Settlement Performance of Juvenile Green Shell (Perna viridis) on Different Substrates Based on Attachment, Byssus Production and Byssus strength

Dyahruri Sanjayasari[1,2]*, Maria Dyah Nur Meinita[1,2], Amron[1], Sesilia Rani Samudra[1,2]

[1]Fisheries and Marine Science Faculty, Jenderal Soedirman University, Purwokerto, Jawa Tengah, Indonesia

[2]Center for Maritime and Bioscience, Research and Community Service Institute, Jenderal Soedirman University

*Corresponding author: dyahruri.sanjayasari@unsoed.ac.id

Abstract

The Asian Green shell (*Perna viridis*) attach to the substrate by producing byssus known as bysogenesis. The success of mussels in choosing the right substrate during the early juvenile phase may possibly increase the survival and optimize their growth. The substrates that have been used in green mussels' cultivation in Indonesia are bamboo stack, and nylon rope. However, these two types of substrates do not have filaments which may reduce larval attachment and increase predation on shellfish. Therefore, considering the importance of understanding the attachment profile based on the characteristics of byssus production of mussels to the substrate, current study tested five (5) types of substrates consisted of coconut coir rope (CC), brown linen rope (BL), rayon yarn rope (RY), ex-garment rope (CR), and nylon rope (NR) as attachment substrates of juvenile mussels with two observation periods *(i.e.* 24 and 48 h). The result revealed that nylon rope had the lowest percentage of attachment to the rope in both 24 and 48 h observation periods among all substrates. The highest production on the number of byssus were observed for RY rope, BL rope and CC rope regardless the observation periods.

Keywords: Asian green shell, juvenile, byssus, substrate, attachment

Introduction

Green mussel (*Perna viridis*) is one of the commodities that support the blue economy in Indonesia. This shellfish species is quite popular in Indonesia and several Asian countries such as India, Malaysia, the Philippines, Thailand, Vietnam, Hong Kong to Japan. Besides having good nutritional value (Chakraborty et al., 2016), it has become economical important for the coastal communities through mussel cultivation (Rejeki et al., 2020). Many coastal communities cultivate green mussels, because of the mussel seeds are available throughout the year, require minimal costs for cultivation and do not require complicated technology in the cultivation process (Litasari, 2002). The success of green mussel cultivation is highly dependent on internal and external factors. Internal factors such as the size of the shellfish may affect the initial stage of substrates selection and the strength of their attachment to the substrate (Newell et al., 1991; Jose et al., 2008; Sanjayasari, 2021). While external factors such as tides, currents, hydro-dynamic action, dissolved oxygen in the waters, food availability may also affect the mussel attachment to the substrate, attachment strength, seed survival, and growth profile of grow-out shellfish in the cultivation area (Sanjayasari and Jeffs, 2019; Supono et al., 2020; Sanjayasari et al., 2021).

There are two methods of cultivating green mussels in Indonesia, *i.e*. by using bamboo stack and longlines (Lymer et al., 2010). The seeds of green mussels with shell length (<1 mm) prefer to attach on a filamentous substrate resembling a seaweed, as it is providing shelter from predators (Alfaro and Jeffs, 2003; Alfaro, 2006). *Mytilus californianus* was found attached to the surface of seaweed, especially filamentous substrates, mussel beds and corals (Soares et al., 2008). The success of mussels in choosing the right substrate in the juvenile phase will possibly increase the survival rate and optimize the growth of mussels. Green mussels attach to the substrate by producing byssus. Byssus is a sticky protein produced externally by mussels' glands near the their feet and plays an important role in attachment to the substrates (Babarro et al., 2008). The duration of byssus attachment and the strength of its attachment to the substrate are related to the type of byssus protein produced chemically and biochemically, to the type of substrate and its environment (Babarro et al., 2014).

Exploration of the environmentally friendly substrates in order to increase attachment and growth of green shell by mussel farmers has not been widely carried out. One of the countries with the highest green mussel culture of exports commodity is New Zealand. The mussel farmers in New Zealand use Christmas tree rope which is an ideal substrate for spat collector ropes (Brenner and Buck, 2010). Meanwhile, the nylon rope and bamboo stack have been used widely by Indonesian mussels' farmers to cultivate the mussels. Since these types of substrate do not have filaments, this may increase mortality, reduce larval attachment, and increase predation on shellfish (Filgueira et al., 2007). Therefore, considering the importance of knowing the characteristics of byssus production in the mechanism of attachment of mussels to the substrate, it is necessary to explore artificial substrates for optimization of attachment media of juvenile green shell. This study aims to determine the attachment profile, characteristics of byssus production, and byssus strength of juvenile green shell on different types of substrates. The results of this study are expected to stimulate the development of green mussel cultivation technology innovations so that the optimization of green mussel production in Indonesia can be achieved.

Materials and Method

The experiments were carried out in the laboratory by testing five substrates rope *i.e.* Rayon yarn rope, ex-garment rope, brown linen rope, coconut coir rope, and nylon rope (Fig. 1) in two period of observation times, *i.e.* 24 and 48 h. Each of the treatment had four replications.

Figure 1. Types of substrates in the experiment took in actual visual (top) and SEM (bottom) with 300x magnifications, a. Rayon ropes (RY), b. Ex-garment rope (CR), c. Brown linen rope (BL), d. Coconut coir rope(CC), and d. Nylon rope(NR).

Juvenile mussel preparation

The juvenile green shell was collected from the North coast of Java Sea (Brebes Regency) (Fig. 2) by using collector rope of 16 mm diameter with 5,5 m long. The rope was submerged for 35 days, and after 35 days the collector rope was collected and brought to the Pescica Marina Laboratory of Fisheries and Marine Faculty, Jenderal Soedirman University, Indonesia.

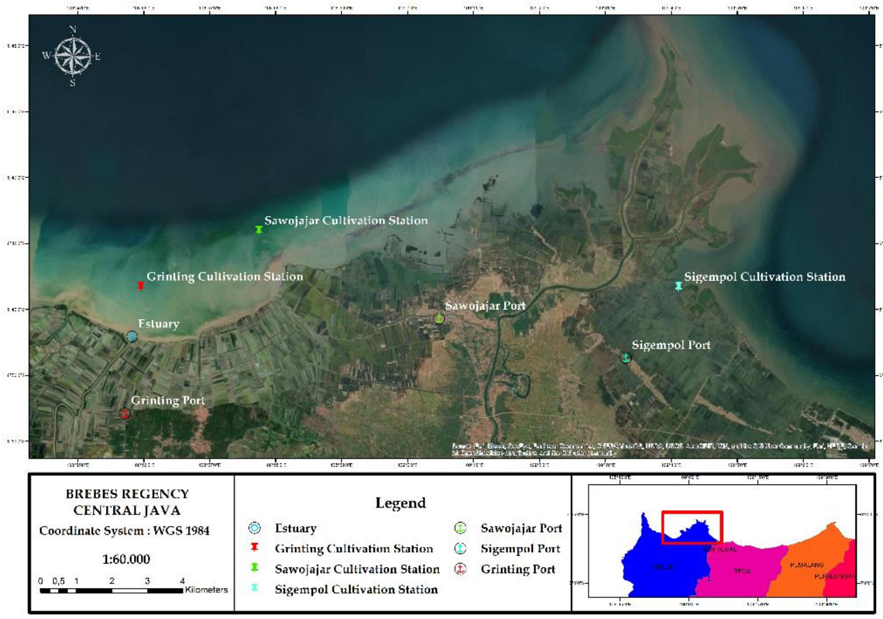

Figure 2. North Coast of Java Sea (Brebes Regency)

The juvenile mussels were size graded in to 1-1.5 mm of shell length by using graded sieve and observed by microscope. The juvenile mussels were photographed, and the shell length were measured by using image J (Fitzpatrick et al., 2013). The selected size of juvenile mussels were then put in a container with 20 L of filtered sea water (Sanjayasari and Jeffs, 2019). The juvenile mussel was acclimatised for 48 h in the container and fed with *Nannochloropsis oculate* with cell concentration ~2 × 10^5 cells $mussel^{-1}$ day^{-1} (Gui et al., 2016; Sanjayasari and Jeffs, 2019).

Experimental design

Forty conical tanks of 1.5 l volume were used as the experimental chamber (Sanjayasari and Jeffs, 2019). These experimental chambers were equipped with round air stone ~ 2 cm in diameter on the bottom of the tank to provide bubble air inside it with ~33 ml min^{-1}. Each of five types of experimental substrates ~20 cm were weighed down suspended within each tank by using a stainless steel nut.

Twenty of juvenile green mussels were put inside each of the experimental tank, and left alone for 24 h and 48 h after being fed with microalgae cell ~2×10^5 cells $mussel^{-1}$ day^{-1} (Gui et al., 2016; Sanjayasari, 2021). After the 24 and 48 h contact with the substrates inside the tank, the number of mussel attached on the substrates was counted without disturbing the mussels and ten randomly chosen settled mussels were selected from each experimental tank to count the number of byssal threads formed during the experimental period by using stereo microscope. The observation was carried out as quickly as possible (within minutes) to minimize the chance of further byssus development or detachment. The characteristic of the byssus production after strong aeration was observed by using *Scanning Electron Microscope* (SEM). Juvenile mussel attachment was the percentage of measurement on the number of juvenile mussels attached on the substrate divided by initial number of juvenile mussels inside each tank, these applied for two observation time i.e. 24 and 48 h.

Statistical analysis

A two-way ANOVA was used to compare mean attachment and mean byssus production of juvenile mussels among treatments for the experiment. The percentage data (i.e., attachment) were firstly square roots transformed prior to analysis. All data were assessed normality and homogeneity of variance test by using Shapiro-Wilk’s test and Levene’s test. Where an ANOVA was significant, the difference between pairs were then tested by using pairwise Tukey’s HSD post-Hoc test. The statistical analysis were performed using Minitab version 19, standard error were provided to show the variation of the mean throughout this manuscript.

Results

Attachment

After the 24 and 48 h of experimental period, there were differences in the mean of attachment percentage of juvenile mussels as a result of the different types of substrates ($p < 0.01$), however there were no difference on the number of mussels attach on the substrates with different aeration speed in 24 and 48 h ($p > 0.05$).

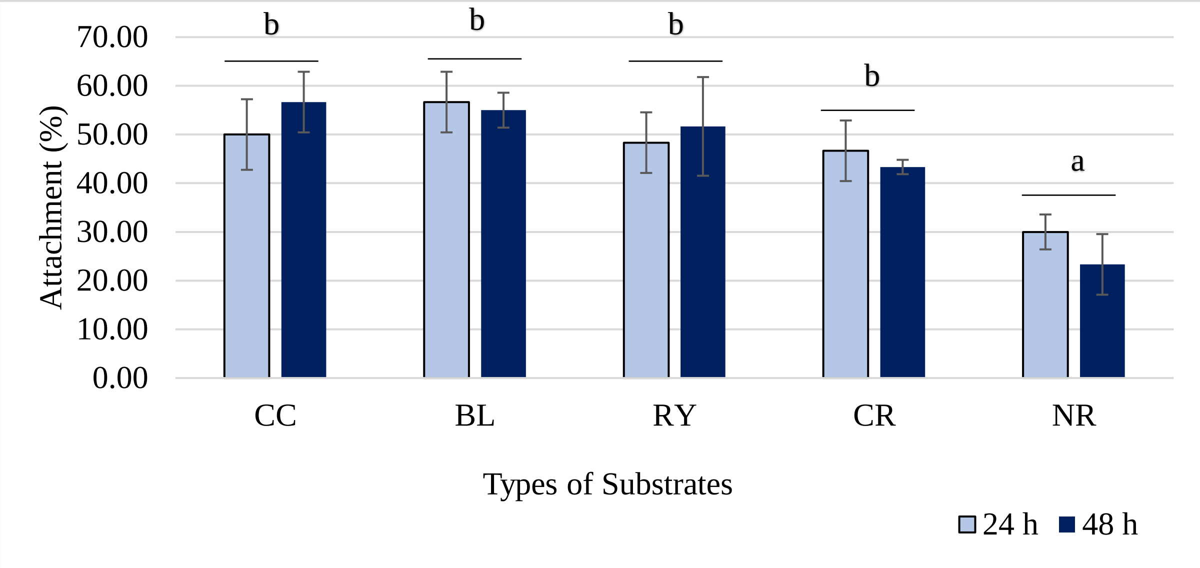

Figure 3. Mean percentage on the number of juvenile mussels attached on various substrates in 24 and 48 h experimental period. Abbreviation of substrates type; CC (Coconut coir), BL (Brown Linen), RY (Rayon Yarn), CR (Ex-garment Rope), NR (Nylon Rope). Different letters above the bar graph indicate significant difference between means for percentage of attachment on types of substrates (Tukey HSD, $p < 0.005$).

Among the types of substrates, the lowest mean on the number of juvenile mussel attachment was observed in the nylon rope, i.e. 30±2.89 % and 25±4.41% respectively for 24 and 48 h experimental period. Greater percentage of attachment were identified in the four other substrates (i.e. CC, BL, RY, CR) with the mean ranging from 43±1.67 % to 58±4.41% for both 24 and 48 h experimental period (Tukey HSD, $p < 0.005$).

Byssus production

There was differences on the number of mean byssus production after 24 and 48 h of experimental period ($p < 0.01$) regardless the types of the substrates. The longer experimental period (i.e., 48 h) showed the higher production of byssus at 21±3.84 compared to shorter experimental period (i.e., 24 h) 16±1.62 (Tukey HSD, $p < 0.05$) (Table 1).

Table 1. Byssus production from juvenile green mussels in the 24 and 48 h of experimental period on five types of substrates

Rope	Byssuss Production		Mean±SE of byssuss production on rope
	24 h	48 h	
CC	18±3.38	21±1.20	20 ± 1.97^{a}
BL	24±0.33	25±2.60	25 ± 0.57^{a}
RY	16±3.21	27±3.53	21 ± 5.39^{a}
CR	13±1.45	21±3.48	17 ± 3.57^{ab}
NR	11±1.20	8±0.88	10 ± 0.13^{b}
Mean±SE of byssuss production at each observation time	16 ± 1.62^{A}	21 ± 3.84^{B}	**19±1.44**

Different letter following the mean of byssus production value within column and row showed significance (Tukey HSD, $p < 0.05$). Abbreviation of substrates type; CC (Coconut coir), BL (Brown Linen), RY (Rayon Yarn), CR (Ex-garment Rope), NR (Nylon Rope).

After the 24 and 48 h of experimental period, there were differences in the mean of byssus production on five types of substrates ($p < 0.01$). The observation revealed the juvenile mussel which attached to nylon rope (NR) had the lowest mean of byssus production at 10±0.13 than the juvenile mussels attached to coconut coir rope (CC), brown linen (BL), and rayon yarn rope (RY) with the mean value of byssus production range from 20 to 25 (Tukey HSD, $p < 0.05$). However, the number of byssus produced from the mussels attached on NR was not different with the mean of byssus produced from the mussel attached on ex-garment rope (CR) 17±3.57, this value also statistically no difference to the number of byssus attached on CC,BL and RY (Tukey HSD, $p < 0.05$) (Table 1).

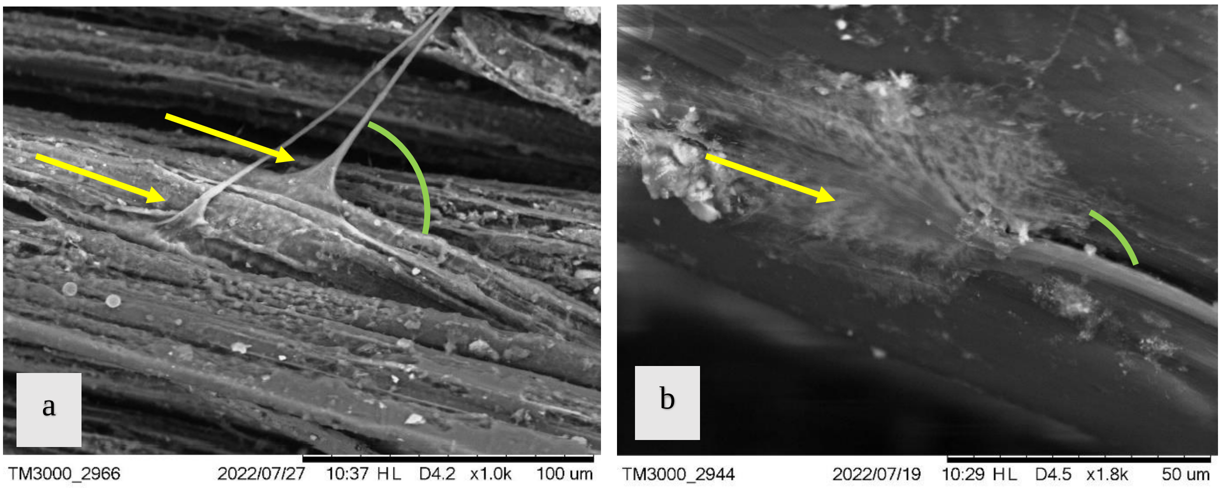

Figure 4. Characteristics of adhesive plaques on two types of substrates a) brown linen rope (BL), and b) nylon rope (NR), observed in 48 h experimental period. Increased number of plaques indicated stronger attachment of juvenile mussels.

After deposition, byssus thread production was increased to adhesive plaques formation (yellow arrow). The types of plaques produced differently according to the types of substrates (Fig. 4 a and b). The adhesive plaques produced by juvenile mussels attached to NR tend to have transparent and less anchor adhesive plaques (Fig. 4b) than the byssus plaques produced by juvenile mussels attached on BL rope (Fig. 4a). In addition, the angle of the adhesive plaques formation could be and indication of the byssus strength. The adhesive plaques of juvenile mussels produced wider angle than the adhesive plaques produce on the NR substates (green line, Fig. 4 a and b). This may be as a causal effect on the least number of juvenile mussels which attached to NR.

Discussion

The attachment of juvenile *Perna viridis* are influenced by various factors such as, water motion, food availability, potential substrates to settle, and the life cycle of the mussels. The present study revealed that attachment on the juvenile green mussels may possibly influence by the types of substrates, however the longer of experimental period (i.e. 48 h) did not indicate on the addition of the juvenile mussel attachment. These results have significant implications for early stage of mussels life cycle on the settlement process under natural and farm condition.

The early stage of mussels have secondary settlement behaviour (Alfaro and Jeffs, 2003; Sanjayasari, 2021), which allow them to attach and the detach themselves on various substrates for several times until the mussels achieve certain size (i.e. ~6 mm). The juvenile mussels may be found to settle on hydroids, filamentous seaweed, debris (Alfaro and Jeffs, 2003) which able to protect them from predators and external mechanical action (i.e., wave action). Since, this size class of mussels are highly mobile through their byssus-pelagic drifting behaviour and selective of their attachment substrates, hence, it would be necessary to find an optimum substrates as their attachment substates. Because it plays an important role for establishing juvenile *Perna canaliculus* into adult mussels. Approximately 70% of juvenile green-lipped mussel were found attached on hard substrates (Wilcox and Jeffs, 2017). Juvenile of *Pinctada maxima* was observed to have 3× higher settlement on the synthetic materials (i.e., garden shade spiral) at ~75 spat per string compare to natural collector rope (i.e. bamboo matting) ~25 spat per string (Libini et.al., 2013). In contrast, current study revealed that juvenile *P. viridis* preferred to attach on substrates which natural material (i.e., coconut coir rope (CC), brown linen (BL) and rayon yarn rope (RY)) which has ~2.5 × higher percentage of attachment compared to the juvenile mussels attached on synthetic material (i.e., nylon rope (NR)) (Fig. 3 and 4). The study on *P. viridis* showed that ~92% of mussels attached on natural material of rope and only 30% of juvnenile mussel attaches on synthetic material of rope after 8 weeks of experimental period (Sulvina et al., 2015). The explanation of the juvenile mussels tends to have higher attachment on the natural materials could be due to the filamentous surface of these materials (i.e., RY, CC, and BL) (Fig. 1a, c, d). Previous study reported that juvenile *P. canaliculus* like to attached on filamentous substrates (i.e., seaweed) due to the structural stability related on the degree of macroalgae branching which may protect the mussels from micro-environmental conditions (i.e., waterflow, salinity, oxygen concentration, substrate stability) (Alfaro et al., 2004).

During the repositioning or settlement, byssal attachments play an important role in the early stage of mussels, as it may determine the future development of mussel (Wilcox and Jeffs, 2017). The present study showed that the byssus production of juvenile *P. viridis* increased during the experimental period (i.e., 24 versus 48 h) regardless the types of substrate (Table 1). In addition, the juvenile mussels produced higher number of byssus production when attached on substrates made from natural materials (i.e., CC, BL, RY) compared to the synthetic substrate (i.e., NR). Previous study reports that the mussels will increase the number of byssus thread in order to enhance their attachment strength (George and Carrington, 2018). The attachment strength is a function of both biochemical properties and mechanical ability of the mussels with the habitat in which the mussels settled (Babarro et al., 2008). Beside the water motion, there are several factors which may trigger the mussel attachment, i.e., size of mussels and chemical interaction between byssal thread and substrates (Carrington et al., 2015). The smaller the size of juvenile mussels, the higher byssal thread production (George,and Carrington, 2018). The juvenile mussels size in current study < 3mm in shell length, with byssus production maximum 25 byssi on CC substrate within 24 h experimental period. On the other hand, other study in juvenile *P. canaliculus* showed maximum byssus production was at 18 under three water flow treatments within 24 h observation time (Alfaro, 2006). These results indicate that the result of byssus production may vary under different experimental treatments.

Chemical interactions between the byssus and the substrates in which the mussels reside may also trigger the attachment stimulation. The present study revealed that the juvenile mussels prefer to settle on the natural material of substrate (i.e., CC, BL. RY). Previous study reported that the protein compound in the byssal thread will develop strong binding attachment when interact with certain chemical cues as DOPA (dihydroxyphenylalanine) (Lee et al., 2007). DOPA is an amino acid which usually contain in some plants and animals. However, there is still limited study which explaining the material of substrates that used in the study containing DOPA or its derivates. Other experiments on testing six artificial substrates (i.e., acrylonitrile butadiene styrene (ABS) plastic, high density polyethylene (HDPE), concrete underlayment board (CUB), aluminium, stainless steel and fiberglass to collect quagga mussel (*Dreissena bugensis*) revealed that the mussels had better attachment to natural surfaces (concrete board), fairly strong attachment on metallic surfaces (aluminium and stainless steel), less strong attachment to polymetric surfaces and the weakest attachment showed the HDPE plates (Mueting et al., 2010). The reason of the juvenile mussels tend to attach on substrates which made from natural materials compared to the synthetical substrates is possibly due to the conductivity characteristic of the substrates (Tamarin et al., 1976). The substrates which are made from natural material had better conductivity than the synthetical materials. This may be the reason of juvenile mussels' preferences in selectively choosing their attachments substratum on the natural materials (i.e., CC, RY. BL).

Conclusions

The increase in the strong attachment may contribute to minimize juvenile *Perna viridis* loss in the ocean during their repositioning and settlement in the early stage of their life. Present study suggests that providing substrate which made from natural materials may potentially improve juvenile mussel attachment through the increase in the number of byssal threads and plaques production. Substrate choice by mussel farmers could have bring benefit to improve the mussels seed recruit in the nature and reducing the loss of the mussel due to secondary settlement behaviour. However, such information required further investigation in the near future. The result of current study is a first step to develop collector substrate rope to optimize Asian green mussel production in Indonesia.

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