

Original Paper

THE EFFECT OF VARIOUS SPAT COLLECTOR MATERIALS FOR SPAT ATTACHMENT OF PEARL OYSTER (*Pinctada maxima*)

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Received : April, 20, 2011 ; Accepted : June, 30, 2011

ABSTRACT

Early stage development of pearl oyster spat was very critical, hence spat required suitable substrates for their settlement to complete their metamorphosis. In this stage, byssus for the attachment is vulnerable and easy to break if there are some disturbance e.g. water movement, hence spat easily falling onto the bottom layer which may have various dirt materials leading to the dead of the spat. In the pearl oyster culture spat collector is important. The aim of this study is to study the effect of different collector materials to the number of spat settled, to reveal the best collector materials and the endurance of spat at the collector. This study was conducted in PT Autore Pearl Culture, at Sumbawa island, Nusa Tenggara Province. Materials used in this research were collectors of different substance i.e. asbestos (A), bamboo (B), polyethylene and roof ceramic (D) substance. The method used was laboratories experiment with Completely Random Design by 4 treatments and 3 replications. Data taken were the number of spat settlement, endurance test, and water quality. Data tested with Analysis of Variance (ANOVA) and further tested with Duncan Duplicate Regional test. Water quality was analyzed descriptively. The results showed that different collector materials performed a high significantly difference ($p < 0.01$) to the number of spat settled. The best settlement reached by polyethylene [106 ± 3.61 ind. (dm^2)⁻¹] followed by bamboo [40 ± 3.61 ind. (dm^2)⁻¹], asbestos [16 ± 2.52 ind. (dm^2)⁻¹] and roof ceramic [15 ± 3.61 ind. (dm^2)⁻¹]. The endurance test indicated that percentage of lowest detached of spat from collector reached by treatment B (10.23%), followed by C (11.12%), D (46.95%) and A (50.60%). Water qualities during study were appropriate for the life and growth of pearl oyster spat.

Keywords: Pearl Oyster (*Pinctada maxima*); spat; collector; settlement; endurance Test.

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INTRODUCTION

Pearl oyster *Pinctada maxima* is one of bivalve which have a high potential to be developed. This was indicated by the recent-establishment of some companies dealing with pearl oyster culture which can be found mostly all around Indonesian waters, especially the eastern part of Indonesia (Winanto *et al.*, 1999), for 81 pearl's companies either local or foreign capital investment, mostly working with pearl oyster of *P. maxima* (Antoro and Erawati, 2001). In some cases, pearl oyster culture industries were still depending on natural seed. In some places of Indonesia oyster cultures were commonly focused on raising the number of oyster and pearl production. Nevertheless, oyster hatchery

activities were only focused on internal company need instead of commercial production. These cases were to be a reason that successful of pearl oyster hatchery becomes an important factor for a continuous pearl production.

Kinne (1997) explained that settlement process of bivalves larvae as well as in the case of *P. maxima* would be depend on many factors, i.e. age, light, temperature, nutrition, and suitable substrates. By fast development of pearl oyster hatchery, effective method of spat production is needed. One of many other effort in finding an appropriate method is by means of collecting *P. maxima* spat using different

collector materials.

Pearl oyster as a benthic organism (sessile) needs a suitable substratum for spat attachment. Spat attachments process of pearl oyster is normally starts from pediveliger stadium, which normally has 18 – 20 days in age. In the beginning, larvae use their vellum for swimming, then crawling slowly with their paddle before attaching to any substrates. If the substrate is suitable for the larvae, they will attached and live on it, then continued to perform metamorphosis in the spat stage. But, if they cannot found a proper substrate, they will delay their stage of metamorphosis (Winanto *et al.*, 2001). Early spat condition (plantigrade stadium) is very critical due to unstable byssus conditions; hence, if there was any vigorous water movement, byssus will be broken. Even though a temporary byssus can be re-excreted, spat may fall into the concrete base and will possibly die. This was due to the mixing with many dirt's materials which was settled down on the bottom *i.e.* faeces, dead spat and other unexpected debris. Organic matter in the debris will change through a decomposition process, and produced some poisonous and hazard substances (*i.e.* NO₂, NH₃) which is threatening to the spat life (Erawati *et al.*, 2001).

Spat collectors were placed for plantigrade to be attached then developed into spat. Materials for collectors will be better if they were easily to get, cheap (in price), tough, and effective for spat attachment. Every material basically can be used for spat collector, but some of them are not effective for good attachment of spat. This study was aimed to evaluate the materials of collector commonly used by some companies for attachment of *P. maxima* spat. Hence, materials of asbestos, bamboo, polyethylene and roof ceramic had been used in this study. Winanto (2004) stated that materials which can be used for spat collectors are: polyethylene, plastic thread, asbestos, roof ceramics and rigid pipe of polyethylene. Nevertheless, roof ceramic materials from soil can also be used for trials (Walne, 1979).

In Indonesia there are some species of pearl oyster *i.e.* *P. maxima*, *P. margaritifera*,

Pteria penguin, *P. fucata*, and *P. chemnitis*, in which the first three species are important pearl producer (Sutaman, 1993; Taufiq, *et al.*, 2007). In general, the organs of *P. maxima* consists of three main parts *i.e.* foot, mantel and internal organ (Dhoe *et al.*, 2001). Nevertheless, the foot is the most important organ for attachment. This organ is very elastic and form like a tongue composed of muscular fibre and can be penetrated by blood pressure to protrude the foot (3 times longer than normal length (Dhoe *et al.*, 2001). Cahn (1949) explained that young spat using their foot for movement until finding an appropriate substratum for their attachment. This foot also has a function to clean debris or other unexpected particles on gills or mantel. Part of the foot have a byssus like a black fiber and have an important role in attachment process (Dhoe *et al.*, 2001). Perforated foot excreted a gland liquid and will become a compact fiber (byssus) after reacted with saline water (Winanto, 2004).

Every single byssus can strongly stick to the substratum and will hold out in extreme condition (Figure 1). Haris (1990) stated that, the byssus stalks are very compact and very deep embedded into the posterior foot. For attachment, byssus a plaque composed of polyphenol protein which is not dissolved into saline water (Waite, 1983 *in* Haris, 1990). Biochemical and X-ray analysis of byssus fiber is composed of collagen fibers. On the outer layer (cuticle), the fibers consist of protein which has a function like β -carotene hence give a protection to the collagen from micro organisms. Collagen as a tissue bunch and β -carotene as an anti-oxidant can stimulate the immune system for attacking diseases and infection (Winanto, 2004).

After pediveliger stage has been attained, *P. maxima* larvae start to look for a place of settlement (Winanto *et al.*, 2001). Attachment process of pediveliger larvae is started by swimming down movement, then followed by protruding byssus fiber for attaching on the substratum. If the substrate is suited, larvae will settle then spat metamorphosis (Winanto, 2004).

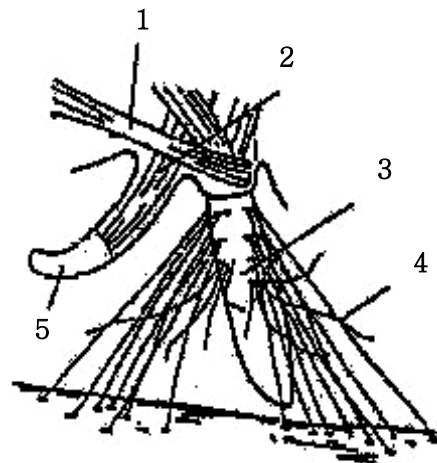


Fig. 1. Settlement Organ of *P. maxima* Spat (Haris, 1990): (1) Anterior retractor byssus muscle; (2) Foot retractor muscle; (3) Byssus stalk; (4) Byssus fiber; and (5) Foot.

Spat attachment on substrates divided into two phases *i.e.* temporal and permanent. Temporal byssus are longer, thinner, fewer, and the form of plaque flatter than permanent byssus. The temporal byssus is very useful in terms of stabilizing position of spat attachment while permanent byssus is secreted (Nalepa and Schloesser, 1993). When sessile life started, pediveliger larvae has to be attached or then unattached (fall off) for several times until they found suitable substrates (Nalepa and Schloesser, 1993).

Every organism have tolerance limit to the changes of environmental condition, temperature range of 25 – 30°C can be tolerate by *P. maxima* (Cahn, 1949). Nayar & Mahadevan (1987); Alagarswami (1983a) in Sudjiharno *et al.*, (2001) explained that in the laboratory condition, the variation of temperatures will affect the attachment time of pearl oyster larvae. On temperature range of 28.2 – 29.8°C larvae will attached when they were 24th days old and at the range of 24.3 – 27.2°C larvae will attached after 32 days old. Hereafter, at low water temperature, larvae will use the time to complete their metamorphosis followed by their attachment process for settlement. Regarding other water quality parameters, pearl oyster will tolerates hydrogen ion concentration (pH) in a range of 7.8 – 8.6 (Matsui, 1960 in Sudjiharno *et al.*, 2001), dissolved oxygen (DO) 5.2 – 6.6 mg L⁻¹ (Imai,

1982 in Sudjiharno, 2001), and salinity 32 – 35 ‰ (Sutaman, 1993).

There are two types of spat collectors *i.e.* natural and artificial. In the natural substrate, pearl oyster normally attached on corals (Asikin, 1962). Artificial spat collectors normally used by pearl oyster hatchery. Wada (1984) stated that, a plastic film, scallop shell and other oysters shells are normally used as artificial spat collectors. Nevertheless, Malaysia and Philippines used materials from asbestos (Chin & Lim, 1976). This study is therefore aimed to find out the most effective collector in term of survival rate and attachment endurance. The materials tested were asbestos, bamboo, polyethylene, and ceramic roof.

MATERIALS AND METHODS

The treatments consist of treatments A (asbestos collector), B (bamboo collector), C (fibrous filamentous polyethylene collector) and D (roof ceramic collector). All of spat collectors are in square form with similar size of 2.5 x 1 dm (**Fig. 2**). The larvae used were in the pediveliger stage (18 days old) with Dorsal-Ventral (D-V) size of 212 ± 16.19 µm and Anterior-Posterior (A-P): 242 ± 17.51 µm. On this stage, as an indication of settlement time, larvae have tried to stick each other in vertical form. Data taken are the number of spat settlement, endurance test, and water quality.

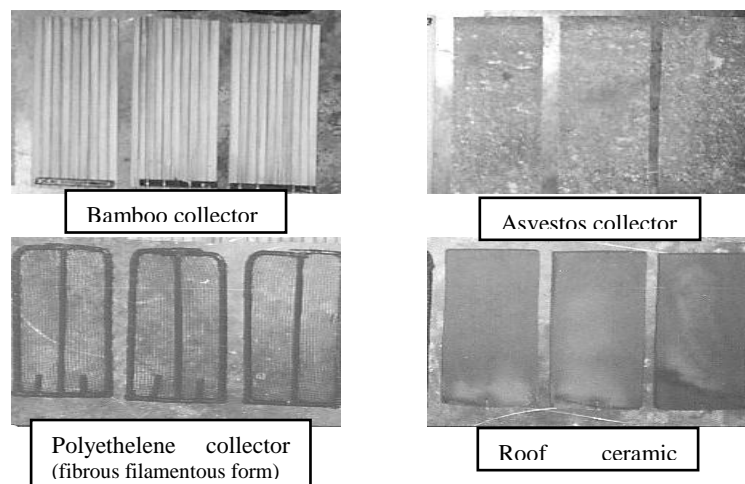


Fig. 2. Spat collectors of *P. maxima* used in this study

In order to hang the collectors, 3 fiber glass tanks with a volume of 40 L had been used. Collectors (A, B, C & D) were sets vertically and placed randomly in every tank and the 4 collectors have a similar distance each other in each tank (**Fig. 3**). The tanks were filled with saline water of 35 ‰ closed to 30 L and

aeration was administered in the middle of the tank. Since the larvae were poured into the tank, with density of 500 larvae L⁻¹ (DVM 212±16.19 µm and APM 242±17.51 µm), the saline water was filled up to 30 L in volume (**Fig.3**).

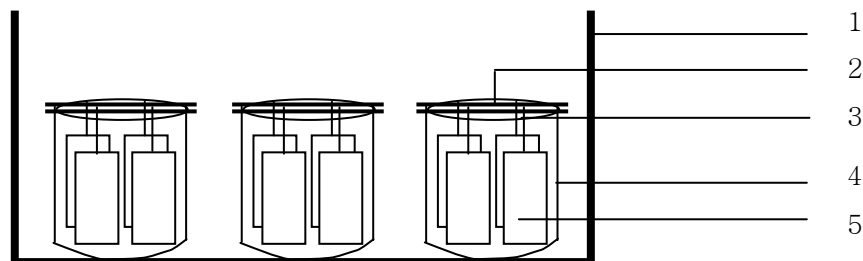


Fig. 3. The scheme of rearing tank for *P. maxima* spat. (1) Outer tank; (2) cover for hanging collectors; (3) bundle of string; (4) rearing tank; and (5) spat collectors (asbestos, bamboo, polyethylene, and roof ceramic).

Water medium was replaced everyday close to 100 % of the volume, unless on the first and second day of rearing, in order to give a chance to larvae for attachment. Water quality test was conducted every day *i.e.* pH, salinity, dissolved oxygen and temperature. The first three parameters were measured every morning, only water temperature is measured 3 times during the day.

Isochrysis galbana and *Pavlova lutheri* were used to feed the larvae with density of 12,000 cell mL⁻¹ d⁻¹ which divided into two phases *i.e.* morning and afternoon. As the larvae developed, the algae concentration is increased by 3,000 cells mL⁻¹ every 3 days.

After 35 days the spat can be observed

clearly, the size was between 1 to 3 mm, hence the spat of *P. maxima* can be counted easily. To avoid the death of spat during counting, the collector was submerged into the water periodically (every 2 minutes) for about 3 seconds, before counting can be continued.

Spat endurance test

Spat endurance test is to assess the effectiveness of collector in term of spat attachment. Spat attached to the collector was served as a first data for endurance test. The test was conducted by allowing a vigorous aeration as current or even waves in natural condition. This aeration using a blower (AC

240 volt, 50 Hz, 220 Watt) for 2 hours continuously. After aerated, the number of settled spat were counted, hence the percentage of survivals can be known.

Data collection

Formula used for counting for the percentage of attached spat is:

$$\% \text{ attached spat} = \frac{\sum \text{ spat attached on each collector}}{\sum \text{ inoculated spat}} \times 100\%$$

Formula for the percentage of fall off spat:

$$\% \text{ spat fall off} = \frac{\sum \text{ inoculated spat} - \sum \text{ survived spat}}{\sum \text{ inoculated spat}} \times 100\%$$

Data analysis

Normality, additive and homogeneity test were used before Analysis of Variant (ANOVA) is administered, followed by Duncan Duplicate Region test. Water quality data was analyzed descriptively.

RESULT AND DISCUSSION

Spat attachment of P. maxima

The result shows that excessive spat attachment was on collector made of polyethylene material (treatment C): $106 \pm 3.61 \text{ spat (dm}^2\text{)}^{-1}$, followed by treatment B (bamboo): $40 \pm 3.61 \text{ spat (dm}^2\text{)}^{-1}$ treatment A (asbestos): $16 \pm 2.52 \text{ spat (dm}^2\text{)}^{-1}$ and the lowest was treatment D (roof ceramics): $15 \pm 3.61 \text{ spat (dm}^2\text{)}^{-1}$ (**Fig. 4**)

Table 1. Spat Attachment of *P. maxima* on Each Collector [$\text{spat (dm}^2\text{)}^{-1}$] after treatments

Repli-cation	Treatment			
	A (asbestos)	B (bamboo)	C (polyethylene)	D (roof ceramic)
1	19	43	103	16
2	14	41	110	18
3	16	36	105	11
Σ :	49	120	318	45
Mean :	16	40	106	15
\pm SD :	2.52	3.61	3.61	3.61

Exp : Initial density of *P. maxima* larvae in the water column is $500 \text{ pediveligers l}^{-1}$

Every material basically can be used as collector, but it does not mean will give a good settlement for larvae. Quayle (1980) in Erawati *et al.*, (2001) stated that one of the factor

influencing or give preferences to *P. maxima* pediveliger is the type and the form of the collectors.

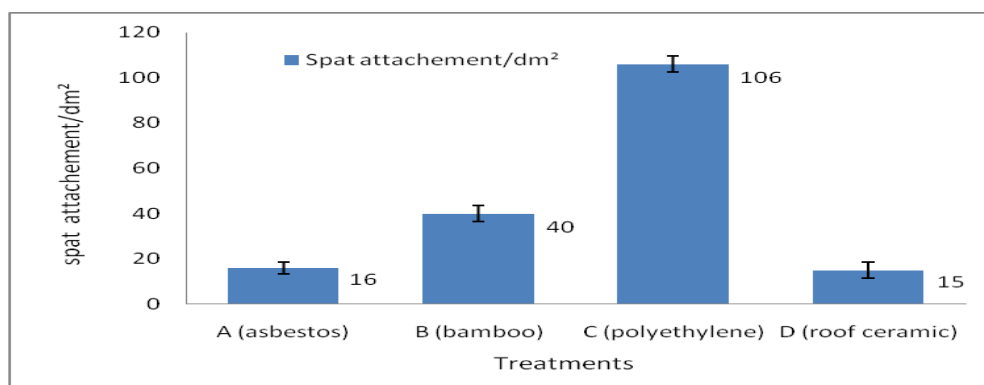


Fig. 4. Number of *P. maxima* spat attachment ($\text{dm}^2\text{)}^{-1}$ on each type of collector, where initial density of larvae in water column was $500 \text{ pediveligers L}^{-1}$.

Attachment process of spat is also influencing by bacterium which forming a bio-film. The bio-filming process is by degrading protein to be N (Nitrogen), P (Phosphor) and K (Kalium). This bio-film layer will be used by periphyton *i.e.* phytoplankton, zooplankton, protozoa for their life. The existence of periphyton will be used as feed, hence *P. maxima* spat attracted to settle. Bio-film forming process on polyethylene is estimated faster than other materials. Hereafter, spat settlement acceleration was also faster on treatment C (Fig. 4). From the percentage view of spat attachment to the pediveliger initial density, none of the treatments attaining 1 %

attachment. Nevertheless, polyethylene (C) have a highest percentage of spat attached: 0.71 ± 0.02 % followed by bamboo (B: 0.27 ± 0.03 %), asbestos (A: 0.11 ± 0.02 %) and roof ceramics (D: 0.10 ± 0.03 %).

Analysis of Variance (ANOVA) shows that among different materials of collectors have significantly different ($p < 0.01$) in term of pearl oyster spat attachment. The existent of bio-film will attract the larvae to settled, as Zhao *et al.*, (2002) stated, the existence of bacterial activity in bio-film formation will attract *P. maxima* spat for attaching onto collector.

Table 2. Duncan Duplicate Region Test for *P. maxima* Spat on Collectors

Treatments	Mean				
C (Polyethylene)	106,267	C			
B (bamboo)	39,867	66,4**	B		
A (asbestos)	16,133	90,134**	23,734**	A	
D (roof ceramic)	14,933	91,334**	24,934**	1,2	D

Note.: ** very significantly different

Duncan Duplicate Region Test also shows that treatment C (polyethylene) performed a very significantly different ($p < 0.01$) to spat attachment on B (bamboo), A (asbestos) and D (roof ceramics). While for B treatment also significantly different ($p < 0.01$) to treatments A and D. Only asbestos and roof ceramics did not show the different. Pediveliger stage (18 – 20 days old) *P. maxima* will searching place for settlement, and this will continuous until plantigrade stage (20-22 days old). This phase is a wean condition of pearl oyster larvae, where they changed from a planktonic life to sessile (Winanto *et al.*, 2001).

Polyethylene collector which had been used formed of synthetic filament. These polyethylene fibers have a relatively small diameter ($\pm 500 \mu\text{m}$), and has a small curve surface form. Nalepa and Schloesser (1993) explained that, the plaque on temporal byssus fiber is flatter than permanent byssus fibers which tend to be curve. This curved plaque will give a stronger attachment of spat to the filamentous collectors. This is due to the byssus plaque can attached on all surface of curve collectors than flat substratum. The byssus plaque of *P. maxima* is composed of polyphenol protein; it is an organic compound which has the ability to stick very strong on substrates and

will not soluble when reacted with saline water (Waite, 1983 in Haris, 1990). On the other hand, the filamentous form of polyethylene made a more extensive and wider surface than other collectors. This condition is estimated that polyethylene collector can catches more bacterium, hence will effect in performing bio-film as well as attracting lots of larvae to settle. Davis (1972) in Erawati *et al.*, (2001) stated that, materials from synthetically filaments are very suitable for monitoring of *Mytilus edulis* attachment.

Other reasons, bamboo, asbestos and roof ceramics have a bright color than polyethylene, whereas during settlement phase *P. maxima* is photo-taxis negative, hence the dark color of polyethylene perhaps served as a settlement attractant. Prytherch (1934) and Hopkins, (1937) and Imai (1971) recorded that, during attachment process, the spat responding passively to the light and bright color.

Endurance test of pearl oyster span on different collectors

Endurance test was conducted after counting numbers and percentage of spat attachment on each collector. The highest percentage of the fall off spat was occur on asbestos collector

(50.6 %), followed by polyethylene and bamboo with means of 6.95%, 11.12% and 10.23% respectively (**Table 3**).

Table 3. Percentage of *P. maxima* Spat Fall Off from Different Collectors.

Replication	Treatment			
	A (asbestos)	B (bamboo)	C (polyethylene)	D (roof ceramic)
1	45.16	14.81	17.05	38.46
2	54.00	2.00	13.24	54.55
3	52.63	13.89	3.06	47.83
ΣX :	151.79	30.7	33.35	140.84
Mean :	50.60	10.23	11.12	46.95
$\pm SD$:	4.76	7.15	7.23	8.08

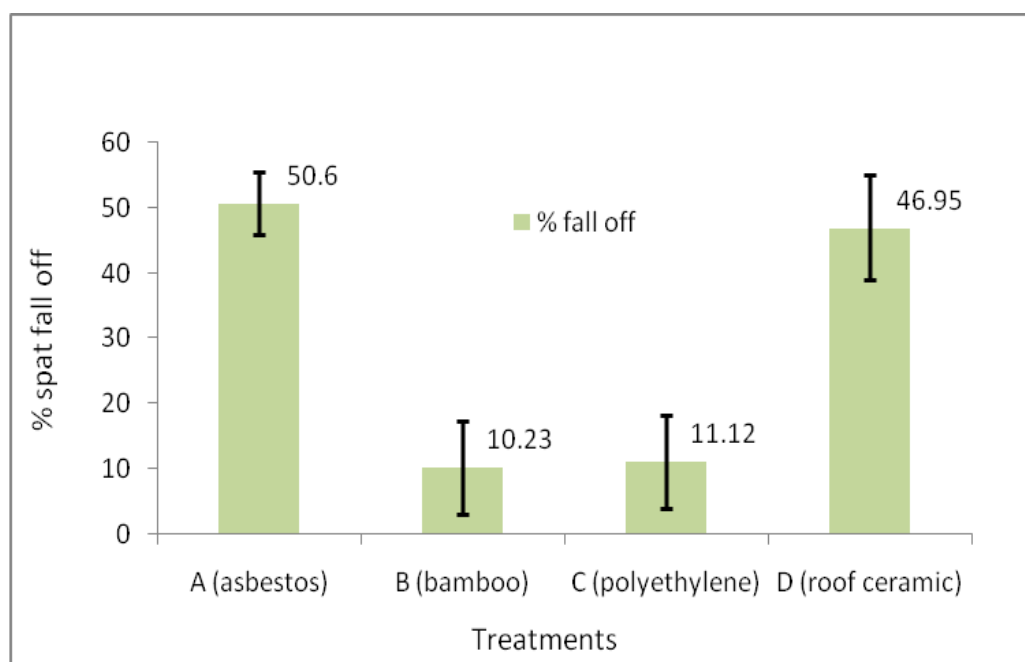


Fig. 5. Histogram percentage of *P. maxima* Spat Fall off from different collectors.

Table 4. Random Analysis of Percentage *P. maxima* Spat Fall Off from Different Collectors.

SK	DB	JK	KT	F count	F table
Treatments	3	4376.00	1458.67	30.56**	0.05: 4.07
Error	8	381.84	47.73		0.01: 7.59
Σ	11	4757.84			

Legend: ** very significantly different

Based on the Random Analysis shows that different collector materials affected were found very significantly different ($p < 0.01$) in spat fall off (**Table 4**). Duncan duplicate region test for spat fall off from different treatments

shows that in between A and C treatments, A and B, C and D, and B and D have significantly different in percentage of *P. maxima* spat fall off, meanwhile A and D, and C and B did not show any different (**Table 5**).

Table 5. Duncan Duplicate Region Test for percentage spat fall off from different treatments.

Treatment	Mean				
A (asbestos)	50.60	A			
D (roof ceramics)	46.95	3.65	D		
C (polyethylene)	11.12	39.48**	35.83**	C	
B (bamboo)	10.23	40.37**	36.72**	0.89	B

** very significant different

The endurance test shows that the highest percentage of spat fall off occurred on asbestos treatment ($A=50.6\pm 4.76\%$), followed by roof ceramic treatment ($D=46.95\pm 8.08\%$), polyethylene ($C=11.12\pm 7.23\%$) and the lowest is in bamboo treatment ($B=10.23\pm 7.15\%$). Random analysis also shows that collectors from different materials have very significantly different ($p < 0.01$) to the percentage spat fall off. Duncan test does not show any difference between bamboo (B) and polyethylene (C), as well as asbestos (A) and roof ceramic (D) ($p > 0.01$) in terms of percentage of spat fall off. On the other hand, asbestos treatment (A) with polyethylene (C); asbestos (A) with bamboo (B); polyethylene (C) with roof ceramics (D); and bamboo (B) with roof ceramics (D) shows very significantly different in endurance test of spat fall off.

From the data above, it can be concluded that bamboo and polyethylene were the best materials for *P. maxima* spat collectors. This was indicated by relatively small of spat fall off during the endurance test. On the other hand, collectors from asbestos and roof ceramics did not give good hold, hence spat fall off are very high. Due to synthetically and filamentous form with relatively small diameter ($\pm 500 \mu\text{m}$) of polyethylene, it gives a good hold to the byssus. Meanwhile the bamboo have a proper porous for sticking the byssus. Nalepa and Schloesser (1993) explain that the plaque form of temporal byssus fiber is flatter than permanent byssus which has curve form. Hence the curve form will give a strong stick on filamentous collectors. Nevertheless, the bamboo also have porous surface which more suitable for byssus attachment, hereafter, quantitatively bamboo is better than polyethylene.

The other things, polyethylene and bamboo collectors are the materials which not easy to be rotten in the water for long period. This condition will give a good effect on polyphenol protein which composed the plaque of byssus in terms of optimum attachment. This is different to asbestos and roof collectors; two

collectors suffering scraped partly on the surface during dipping in the water. Even though spat can strongly attached, but if the substrates which used for stick easy to scrape by water, hence the spat will also easily to fall off to the basin. In general, the results of this study shows that, the best material used for collector is polyethylene, in terms of total spat attachment and spat endurance test.

Water quality

Range of water quality during spat rearing was found in the optimum ranges (**Table 6**), hence gave support in the development as well as growth of pearl oyster. These due to a good system in oxygen supply and proper management of food quantities. The other things is water exchange closed to 100 % was conducted every (other) day.

Temperature of the water medium has an important role in aquaculture, this will affected the metabolic rate of species cultured. Higher temperature will push the metabolic rate in the body. The ranges temperature during rearing was $26.4\text{--}30^\circ\text{C}$. As mentioned by Cahn (1949), a good temperature range for *P. maxima* live is $25\text{--}30^\circ\text{C}$. Hydrogen ion concentration (pH: *puissance negative de H*) also shows a stable ranges of $7.8\text{--}7.9$. Matsui (1960) in Sudjiharno *et al.*, (2001) stated that, the best pH for *P. maxima* were in the range of $7.8\text{--}8.6$. Hence, pH medium do not influence the chemical reaction in water especially NO_3 or NH_4 (Forteath, 1990).

Salinity was also in an optimum ranges for rearing pearl oyster spat ($34 - 35\text{‰}$). As Sutaman (1993) said, sea water salinity for pearl oyster culture are $32 - 35 \text{‰}$. **Table 6** showed that, no extreme ranges of salinity occurred. But if any salinity had changes, the bivalve will give a response by closing their shell and re-adjust ion concentration, amino acid and other molecule to stabilize cell volume

Table 6. Water Quality during Rearing of Pearl Oyster (*P. maxima*) Spat.

Parameters	Measurements	Optimum range
Water t (°C)	26.4 – 30	25 – 30 (Cahn, 1949)
S (‰)	34 – 35	32 – 35 (Sutaman, 1993)
pH	7.8 – 7.9	7.8 – 8.6 (Matsui, 1960 cited by Sudjiharno, et.al., 2001)
DO (mg L ⁻¹)	5.0 – 5.6	5.2 – 6.6 (Imai, 1982 cited by Sudjiharno, et.al., 2001)

This condition followed by declining filtration and respiration rate, but will recover after osmotic homeostasis has been attained (Taufiq et.al, 2007). Dissolved oxygen measured during rearing was in between 5.0 – 5.6 mg L⁻¹. This high condition was due to a good aeration system and water exchange every second day. Nevertheless Imai (1982) cited by Sudjiharno (2001) stated that optimum requirement for *P. maxima* culture are in between 5.2 to 6.6 mg L⁻¹.

CONCLUSION

Different collector materials revealed to have a very significant difference to the number of pearl oyster (*P. maxima*) spat attachment. The best material used for collectors was polyethylene, where this was indicated by highest spat attachment (106 ± 3.61 spat (dm²)⁻¹ whilst percentage of spat fall-off during endurance test is relatively small ($11.12 \pm 7.23\%$).

ACKNOWLEDGEMENT

The author really like to thanks to Mr. Justin Cullen in giving permission for using facilities of Autore Pearl Culture at Sumbawa Island. Appreciation and thanks to Mr. Sutarno who had help in the preparation and collection of data, also to all staff of Hatchery at Dompu, Sumbawa. This manuscript has also been Presented on international conference on Natural Science at Machung University Malang on July 2011 for this presentation, we also thanks to Ms. Limantara and Alexander von Humboldt Foundation.

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