Effectiveness of seaweed (*Caulerpa lentillifera*) as biofilter in vanamei shrimp (*Litopenaeus vannamei*) culture

[1]Margono, [2]Jana T. Anggadiredja, [3]Mochammad Nurhudah

[1] Department of Aquaculture, Faculty of Fisheries Resources Utilization, Jakarta Technical University of Fisheries, Jakarta, Indonesia; [2] National Resilience Institute, Republic of Indonesia; [3] Marine and Fisheries Polytechnic of Karawang, Indonesia. Corresponding author: Margono, margono.bappl.stp@gmail.com

Abstract. Increased production of vanamei shrimp farming to meet market demand has an impact on increasing stocking densities and feed usage during aquaculture activities. This increases the residual waste of aquaculture, in the form of organic and inorganic materials in the maintenance media. The waste causes the quality of water to decline, triggering various diseases that result in low production. The efforts made to overcome water pollution include using different seaweeds as a biofilters. *Caulerpa lentillifera* is a seaweed that can act as a biofilter, absorbing pollutants in the culture media. This study aimed to analyze the effectiveness of *C. lentillifera* as a biofilter and an economically valuable cultivation commodity. The study used a completely randomized design (CRD) with three treatments and three replications: vanamei shrimp (*Litopenaeus vannamei*) and *C. lentillifera* separateed from the cultivation media (separate biofilter, A); shrimp and *C. lentillifera* maintenance in one shrimp culture media container (biofilter combined/polyculture, B); shrimp without *C. lentillifera*, as control (monoculture, C). The parameters measured were water quality (turbidity, total organic matter and ammonia) and production performance (survival rate, average daily growth and biomass). The turbidity values in treatments A, B and C were 2.48±0.3, 3.18±0.62 and 3.55±0.67 NTU, respectively. The total organic matter was 120.01±0.15, 98.46±0.17, and 134.50±0.15 mg L^{-1}, in treatments A, B and C, respectively. Ammonia values were 0.42, 0.42 and 0.56±0.01 mg L^{-1} in treatments A, B and C, respectively. The turbidity, total organic matter and ammonia values in treatment A and B were lower than in C. The survival rate of shrimp in treatment A was higher (78.67±1.22%) than in treatment B (77.77±1.00%) and C (66.66±1.44%). The average daily growth of treatment B shrimp was the highest, with a value of 0.299 g day^{-1}, with the lowest feed conversion ratio value, of 1.73. The final biomass of *C. lentillifera* was higher in treatment B, 342.98 g, while the final biomass of *C. lentillifera* in treatment A was 157.33 g.

Key Words: inorganic matter, organic matter, polyculture, quality of water, residual waste, vanamei shrimp.

Introduction. Shrimp is the main commodity in Indonesia (Indonesian Fishery Producers Processing and Marketing Association 2019). Shrimp production increased from 147000 tons (1.42 billion USD) in 2017 to 180000 tons (1.8 billion USD) at the end of 2018 (Ministry of Marine Affairs and Fisheries 2018). One species of shrimp that is cultivated is vanamei shrimp (*Litopenaeus vannamei*). Shrimp present some advantages, including high resistance to diseases, high levels of productivity, and lower protein requirements than for some other species of shrimp, like tiger shrimp (*Penaeus monodon*) (Cholik et al 2005).

Intensive vanamei shrimp culture has an impact on the use of artificial feed in large quantities compared to traditional or semi-intensive systems. This results in a decrease in the quality of water (Cholik et al 2005). Of the feed administered in the cultivation process, 10% will dissolve in the water medium, 90% is eaten by the shrimp, out of which 15% remains undigested and is delivered back into the water medium; the remaining 75% is considered digested (Lin et al 1993). However, 50% of the digested feed will experience excretion into metabolic waste (Troell et al 1999). The excreted compounds undergo chemical processes assisted by bacteria, resulting several compounds, including ammonia (NH_3). These compounds are toxic and threaten the survival of shrimp. More intensive cultivation activities will lead to higher ammonia production as well. This is because the amount of feed administered is directly proportional to the shrimp stocking density (Amri 2004). The stocking density of 50 vannamei shrimp per m^{-2} in 2500 m^{-2} cultivation area can produce 108.49±1.53 kg N and 56.13±6.56 kg P waste (Syah et al 2006). If the stocking density is increased, more waste will be generated. This is shown by Syah et al (2014), where at a stocking density of vanamei shrimp of 500-600 per m^{-2}, in an area of 1000 m^{-2}, and a water depth of 1.75-1.8 m, the resulting N retention value was 30.47-33.34% and the P retention was 16.59-18.05%. This shows that the load of N and P waste generated during 105 days of cultivation was 406.57 - 532.20 kg N and 100.33 - 119.50 kg P. According to Atjo (2013) and Suwardi et al (2014), the value of the feed conversion ratio in the cultivation of vanamei shrimp ranges from 1.3-1.6. Boyd & Massaut (1999) shows that if a farming activity has a feed conversion ratio value of 1.5, it produces N waste between 12.6-21 kg and P waste of 1.8-3.6 kg for each ton of cultivated shrimp biomass.

The accumulation of organic matter in the form of leftover food, urine and feces has an impact on eutrophication and hypernutrification, which causes a decrease in water quality, which subsequently causes disease and death in shrimp (Lin et al 1993; Boyd 1999; Horowitz & Horowitz 2000; Montoya & Velasco 2000; Pilay 2004; Palayukan et al 2016). Shrimp disease is caused by viruses, bacteria, fungi, protozoa, nutrients, toxic and environmental diseases (Schnieszko 1974). Other factors are stress due to high density, malnutrition, poor handling, parasitic infections, high organic matter, low oxygen, and poor water quality (Maskur et al 2014).

Efforts are being made to improve the quality of aquaculture water by utilizing seaweed as a biofilters. *C. lentillifera* is a seaweed consisting of stolons and ramuli. Ramuli have shapes resembling grapes and contains bioactive compounds that can absorb cultivation waste. In this research, *C. lentillifera* seaweed was used as a biofilter for vanamei shrimp culture. The economic benefits of using seaweed can provide added value to increase productivity in aquaculture activities.

This study was conducted to determine the effectiveness of *C. lentillifera* in improving water quality in shrimp culture, due to the lack of research on the use of *C. lentillifera* seaweed as a biofilter in semi-intensive vanamei shrimp culture.

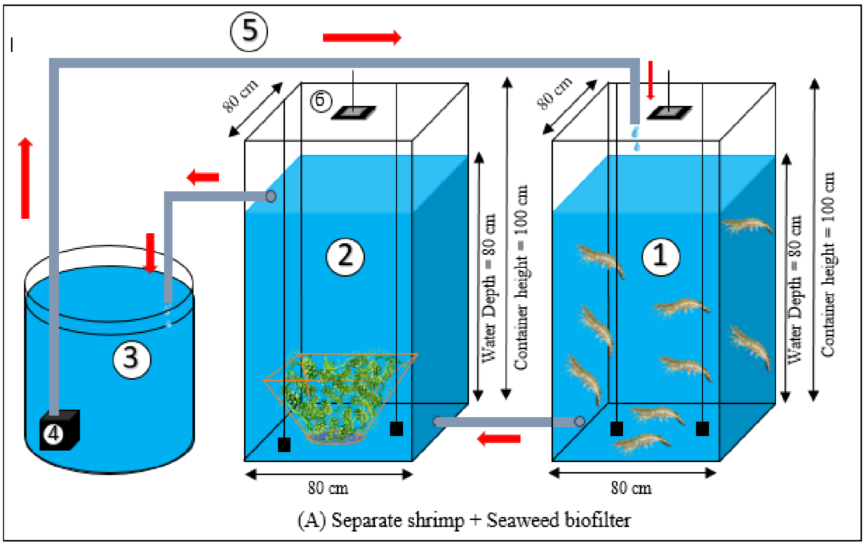
Material and Method

Time and place. This research was conducted from September to November 2019, at the Jakarta Fisheries Institute Laboratory Serang, Banten, Indonesia.

Experimental design. A completely randomized design (CRD) was used, with 3 treatments and 3 replications: A - separate biofilter (maintenance of vanamei shrimp + *C. lentillifera* seaweed separated in maintenance container, and water recirculated periodically) (Figure 1); B - combined biofilter (maintenance of vanamei shrimp + seaweed combined in a maintenance container) (Figure 2); C - control (maintenance of vanamei shrimp without seaweed) (Figure 2).

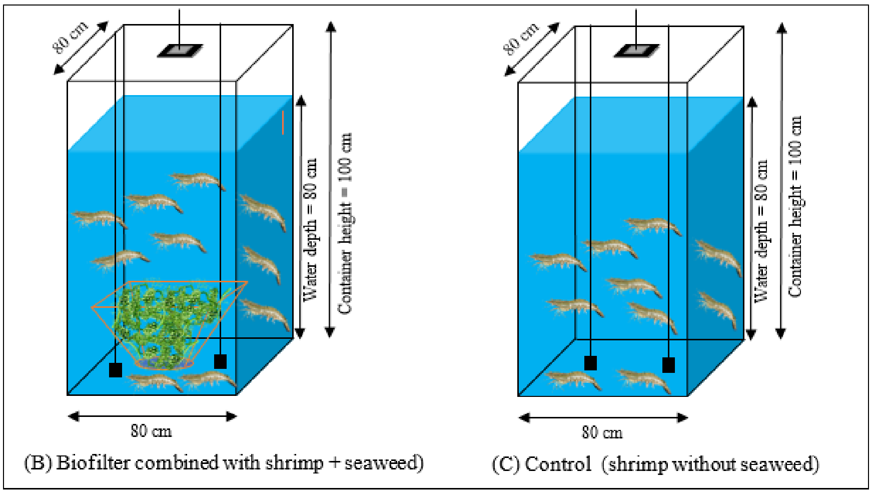
Equipment and materials. The equipment used in this study included pumps, digital scales, refractometers, thermometers, pH meters, DO meters, Lux meters, Hi-blow, LED lights. The ingredients used included vanamei shrimp, seaweed (*C. Lentillifera*), and brackish water at a salinity of 30 ppt.

Containers and media. Maintenance tanks were made of wooden planks and coated with 0.05 mm thick HDPE (high density polyethylene) plastic. There were 12 maintenance tanks, each with a dimension of 80x80x100 cm. Maintenance containers were washed thoroughly using clean water before use. The maintenance containers were prepared according to the experimental design, then each installed with 2 aerated units and an LED lamp with 100 watts for the needs of seaweed. The maintenance media was first collected in a reservoir and sterilized using chlorine at a dose of 50 ppm. After the water was considered sterile, it was placed into the maintenance containers. The water depth was 80 cm, with a volume of 512 liters per container. Salinity of aquaculture media was 30 ppt.



(A) Separate shrimp + Seaweed biofilter

Figure 1. Biofilter system design (A).



(B) Biofilter combined with shrimp + seaweed)

(C) Control (shrimp without seaweed)

Figure 2. Design drawings of combined biofilter treatment (B) and treatment control (C).

Shrimp and seaweed rearing. Vanamei shrimp used were juveniles with an average weight of 4.38±0.02 g. The stocking density of each container was 25 shrimp per 512 L. *C. lentillifera* with a weight of 500 g was used in the 512 L of water. Before the shrimp were stocked, they were acclimatized to the new environment. Shrimp stocking was done in the afternoon, to avoid stress. Likewise, seaweed was distributed before acclimatization in the cultivation media, in the afternoon.

Vanamei shrimp were administered commercial feed in the form of crumble size pellets with 36% crude protein, 4% of total biomass. Feeding was carried out 4 times a day, at 07.00 am, 12.00 am, 04.00 pm, and 09.00 pm. Growth sampling was conducted every 10 days in the 90 days of cultivation to determine the average daily growth (ADG), survival rate (SR), food conversion ratio (FCR), and biomass. Water replacement was carried out every 10 days with a turnover volume of 10%.

Test parameters. The physical water parameters observed were: temperature, using a thermometer; salinity, using a refractometer; pH, using a pH meter; dissolved oxygen (DO), using a DO meter; light intensity, which was measured at the time of the initial preparation of the maintenance using a Lux meter. The chemical parameters measured included ammonia, nitrites, nitrates, total organic matter (TOM), and turbidity, which were measured every 10 days during the study. Biological parameters measured include bacteria (total vibrio and total bacteria). Cultivation performance parameters include ADG and SR.

Average daily growth. Analysis of ADG of *C. lentillifera* and vanamei shrimp was calculated using the following formula (Effendie 1997):

$$ADG = \frac{Wt - Wo}{t}$$

Where: Wt - final sampling weight; Wo - initial sampling weight; t - time.

Survival rate. The survival rate of vanamei shrimp during maintenance was calculated using the formula (Effendie 1997) as follows:

$$\mathrm{SR} = {}^{Nt}/_{No} \times 100\%$$

Where: Nt - the number of shrimp on the t-day; No - number of shrimp on initial stocking.

Food conversion ratio. FCR is the ratio of the amount of feed (kg) administered to produce 1 kg of shrimp. FCR can be calculated using the formula (Farchan 2006) as follows:

$$\mathrm{FCR} = \frac{\text{Amount of feed given (kg)}}{\text{Increased shrimp biomass (kg)}}$$

Data analysis. The effect of the treatment of the parameters to be observed was tested using ANOVA, with the F test at a 95% confidence interval.

Results and Discussion. The TOM values in the three treatments (Figure 3) showed significant differences. TOM consists of suspended, colloidal and dissolved material in the maintenance media (Syandri et al 2017). Based on the results of the study, the highest TOM concentration was in treatment C (135.51 mg L^{-1}), while treatment B showed the lowest TOM value, 98.49 mg L^{-1} (Figure 3). The highest TOM value was found in treatment C; this indicates that the organic matter suspended in the water has a high concentration. The cause of high TOM value is organic material such as the excretion of shrimp in the form of feces and urine from metabolic waste (Andriani et al 2017). Another factor that influences the value of TOM is the presence of seaweed that acts as a biofilter (Haq et al 2011; Silkin et al 2012).

The feces, urine, and food waste that are not consumed are decomposed by nitrifying bacteria, resulting ammonia, among others (Sichula et al 2011). Ammonia undergoes a nitrification process into nitrite and nitrate compounds. The conversion of ammonia compounds from the metabolic waste of shrimp and uneaten feed to nitrite is carried out by *Nitrosomonas* sp. Nitrite is then converted by *Nitrosococcus* sp. to nitrate (Feliatra 2001). Nitrate is used by seaweed as an ingredient or nutrient that supports the growth process. Seaweed acts as a biofilter of TOM, ammonia, and nitrites. Therefore, the TOM values in treatments A and B are lower than those of treatment C, because both treatments use seaweed as biofilter. This causes the rest of the metabolism products of the test organisms to decompose better than just using nitrifying bacteria. Nitrification results in the form of nitrate compounds are absorbed by seaweed and react with other compounds in the form of carbon and water in the process of making food (Haq et al 2011; Silkin et al 2012). However, the TOM values in treatments B and C differed significantly ($p<0.05$), but treatment A did not differ significantly from treatments B and C ($p>0.05$).

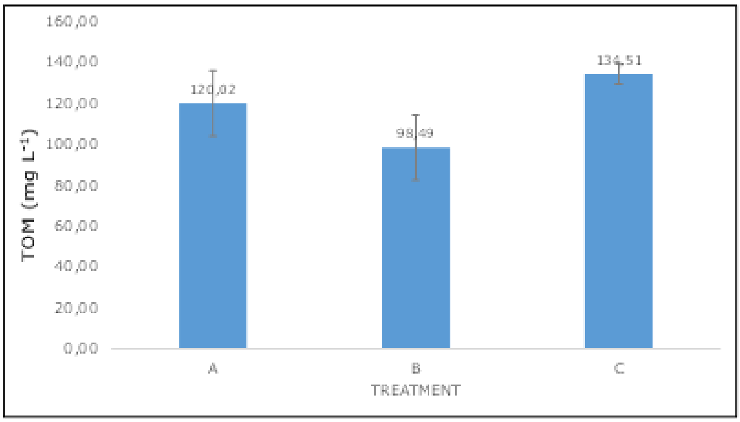


Figure 3. Total organic matter (TOM) concentrations in the maintenance systems; the whiskers represent the value range of TOM in mg L^{-1}.

Ammonia concentration is presented in Figure 4a. Treatment C has the highest concentration of ammonia, 0.55 mg L^{-1}. The lowest ammonia concentration is in treatment B, with 0.42 mg L^{-1}. Ammonia is derived from organic materials such as feces, urine and leftover food that has been decomposed by bacteria (Bhakta 2006). Therefore, high ammonia concentrations in treatment C are thought to be due to low assimilation rates or decomposition of ammonia compounds. Decomposition of ammonia compounds is carried out by bacteria (Ekasari 2009). In addition, the high concentration of ammonia in treatment C is suspected to have occurred because there is no seaweed as a biofilter, so the nitrites and nitrates were not utilized and converted back into ammonia during anaerobic conditions. Treatment B had the lowest ammonia concentration, presumably because the seaweed utilized nitrate, so that the concentration of inorganic compounds in the media was influenced by this. This is presented in Figure 4c, where the nitrate concentration of treatment B (8.53 mg L^{-1}) is the highest concentration. The lowest ammonia concentration in treatment B shows that the ammonia compound has been converted into nitrate due to the presence of seagrass.

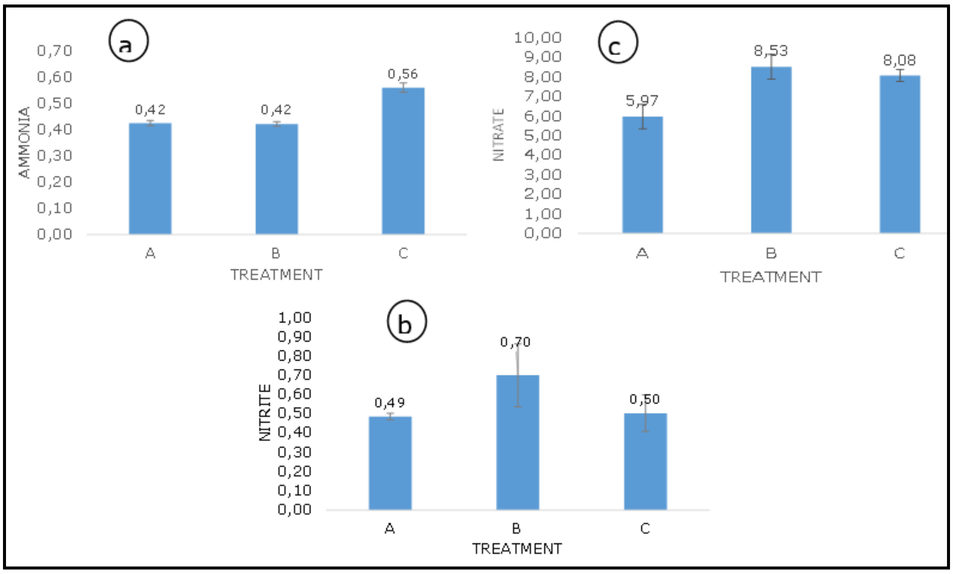


Figure 4. The concentration (mg L^{-1}) of ammonia (a), nitrite (b), nitrate (c) in the maintenance systems; the whiskers represent the value ranges of ammonia, nitrite and nitrate in mg L^{-1}.

Figure 5 shows that the total bacteria present in the water is directly proportional to the amount of organic material utilized and the ammonia formed. This occurs because chemical reactions that take place in the formation of bacterial biomass are supported by the presence of ammonia, carbon elements derived from carbohydrates, bicarbonate ions, and DO (Ekasari 2009). Ammonia can be nitrified in aerobic conditions where there is DO in the water. The nitrification process is the process of converting ammonia to nitrite and nitrate in an aerobic state (Burghate & Ingole 2014). Therefore, if the concentration of ammonia is high, the nitrite found in the maintenance media is low, because ammonia has not been yet converted to nitrite (Figure 4). The lowest value of total bacteria was in treatment C. The total bacteria value in a maintenance medium is determined by several factors, including the availability of nutrients for the process of assimilation to form biomass (Mekala et al 2016). Thus, the high total bacteria in treatment B shows that the ingredients or nutrients needed for bacterial growth are at high concentrations. This is indicated by the highest total bacteria present in treatment C media (Figure 5).

Seaweed used in this research was *C. lentillifera*. This seaweed is a macroalgae that grows well in the tropics (Nurjanah et al 2019). Seaweed growth performance is influenced by several factors, including light intensity affecting photosynthesis (Guo et al 2015; Iskandar et al 2015), heterotrophic bacteria, and turbidity (Darmawati et al 2016). In this study, all treatments used a light bulb with an intensity of 2500 lux. According to Iskandar et al (2015), light intensity in the range of 400-3500 lux is optimal for *Caulerpa* sp. Total bacteria and turbidity are presented in Figures 5 and 6, respectively.

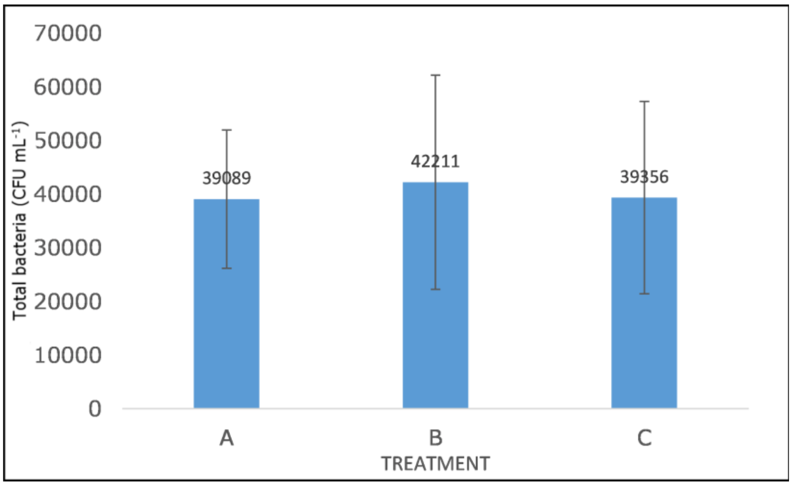


Figure 5. The total bacteria in the maintenance systems; the whiskers represent the value range of total bacteria in CFU mL^{-1}.

Turbidity represents the ability of light to penetrate water to a certain depth (Ngueku 2014). Figure 6 shows that the turbidity value in treatment C was the highest among treatments. That is caused by treatment C not applying the polyculture system with *Caulerpa* sp. as a biofilter for the remediation process, so that organic material that causes turbidity is not utilized (Huey & Meyer 2010). Treatment A shows the lowest turbidity value. As stated by Ekasari (2009), bacteria need ammonia and other organic materials for body mass formation through assimilation. The optimal value of turbidity in the maintenance of vanamei shrimp is lower or equal to 30 cm (Santosh & Singh 2007). Therefore, treatment C showed the highest turbidity, with the lowest light intensity. Treatment B had a higher turbidity compared to treatment A, so it appears that treatment B received less light compared to treatment A. The incoming light affects the growth of seaweed, influencing photosynthesis (Poespowati et al 2013).

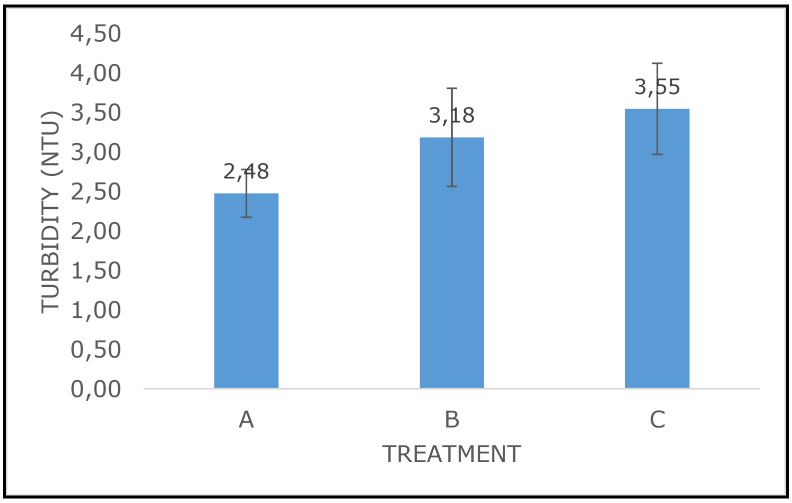


Figure 6. Turbidity values in the maintenance systems; the whiskers represent the value range of turbidity in NTU.

The optimal temperature, DO and salinity values for the maintenance of vanamei shrimp are 27-29°C, 4-6 mg L^{-1} and 28-32 mg L^{-1} (Buckle et al 2006). Optimal temperature, DO, pH, and salinity values for *Caulerpa* sp. growth are 25-32°C, 3.2-6.4 mg L^{-1}, 6-9, and 25-30 mg L^{-1}, respectively (Iskandar et al 2015). The optimal concentration and quality of water in the maintenance media of all treatments facilitated biological and chemical processes. However, there are differences in the systems implemented (Table 1). The feces and urine of shrimp, as well as uneaten feed can be reused by seaweed as a nutrient for the growth of seaweed (Monagail et al 2017).

Table 1
Temperature, dissolved oxygen, pH, and salinity of the maintenance media with different systems

Treatment	*Parameter*			
	Temperature	*Dissolved oxygen*	*pH*	*Salinity*
A	28.7-30	3.7-4.8	7.1-7.3	30
B	28.8-29.8	3.8-5	7-7.4	30
C	28.5-30	3.9-5	7-7.3	30

Caulerpa sp. has antibacterial compounds, including alkaloids, flavonoids, phenols, and saponins (Saputri et al 2019). The utilization of *Caulerpa* sp. in the polyculture system as a biofilter produces a higher survival rate. Figure 6 shows that treatment C, which did not apply the polyculture system and seaweed as a biofilter, had the lowest survival rate. The *Vibrio* sp. in the highest amount was in treatment A, with 27066 CFU mL^{-1}, while treatment C had the lowest total *Vibrio* sp. (Figure 7). The high concentration of *Vibrio* sp. in treatment A is suspected to occur because the system applied a separate polyculture, with the possibility of organic matter accumulation that could have stimulated the growth of *Vibrio*. *Vibrio* is a genus of pathogenic bacteria that spreads rapidly and can produce a mortality rate of 85%, causing vibriosis in aquatic animals, including shrimp (Sarjito et al 2015).

Good or optimal water quality is positively correlated with the stress level of the test organisms (Yildiz et al 2017). This can be known through a number of growth performance parameters, including SR, FCR, ADG, and biomass growth.

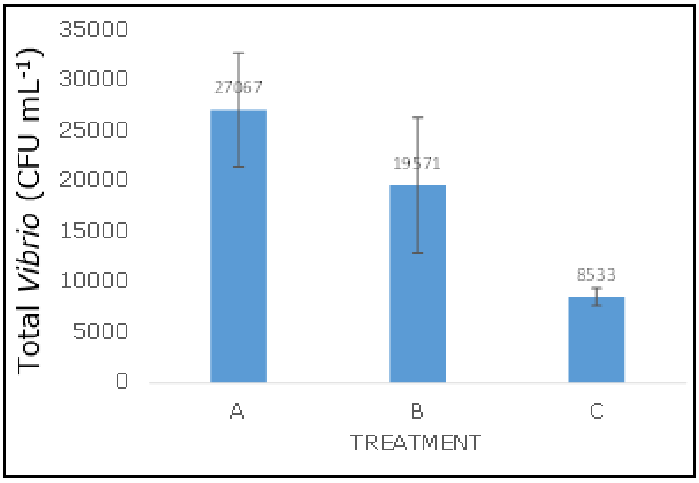


Figure 7. Total *Vibrio* in the maintenance systems; the whiskers represent the value range of total *Vibrio* in CFU mL^{-1}.

Figure 8 shows that the highest SR was in treatment A (78.67%), but there was no significant difference ($p>0.05$) between treatments. The value of the SR is influenced by several factors, including the environmental quality (Mannan et al 2012). The SR value in treatment C was the lowest, presumably because treatment C does not apply seaweed as a biofilter. SR is influenced by ammonia, because it is toxic and interferes with the physiological conditions of the test organisms, creating stress and low survival rates (Joel & Amajuoyi 2010).

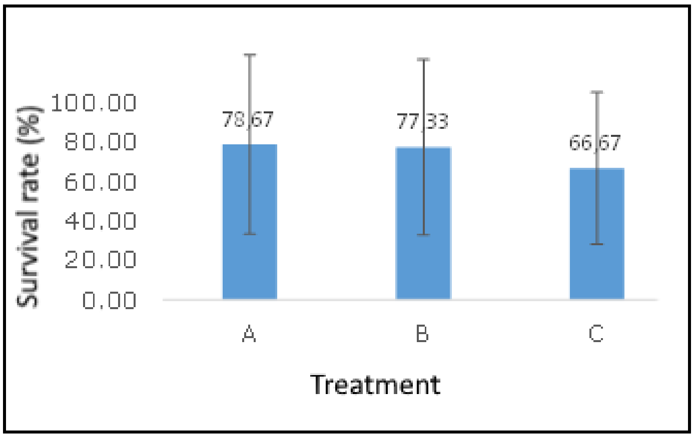


Figure 8. Survival rate of vanamei shrimp (*Litopenaeus vannamei*) in the maintenance systems; the whiskers represent the value range of survival rate (%).

Figure 9 shows that the best FCR value is in treatment B (1.73), while the highest FCR value was in treatment C (2.5). Treatment B had the lowest FCR, so it can be concluded that treatment B requires less feed compared to the other treatments in the formation of 1 g of meat. Therefore, treatment B utilizes feed more efficiently. This is also supported by the total bacterial in treatment B, where the highest value was found (Figure 5). This shows that there could be additional digestive enzymes produced by heterotrophic bacteria that help improve digestibility in shrimp. The seaweed biofilter system in treatment B produced a good state of the maintenance media, so that shrimp experienced less stress, had the highest SR, and lowest FCR.

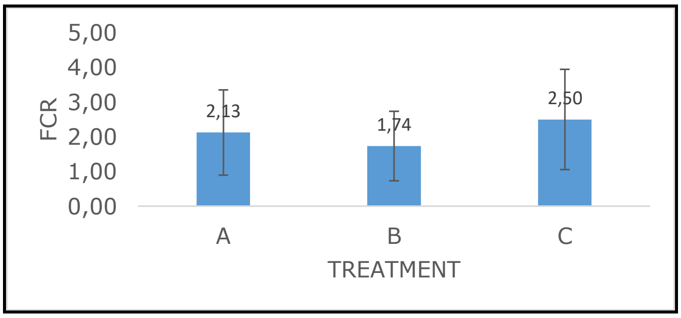


Figure 9. Food conversion ratio (FCR) in the maintenance systems; the whiskers represent the value range of FCR.

ADG is the daily growth measured during maintenance. The highest ADG value is in treatment B (0.299 g day^{-1}) (Figure 10). This is because treatment B has the best FCR (Figure 9). A factors that influences growth is the digestibility of the food consumed (Yeo et al 2017). Thus, the efficiency in the use of feed is directly proportional to the growth obtained. Figure 11 shows the increase in shrimp biomass in each treatment. Initially, all treatments started with the same amount of biomass, but at the end of the study the amount of biomass that showed the highest increase was in treatment B (431.3 g).

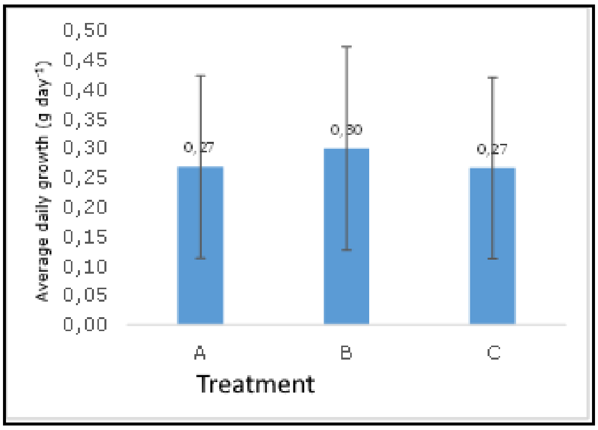


Figure 10. Average daily growth (ADG) of vanamei shrimp (*Litopenaeus vannamei*) in the maintenance systems; the whiskers represent the value ranges of average daily growth in g day^{-1}.

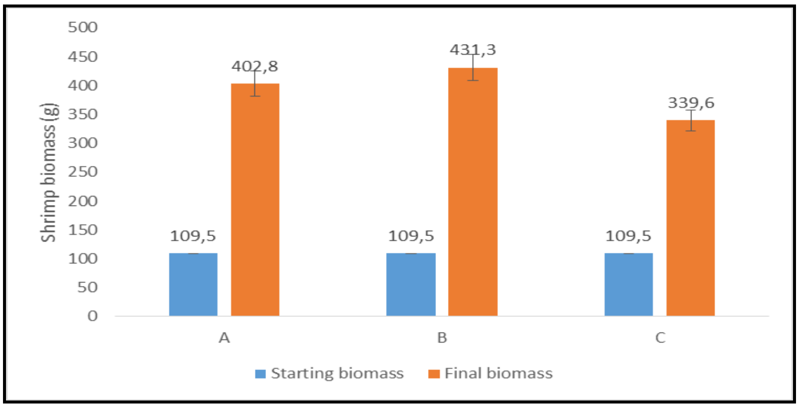


Figure 11. The initial and final biomass of vanamei shrimp (*Litopenaeus vannamei*) in the maintenance systems; the whiskers represent the value rnages of shrimp biomass in g.

Figure 12 shows changes in seaweed biomass. Treatment A showed a decrease to 157.33 g in biomass at the end of sampling. However, treatment B did not show a sharp decrease in seaweed biomass, with a final weight of 399.66 g. This is presumably because the water quality in treatment A did not support the growth of seaweed. According to Guo et al (2015) and Darmawati et al (2016), seaweed growth is influenced by turbidity, total bacteria and the availability of organic matter.

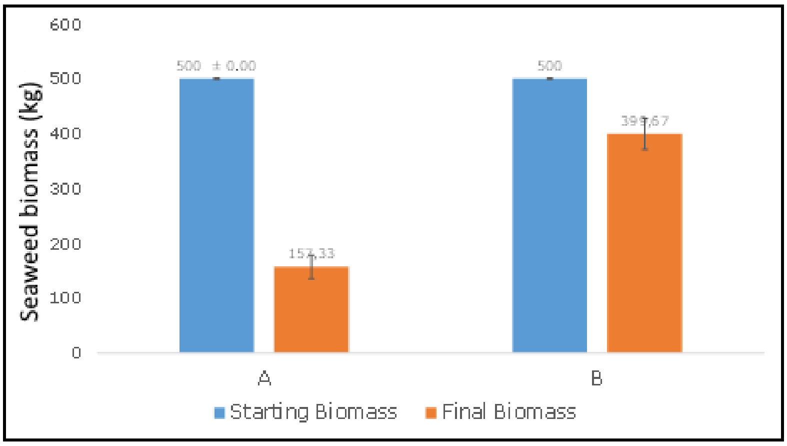


Figure 12. Initial and final biomass of seaweed *Caulerpa* sp. in the maintenance systems; the whiskers represent the value ranges of seaweed biomass in kg.

The decrease in seaweed biomass in treatment A was suspected because treatment A contained the lowest nitrate compound (Figure 4c). According to Sabirin et al (2015), seaweed utilizes nitrate compounds as a supplier of nitrogen for the process of food formation. Therefore, the low concentration of nitrate causes seaweed in treatment A to lack nutrients, so that the biomass decreases. In addition, the presence of heterotrophic bacteria in the treatment is the lowest (Figure 5), so that the nitrification process from ammonia to nitrate is not optimal. Even though optimal light intensity was provided, the final period of maintenance had an increased turbidity value, which affected the entry of lamp lights. This is thought to have inhibited the growth of seaweed.

Conclusions. Seaweed *C. Lentillifera* is incorporated effectively as a biofilter for improving water quality and increasing the growth performance of vanamei shrimp. The use of seaweed as a biofilter can maintain water quality, lower ammonia concentration and improve the growth rate.

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

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Authors:
Margono, Jakarta Technical University of Fisheries, Department of Aquaculture, Faculty of Fisheries Resources Utilization, AUP 01 St., Pasar Minggu 12520, Jakarta, Indonesia, e-mail: margono.bappl.stp@gmail.com
Jana Tjahjana Anggadiredja, National Resilience Institute, Medan Merdeka Sel. No. 10, RT.11/RW.2, Gambir, Kecamatan Gambir, 10110 Jakarta, Indonesia, e-mail: ina_seaweedsociety@yahoo.com
Mochammad Nurhudah, Marine and Fisheries Polytechnic of Karawang, Lingkar Luar Tanjungpura St., Karangpawitan, West Karawang, Karawang Regency, 41315 West Java, Indonesia, e-mail: mochnurhudah@kkp.go.id

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