The spat settlement of the black-lip pearl oyster, *Pinctada margaritifera* (L.) (Bivalvia: Pteriidae) on various cultch materials in Kupang Bay, West Timor, Indonesia

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Abstract. This study aims to determine the potential production of black-lip pearl oyster spat (*Pinctada margaritifera*) and determine the effect of different cultch materials for collecting spat and their production. This is quantitative research with an experimental method using a randomized block design (RBD) with four treatments of the spat collection method, each with three replications. The treatments are the oyster collection methods with collectors such as nylon rope, bamboo, wood and shells hanging from the water column in a location where black-lip pearl oyster spat is found. The research data will be processed to calculate the potential spat production for each method, then will be analysed statistically using the Kruskal-Wallis and Mann-Whitney tests. The results showed that the different types of collectors had a significant effect on the attachment of natural pearl oyster spat. The potential of natural pearl oyster spat production based on the average value of spat attachment to clamshell collectors shows that the production potential is 477 spat m^{-2} for a month or 5367 spat m^{-2} for a year.
Key Words: cultch, potency, production.

Introduction. Black-lip pearl oyster, *Pinctada margaritifera* (L.) (Bivalvia: Pteriidae) is spread from subtidal areas to offshore areas. Most live in sub-tidal areas to a depth of 15 m. This biota inhabits mangrove, seagrass and coral reef ecosystems, found attached to weeds, sponges, hard corals, seagrass, soft coral, rocky substrate, dead shells and sandy bottom. Most often live attached to the substrate on dead coral and weeds (Razek et al 2011). Pearl oyster cultivation in Indonesia has long been developed with the main cultivated species being *Pinctada maxima* which produces round pearls (Andalus & Guntur 2001). Pearl oysters are a fishery product that has promising prospects (Yulianto et al 2016). One location that has the potential for black-lip pearl oysters is the waters of Kupang Bay, Kupang Regency, Indonesia.

Black-lip pearl oyster production has its own level of difficulty and has a greater risk of failure because it is influenced by environmental conditions at the cultivation location. So, it is necessary to analyse the level of suitability of the location for pearl oyster cultivation (Yulianto et al 2016; Rizaki et al 2021). Selecting a location through inventory activities and mapping potential land resources is an important initial stage (Rizaki et al 2021). Including the suitability of the location is the availability of natural spat if the cultivation that will be carried out relies on the natural supply of pearl oyster spat. The availability of pearl oyster seedlings is one of the main requirements in developing the cultivation business. Indicators of young shellfish with good growth are having a protruding shell lip (hasaky), the young shellfish sticking firmly to the substrate, the shell is brightly coloured and if the young oysters are touched, they quickly respond by closing the shell (Hamzah & Nababan 2009).

The development of black-lip pearl oyster cultivation on a community industrial scale generally relies on a supply of oyster seeds from nature, considering the large investment costs to build a hatchery. Therefore, the location chosen for developing this business should have sufficient natural seed production potential to support pearl production. Cultivation of black-lip pearl oysters to produce pearls has been a viable industry in the tropical Pacific since 1976 (Haws & Ellis 2000).

With the considerations above, it is important to carry out research that examines the potential for spat black-lip pearl oyster production with several cultch materials in the waters of Kupang Bay, Kupang Regency, Indonesia. It is hoped that this research will provide important scientific information about the most suitable cultch material for the collection of natural spat black-lip pearl oysters and the potential to produce natural spat black-lip pearl oysters which is important for the development of community-based black lip-pearl oyster cultivation businesses in the future.

Material and Method

Time and place of study. The research was carried out in the waters of Kupang Bay, East Nusa Tenggara Province, Indonesia, from July to October 2023.

Experimental design. This research is a quantitative study, using an experimental method with a randomized block design (RBD) with four treatments and three replications. The cultch of spat that will be used was designed and constructed as a treatment in this research, made using several materials, namely: a) polyethylene rope, b) bamboo, c) wood and d) shells. Cultches as experimental units in this research totalled 12 units with a size of 40 x 30 cm2 each. Each treatment is distributed to each group, then randomization is carried out in each group. Placement of the cultch at the research location uses a raft, with the cultch hanging from the crossbar.

Procedure and parameters measured. During the experiment, the experimental units were kept secure, and after 10 weeks observations were made of the spat attached to each cultch. At the time when oyster spat is more than 1 (one) mm in size, it is possible to visually observe it directly. Black-lip pearl oyster (*Pinctada margaritifera*) spat at this age can already be differentiated from other oyster spat. The number of black-lip pearl oyster spats was counted with the help of a hand counter in each cultch/experimental unit. Data from calculating the number of spats is used to calculate the total attachment of spats from each cultch, in addition it is used to calculate the production potential of spats which is calculated based on data from the cultch with the highest number of spat attachments. Apart from that, during the observation period, water quality measurements, namely dissolved oxygen, temperature, pH, salinity and water currents. The temperature and DO are measured using DO meter (Lutron™ DO-5519, with DO range: 0 to 20 mg/L x 0.1 mg/L and temperature: 0 to 50°C). pH was measured using pH-meter (pH-meter digital ATC™, with range pH: 0.00 – 14.00, resolution: 0.01 accuracy: ± 0.1 at 20°C), salinity was determined measured using a refractometer (salinity refractometer ATC™, with salinity range 0-100 ppt), and current speed was measured using float tracking method. All parameters were assessed six times. Water quality measurements were carried out at the location where the experimental groups were placed and were carried out near the surface. Data on the number of spat attachments is processed to calculate the following variables: a) spat attachment (∑ spat), and b) spat production (∑ spat m^{-2}).

Statistical analysis. The spat attachment calculation data was then analysed using the Kruskal-Wallis test and continued with the Mann-Whitney test with the SPSS 24 software. Next, the highest spat attachment data from the four treatments was used to calculate the potential for spat production by calculating the average number per unit area.

Results. The results of attaching pearl oyster spats to the cultch show that black-lip pearl oyster spats (*Pinctada margaritifera*) tend to choose an attachment position that is relatively protected and has a stable attachment area. Attachment of the spat to the oyster shell cultch generally occurs on the inside for a side of the shell.

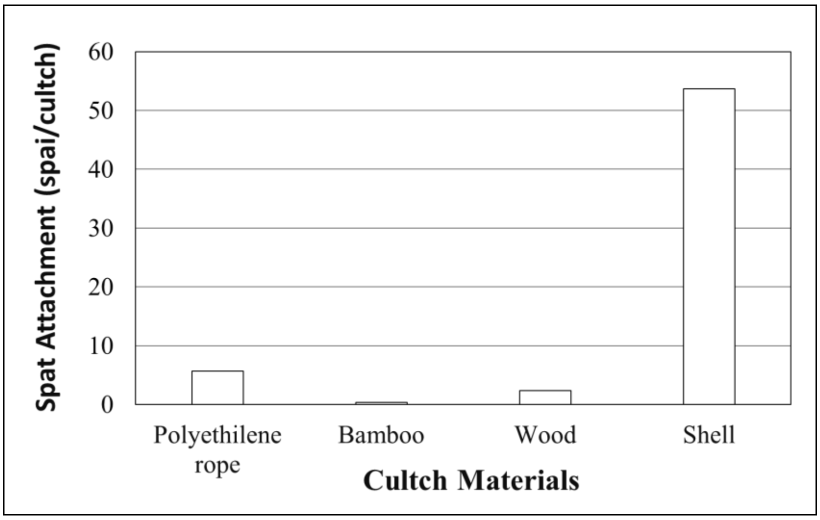

Figure 1. Black-lip pearl oyster (*Pinctada margaritifera*) spat attachment chart.

The results of the Kruskal-Wallis and Mann-Whitney tests showed that the treatment using different cultch materials resulted in the natural spat attachment of pearl oysters being significantly different ($p<0.05$). So H0 is rejected and this means that the different types of cultches have a significant effect on the attachment of the natural spat of pearl oysters. The highest spat attachment occurred in the clam shell cultch treatment with an average attachment value of 53.67±21.59 spat for a cultch and followed by the nylon cultch treatment with an average value of 5.67±1.53 spat for a cultch. The lowest spat attachment occurred in the wood and bamboo treatments respectively with an average value of 2.33±1.15 spat for a cultch and 0.33±0.58 spat for a cultch (Figure 1). The potential for natural spat production of pearl oysters produced based on the average value of spat attachment to the shell cultch is 5367 spats m^{-2} for a year.

As supporting data, several water quality parameters, namely dissolved oxygen, pH, salinity and temperature, were measured in situ, at the location where the experimental unit was placed at a depth of 50 cm from the water surface. The condition of the waters at the water location looks murky because of mud particles which shows that around this location there is a source of fresh water that enters coastal waters so that the mixing of these two types of water masses produces coagulants which cause the water to look murky. At this location there is also a relatively dense mangrove ecosystem which is a source of nutrients that supports the production of phytoplankton which is the main food for pearl oysters.

Table 1

Results of water quality measurements at the research location

Parameter	*Value*			*Quality standards (DKP 2022)*
	Min	*Max*	*Average*	
Dissolved oxygen (mg L^{-1})	5.2	7.5	5.84	> 5
pH	7.3	8.3	7.94	7.2 - 8.2
Salinity (ppt)	27	36	31.86	27 – 38
Temperature (°C)	26.7	30.3	28.86	27 – 32

The results of water quality measurements during the research obtained range and average data for each parameter are as follows: dissolved oxygen (DO) 5.2-7.5 mg/L, pH 7.3-8.3, salinity 27-36 ppt, temperature 26.7-30.3°C (Table 1). Based on data from water quality measurements, it is known that both dissolved oxygen, salinity and water temperature at the research location are within the tolerance range, even from the average values of the pH and temperature parameters in accordance with quality standards (DKP 2022) for cultivating black-lip pearl oysters. However, the large amount of freshwater runoff due to the presence of several estuaries around the bay needs to be considered because it will affect spat production, especially during the rainy season.

Discussion. This research shows that the highest spat attachment for black-lip pearl oysters (*Pinctada margaritifera*) occurs in cultches made from clam shell material. The production potential based on the average value of attachment of spats to clam shell cultivars is 477 spats m^{-2} for a month or 5367 spats m^{-2} for a year. Although several previous studies have shown that polyethylene material has a higher rate of attachment to oyster spats, this is because all the materials used in this research did not undergo prior treatment to remove the chemical effects contained in some of the materials. Materials such as polyethylene, bamboo and wood should be boiled and soaked for some time before being used as cultch materials (Ompi et al 2018; Arini & Jaya 2011).

The development of the early stages of pearl oyster spat is very important, because spat requires a suitable substrate to attach to in order to complete its metamorphosis. At this stage the byssus as an attachment organ is vulnerable and can easily break if there is interference, for example water movement, and the spat easily falls to the bottom layer which results in the death of the spat (Arini & Jaya 2011; Ompi et al 2018). Spat attachment is also related to temperature and chlorophyll-a (Yigitkurt et al 2020). Likewise, locations whose waters are exposed to water runoff from islands produce less spat, because the cultivator placed there becomes very dirty. On the other hand, locations with clear water, moderate currents, and a distance of 35 m from the nearest reef, produce the highest number of spat (Friedman et al 1998).

Before becoming spat, the oyster's life stage after the egg hatches begins with the D larval stage occurring around 24 hours after fertilization, with a size of 79.7±2.3 μm. 10 days old larvae have umbo’s that appear above the hinge axis. On the 22nd day the larvae reach 230.8±4.9 μm in length and begin to develop colour spots before entering the pediveliger larval stage on the 21st day (Doroudi & Southgate 2003). The initial spat stage occurs 45 days after fertilization and the final spat stage occurs 120 days after fertilization. After that, the oyster seeds enter the juvenile stage (Ky et al 2013).

Black-lip pearl oysters are protandrous hermaphrodites, with a spawning season in July and November. This species has two spawning periods a year (Hwang 2007). However, in other locations, spat attachment is found throughout the year except April and May (Yigitkurt et al 2020). In the Solomon Islands, spat can be caught all year round, although collection is most effective between November and March (Oengpepa et al 2006). The onset of reproduction appears to be regulated by dissolved oxygen at the sea surface. Sexual composition in nature shows that the number of female oysters is greater than the number of male oysters during the spawning season (Hwang 2007).

In pearl oyster cultivation, spat collectors are important. Different cultch materials affect the amount of spat that are settles, to determine the best collecting material and spat resistance in the collector. The best reduction was achieved by polyethylene (Arini & Jaya 2011). All pearl farming companies need a stable supply of young pearl oysters (spat) so that the farm can continue to operate. Spat collection is the process of attracting pearl oyster larvae to an artificial substrate, a process commonly used in the pearl industry because it is cheap and simple (Haws & Ellis 2000).

The cultch material and its position influence the attachment of the black-lip pearl oyster spat. The attachment of spats to polyethylene pipes is higher than to plastic baskets (Ehteshami et al 2011). Studies in French Polynesia show that the use of black plate cults is more efficient for *P. margaritifera* spat collections than the plastic shade-mesh cults widely used today (Crusot et al 2021). Another study on Orpheus Island, Australia, succeeded in collecting *P. margaritifera* using a cultch based on polyethylene mesh (Beer & Southgate 2000). Polyethylene tubing placed horizontally in the bottom third of the tank is suitable for housing *P. margaritifera* pediveliger larvae in the hatching area and may also be suitable for rearing in the sea (Ehteshami et al 2011). In the Solomon Islands, spat is most often found in cultch shade mesh (Friedman et al 1998). Meanwhile, in the hatchery, more pediveliger larvae attached and metamorphosed on polypropylene ropes (Ompi et al 2018).

The incidence of high mortality rates while in the cultch on black-lip pearl oyster spat, may be due to predation by ranellid gastropods (Friedman et al 1998; Friedman & Bell 2000), xanthid and portunid crabs, as well as flatworms which also land in the cultch (Friedman & Bell 2000). In the Solomon Islands, spat predator biofouling in cultches is found throughout the year but there are more gastropod predators in January (Oengpepa et al 2006). Apart from that, turbid waters due to the presence of river estuaries will affect the distribution of *P. margaritifera*, where there will be fewer spats in that place (Kishore et al 2018). Other factors also related to spat attachment besides the cultch material are reactions to light and gravitational forces (Ehteshami et al 2011). However, sudden exposure to higher salinity (above 40 ppt) will aid the release of *P. margaritifera* spat from residential tanks in hatcheries to facilitate stress-free transfer of spat to mariculture systems (Libini et al 2018).

The national spat collection program will produce benefits to improve livelihoods and support the expansion of the cultured pearl industry (Kishore et al 2018). Seeds that have been collected, both from hatcheries and from cultch (catching in nature) are put into rearing baskets (Sutaman 1993). Problems in pearl oyster cultivation include predation, disease and biofouling. This can result in a massive loss of productivity. However, the pearl industry has a solution to overcome this problem. For example, by regularly cleaning net bags, biofouling organisms and pearl oysters (Aji 2011).

Oyster growth is negative allometric (weight gain is smaller than length increase) (Kalesaran et al 2018). There is a strong relationship between shell length, shell height and shell thickness (Elamin & Elamin 2014; Kalesaran et al 2018). The growth rate of oysters (*P. margaritifera*) decreases as the age of the oyster increases from an average of 34.6 mm/year in the 2nd year, to 3.7 mm for a year in the 7th year (Aideed et al 2014). Cultured pearl production, and related activities, are of vital social and economic importance to remote coastal communities in Polynesia and the western Pacific (Johnson et al 2019).

In the future, genetic approaches to create faster-growing oysters, disease resistance and high-quality pearl production have yielded promising results. In this way, pearl oyster productivity can be increased (Aji 2011). The back-lip pearl oyster, *P. margaritifera*, can produce the widest range of pearl colours, thanks to donor colour polymorphism in the inner shell, which is mainly responsible for colour transmission (Ky et al 2019). Phenotypic colour selection in *P. margaritifera* spat can be used as a useful indicator in the pearl production cycle and family selection for donor oyster lines with specific colour reproduction (Ky et al 2018).

Several previous studies as well as this research recommend several different cultch materials. This shows that perhaps some culture materials can be applied and become an alternative for use in pearl oyster cultivation. However, currently on an industrial scale, polyethylene cultch materials are more commonly used. Maybe in some locations where it is difficult to obtain polyethylene, other materials can be used, especially if cultivation is carried out in a community industry concept. However, some materials that may contain chemical residues or other active ingredients should be treated first before being used as culturing material.

Conclusions. The results of the research showed that different types of cultches had a significant effect on the attachment of the natural spat of pearl oysters. The highest attachment of spat black-lip pearl oyster (*Pinctada margaritifera*), occurred in shell cultch. The production potential of natural black-lip pearl oyster spat produced based on the average value of spat attachment to the shell cultch shows that the production potential is 477 spats m^{-2} for a month or 5367 spats m^{-2} for a year.

Recommendations. Clam shells can be used as an alternative cultch to obtain natural spat of black-lip pearl oysters (*Pinctada margaritifera*). Material in the form of oyster shells in several locations may be easy to obtain and cheap. Therefore, this material could be an alternative in developing black-lip pearl oyster cultivation, especially on a community industrial scale.

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Conflict of interest. The authors declare that there is no conflict of interest.

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