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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 pediveliger were affected by the substratum. The number of settled and metamorphosed pediveligers on polypropylene rope, natural ropes of *Arenga piñata* 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 *Arenga piñata* and *Cocos nucifera* appeared. A significant effect of time, but not of substratum, on growth occurred. Overall, settling and metamorphosis of *P. maxima* pediveliger larvae were strongly affected by artificial and natural ropes, while metamorphosis occurred haphazardly on the Pyrex glass. Growth occurred after settling and metamorphosis.

1. Introduction

Pearl oysters, *Pinctada maxima* (Jameson, 1901), have been actively cultured and developed to be 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 throphic levels. According to Chae & Joo An (2017), plastic debris, including micro and nanoplastics, 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 (Karlianzah 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 *P. margaritifera* attached on shade meshes than other available substrates, while larvae of *P. maculata* were found more abundant on plastic sheeting than other substrates (Friedman et al 1998). Another example, 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). Interestingly up to now, information on using natural organic ropes from fibers of sugar palm and coconut as pearl oyster spat collectors is not available.

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 *Arenga pinnata* and *Cocos 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 a spat collectors in the future.

2. Materials and methods

2.1. Settling and metamorphosis

These experiments used larvae of *Pinctada maxima* which had been spawned and reared until 18 days 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, *Arenga piñata*, (3) coconut fiber rope from *Cocos 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^{o}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.

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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.

2.2. 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.

2.3. 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 1990). In cases where the ANOVA-test showed significant treatment effects, the means were compared using the SNK-test (Sokal & Rohlf 1981).

3. Results

3.1. Settling and metamorphosis

Larvae of *P. maxima* settled and metamorphosed on substrata of polypropylene rope, natural fiber of *Arenga pinnata* and *Cocos 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, gills developed, hard shell valves produced, and juveniles were attached on the substrata as shown at Fig. 1.

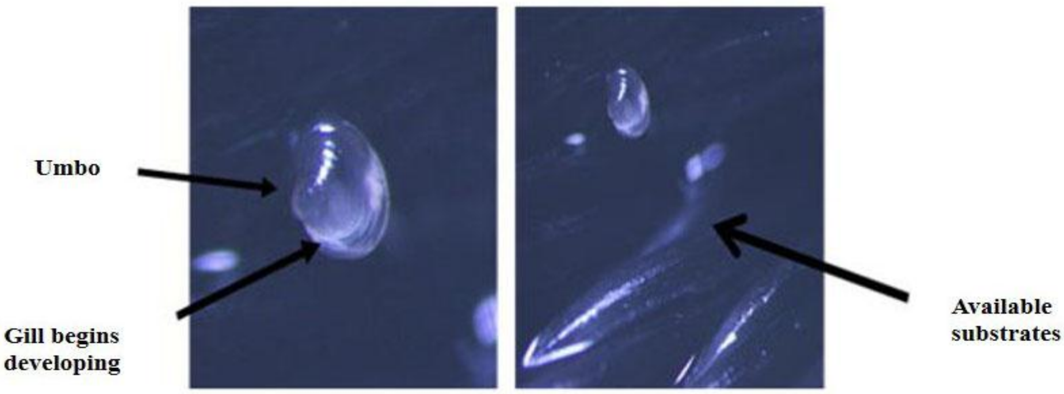

Figure 1. Pediveliger *P. maxima* attached on substrata available

The number of settled and metamorphosed pediveligers were analyzed using One-Way ANOVA with substratum as the main effect (Table 1). The results showed a significant effect of substratum on settling and metamorphosis (One-Way ANOVA, P<0.01).

Table 1. One-Way ANOVA, number of settled and metamorphosed pediveligers, with substratum as the main effect (***: P<0.01).

Sources	DF	SS	MS	F	P
Substrates	3	12.564	4.188	112.272	0.000***
Error	8	0.298	0.037		

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) (Fig. 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) (Fig. 2).

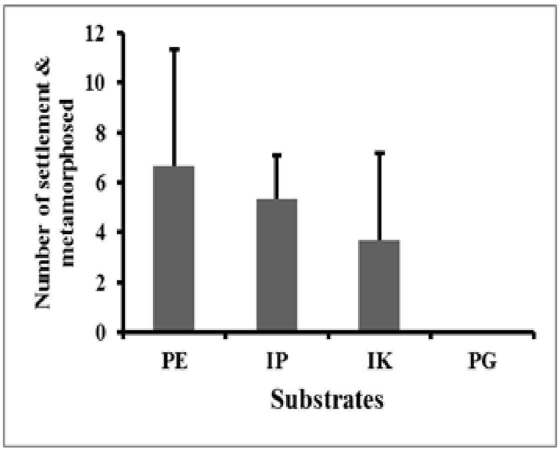

Figure 2. Mean number of settled and metamorphosed pediveligers of *P. maxima* attached on different substrates: PE = polypropylene rope, IP = sugar palm rope, IK = coconut rope, and PG = Pyrex glass (Bars = 95 % Confidence intervals).

3.2. Growth

A pattern of consistently slowly increase in shell length from day 1 until day 4 of pediveligers of *P. maxima* attaching on each type of substrate was observed (Fig. 3).

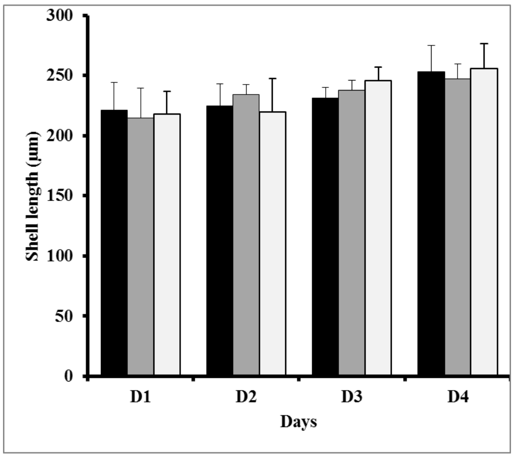

Figure 3. Mean shell length of pediveligers of *P. maxima* settled and metamorphosed on different substrata from day 1 until day 4. D1 = first day, D2 = second day, D3 = third day, and D4 = fourth day. Black bars = coconut fibers, grey bars = sugar palm fibers, and white bars = polypropylene ropes (Bars=are 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 (Table 2). The results showed that the shell length of settling and metamorphosis of pediveliger larvae were affected by time (P<0.05), but not by substratum (P>0.05).

Table 2. Two-way ANOVA, the shell length of settled and metamorphosed pediveligers of *P. maxima*, with time and substratum as the main effects (**:P<0.01; n.s.: not significant).

Sources	DF	SS	MS	F	P
Time	2	4.570.972	1.523.627	17.353	0.000
Substrates	2	508.667	254.333	2.897	0.075 n.s
Time X Substrates	6	939.778	156.630	1.784	0.145 n.s
Residual	24	2.107.333	87.806		

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 (P<0.05) (Tabel 3). Shell length of settled and metamorphosed pediveliger larvae on day 4 was significantly larger than larvae on days 1, 2, and 3 (Fig. 4). However, no difference in shell length among settled and metamorphosed larvae on days 1, 2, and day 3 was determined (P>0.05).

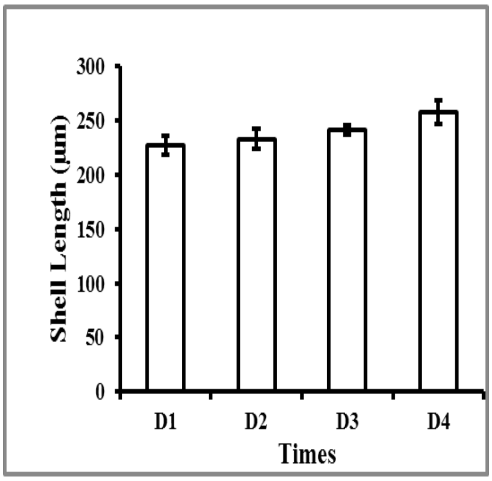

Figure 4. Mean shell length of settled and metamorphosed pediveligers of *P. maxima*. D1 = first day, D2 = second day, D3 = third day, and D4 = fourth day.

Table 3. One-Way ANOVA, shell length of settled and metamorphosed larval *P. maxima* with time as the main effect (**: P<0.01)

Sources	DF	SS	MS	F	P
Time	3	4.570.972	1.523.657	13.712	0.000
Error	32	3.555.778	111.118		

4. 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; Young *et al* 2002; Carl *et al* 2012).

In the present study, *Pinctada 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, *Arenga pinnata* and coconut three, *Cocos nucifera* were suitable substrates for pediveligers to settle and metamorphose. However, Pyrex glass bottom is apparently not a suitable substrate, since pediveligers might search and test a suitable substrate when crawling continuously at the glass bottom, but eventually swam back into the water column. Behavior of attaching, and crawling, and subsequent swimming back into water column has also been reported for other species such as the coral associated, *Lithopaga simplex* and *L. lessepsiana* (Mokadi et al 1993), *P. margaritifera* (Doroudi et al 1999), *M. edulis* (Bayne 1965), *M. galloprovincialis* (Carl et al 2012; Ompi 2010).

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 substrate complexity as suggested by Carl et al (2012) and

Cristensen 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 (Kalianasundaram & Ramamoorthi 1987).

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). Furthermore, the organic materials containing palm sugar and coconut trees might also provide additional attraction to pediveligers to be stimulated to settle and metamorphose. 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 and Munro 1997). Other examples showed that dark colour of plastic sheets attracted more larvae of *P. martensii* to settle than light colour (Su et al 2007). However, in this study, colour did not seem to have any effect as settling and metamorphosis of pediveligers of *P. maxima* on different substrata with various brown, gold, and dark colours were similar 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, pediveligers might not feed and rely upon stored nutrients and energy while velum and cilia disappear and, at the same time, a new feeding mechanisms develops. 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 good substrate to attract larvae to settle and metamorphos and this substrate has been used as spat collector as reported by Taylor et al (1998). Other artificial materials, such as plastic sheets and ropes, have also been reported as spat collectors (Friedmen et al 1998; Su et al 2007; Libini et al 2013). In this study, natural fiber rope from sugar palm, *Arenga pinnata* and coconut fiber rope from *Cocos 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 2012) and coconut hard shell (Libini et al 2013). A better growth performance after metamorphosis might occur when organ and changing feeding mechanisms have been completed.

5. Conclusion

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. There was no significant preference of artificial or natural fiber ropes for settling and metamorphosis. Increased growth in shell length of pediveligers appeared after settling and metamorphosis.

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References

[1] Chae Y and Joo A Y 2017 Effects of micro and nanoplastics on aquatic ecosystems: Current research trends and perspectives *Mar. Poll. Bull.* **115** 20 – 8
[2] Ferita I, Tawarti and Syarif Z 2015 Identifikasi dan karakteristik tanaman enau (*Arenga pinnata*) di Kabupaten Gayo Lues. *Prosiding Seminar Nasional Masyarakat Indonesia* **1 (1)** 31 - 7
[3] Mogea J, Seibert B and Smits W 1991 Multipurpose palms: the sugar palm (*Arenga pinnata* (Wurmb) Merr.) *Agroforestry Systems* 1**3 (2)** 111- 29.
[4] Friedman F and Bell J D 1996 Effects of different substrata and protective mesh bags on collection of spat of the pearl oyster, *Pinctada margaritifera* (Linnaeus, 1758) and *Pinctada maculate* (Gould, 1850) *Journal of shell fish research* **15 (3)** 535 – 541.
[5] Taylor J J, Southgate P C and Rose R A 1998 Assessment of artificial substrates for collection of hatchery-reared silver-lip pearl oyster (*Pinctada maxima*, Jameson) spat *Aquaculture* **162** 219 – 30.
[6] Libini C L, Manjumol C C, Idu K A A, Kripa V and Mohamed K S 2013 Effect of pediveliger densities and cultch materials on spat settlement of black lip pearl oyster Pinctada margaritifera (Linnaeus, 1758) in hatchery, *Marine Biological Association of India* **55 (1)** 30 - 4.
[7] Karliansyah M R, Waluyo H, Zulhasni, Dwiputra Dj, Ardi T, Rusly A, Bayuadji S and Retnoningtyas H 2010 *Green fins guidelines paduan selam dan snorkeling ramah lingkungan* Kementerian Lingkungan Hidup Jakarta p 71.
[8] Fowler J, Cohen L and Jarvis P 1998 *Practical statistics for field biology* (England John Wiley & Son) p 259.
[9] Sokal R R and Rohlf F J. 1981 *Biometry* (New York Free-man and Company) p 859
[10] Svane I and Young C G 1989 The ecology and behavior of Ascidian larvae *Oceanography Marine Biology Annual Review* **27** 45 – 90.
[11] Young C M, Sewell M A and Rice M E 2002 *Atlas of marine invertebrate larvae* New York.
[12] Carl C, Poole A J, Williams M R, and De Nys R 2012 Where to Settle—Settlement Preferences of *Mytilus galloprovincialis* and Choice of Habitat at a Micro Spatial *Scale PLoS ONE* **7(12)** e52358
[13] Ompi M 2010 Settlement behavior and size of mussel larvae from the family Mytilidae (*Brachidontes erosus* (Lamarck, 1819), *Brachidontes rostratus* (Dunker, 1857), *Trichomya hirsutus* (Lamarck, 1819), and *Mytilus galloprovincialis* Lamarck, 1819 *Journal of Coastal Development* **13 (3)** 215-277.
[14] Mokady O, Bonar D B, Arazi G and Loya Y 1993 Spawning and development of three coral-associated Lithophaga species in the Red Sea, Marine Biology **115** 245 - 52.
[15] Doroudi M, Southgate P C and Mayer R 1999 Growth and survival of blacklip pearl oyster larvae fed different densities of microalgae *Aquaculture International* **7** 179-87.
[16] Bayne B L 1965 Growth and delay of metamorphosis of the larvae of *Mytilus edulis* Ophelia **2** 1 – 47.
[17] Christensen H T, Dolmer P, Hansen B W, Holmer M, Kristensen L D ,Poulsen D LK, Stenberg C, Albertsen C M and Støttrup J G 2015 Aggregation and attachment responses of

blue mussels *Mytilus edulis* impact of substrate Composition, time scale and source of mussel seed *Aquaculture* **435** 245- 51.
[18] Kalyanasundaram M and Ramamoorthi K 1987 Larval development of the oyster *Saccostrea cucullata* (Born) Mahasagar Bulletin of the national institute of oceanography **20 (1)** 53 - 8.
[19] Braley R D and Munro D 1997 Preference for spat collector materials in tanks by larvae of *Pinctada margaritifera* at Penrhyn Atoll Cook Islands SPC Pearl Oyster Bulletin **10** 8 - 11.
[20] Su Z H, Liangmin Y Y and Li H 2007 The effect of different substrates on pearl oyster *Pinctada martensii* (Dunker) larvae settlement *Aquaculture* **271** 377- 383.
[21] Videla J A, Chaparro O R and Thompson R J 1998 Role of biochemical energy serve in the metamorphosis and early juvenile development of the oyster *Ostrea chilensis Mar. Biol.* **132** 635 – 40
[22] Friedman K J, Bell J D and Tiroba G 1998 Availability of wild spat of the blacklip pearl oyster, *Pinctada margaritifera* from open reef systems in Solomon Islands *Aquaculture* **167** 283 - 99.
[23] Arini E and Jaya T S P 2011 The effect of various spat collector materials for spat attachment of pearl oyster (*Pinctada maxima*) *Journal of Coastal Development* **15 (1)** 33 - 44