A. pinnata* and coconut tree, *C. nucifera* were suitable and favorable substrates for pediveligers to settle and metamorphose. However, Pyrex glass bottom was apparently not a suitable substrate, since pediveligers might search and test a suitable substrate when crawling continuously at the glass bottom, and swam around and around closely to the substrate available and eventually swam back into the water column. Swam around to the left or to the right were common swimming behavior phenomenon when getting closed to the substrata available in the early settling stage. Behavior of attaching and crawling, and subsequent swimming back into water column has also been reported for other species such as *Mytilus edulis* (Linnaeus, 1758) (Bayne 1965), the coral associated, *Lithopaga simplex* and *L. lessepsiana* (Mokadi et al 1993), *P. margaritifera* (Doroudi et al 1999), *Mytilus galloprovincialis* (Lamarck, 1819) (Ompi 2010; Carl et al 2012).

Physical structure of ropes used for substratum could have been a major source of stimuli for pediveligers to settle and metamorphose in this study. Surface pattern of each of the three substrates might increase the surface area and thus indicated a substrate complexity as suggested by Carl et al (2012) and Christensen et al (2015). Substrate complexity might stimulate metamorphosed pediveligers to penetrate further into the substrate, and at the same time, metamorphosed pediveligers might produce more byssus to attach on the substrate as indication of a strong attachment. A strong attachment on substrata may indicate a new life as a young oyster spat has begun (Kalyanasundaram & Ramamoorthi 1987; Somchai & Cedhagen 2012).

Aside from this, all rope surfaces contained very small hairs, which may also stimulate larvae of *P. maxima* to settle and metamorphose in this study. Fine hairs on filamentous materials was reported to be an attractive and suitable substrate for stimulating larvae to settle and metamorphose for other marine bivalves such as larvae of *M. edulis* (Bayne 1965) as well as larvae of South Australian marine mytilid species (Ompi 2010). Furthermore, the organic materials containing palm sugar and coconut trees might also provide additional attraction to pediveligers to be stimulated to settle and metamorphose. Many researchers have concerned that substrate colour may also be a factor to stimulate larvae to settle and metamorphose. For example, in the Pacific Islands, settling larvae of *P. margaritifera* preferred a dark to a light surface of collectors (Braley & Munro 1997). Other examples showed that dark color of plastic sheets attracted more larvae of *P. martensii* to settle than light colour (Su et al 2007). However, in this study, color did not seem to have any effect, since settling and metamorphosis of pediveligers of *P. maxima* on different substrata with various brown, gold, and dark color were not significant in number.

Growth might not occur in early stages of settling and metamorphosis. In this study, no shell length increment occurred during the first 24 until 72 hours. This could be caused by morphological changes such as loss of velum, reorientation of mouth and foot, including developing of gill filaments such as it was shown for the pediveligers of *Ostrea chilensis* (Videla et al 1998), *Brachidontes rostratus*, *B. erosus*, *Trichomya hirsutus* (Ompi 2010), and *M. galloprovincialis* (Carl et al (2012)). During early settlement and metamorphosis of many marine bivalves, pediveligers might not feed and rely upon stored nutrients and energy while velum and cilia disappear and, at the same time, a new feeding mechanism develops (Videla et al 1998; Ompi & Svane 2018). Feeding mechanisms might improve at the end and after metamorphosis and, as a result, metamorphosed pediveligers begin to feed, which increases growth activities. In this study, the shell length increment of *P. maxima* pediveligers appeared clearly 72 to 96 hours after introducing substratum.

Overall, this study confirms that polypropylene rope is a suitable substrate to attract larvae to settle and metamorphose and this substrate has also been used as spat collector as reported by Taylor et al (1998). Other artificial materials, such as plastic sheets and ropes, had also been reported as spat collectors (Friedman et al 1998; Su et al 2007; Libini et al 2013), concrete, limestone, and estuary rock (Lodeiros et al 2017). In this study, natural fiber rope from sugar palm, *A. pinnata* and coconut fiber rope from *C. nucifera* were also attractive and suitable substrata for pediveligers of *P. maxima* to settle and metamorphose. We recommend that these substrates can be used as spat collectors like other natural spat collectors from bamboo material (Arini & Jaya 2011), coconut hard shell (Libini et al 2013), and oyster shell (Lodeiros et al 2017). A better growth performance after metamorphosis might occur when organ and changing feeding mechanisms have been completed.

Conclusions. Pediveligers of *Pinctada maxima* settled and metamorphosed on polypropylene rope, and both natural fiber rope of *Arenga pinnata* and *Cocos nucifera*, but less frequently settle and metamorphose on Pyrex glass. Pediveliger larvae postponed settling on unfavorable substratum occur. Increased growth in shell length of pediveligers appeared after settling and metamorphosis.

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