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THE ROLE OF SEAWEED *(Gracilaria verucosa)* IN CO-CULTIVATION WITH TIGER SHRIMP (*Penaeus monodon*) AS AN ECOLOGICAL INTENSIFICATION

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ABSTRACT

The use of seaweed as ecological tools for improving environmental condition has been proved to sustain shrimp production. However, adding too much seaweeds caused oxygen depletion and nutritional competition. The objective of this study was to determine the optimum density of *G. verucosa* for supporting the growth of monodon shrimp. The research was conducted in Tambakbulusan village, Demak Regency with 20 fiber semi outdoor tanks. The tanks were used for the experiment without seaweed and with four densities of seaweed (50, 100, 150 and 200 g.m^{-2}) using hanging rope and were co-cultivated with 80 individuals of post larva (2.0-2.9 g) monodon shrimp in 800 L brackish-water system. The concentration of TAN, NO_3, and NO_2 were monitored every week, while dissolved oxygen (DO), pH, temperature, and salinity werechecked daily for 30 days. The highest shrimp survival rate (79.75%) and growth rate (6.6%day^{-1}) were obtained in the 100 g.m^{-2} of *G. verucosa*, significantly higher than in other treatments. Furthermore, seaweed density has a decreasing effect of NO_3 content (g/L) in RL100 from 0.26 to 0.16. and the lowest concentration of NO_2 and NO_3 were determined. Conversely, the highest density of seaweed (200 g m^{-2}) gave the lowest survival rate of shrimp (53.67%) due to high NO_2 content. DO had various values depending on the density and drop to less than 3.5 ppm in the highest density. In all treatments, pH, temperature and salinity werein a good range for shrimp culture. Thus, 100 g.m^{-2} of seaweed was optimal for ensuring the function as nutrient removal in shrimp co-culture.

Keywords: macro algae; ammonia; nitrite; nitrate

INTRODUCTION

Indonesia has been the one of the world's largest shrimp and seaweed producer since the launch of the National Shrimp Program in 1982 (Dahuri, 2013). This is seen from the growing of shrimp commodities around 7.5 million tons in 2020 and 10.99 million tons for seaweed. (KKP 2020). Furthermore, the export of tiger shrimp is still a big opportunity and can be further increased, given the potential in the form of widely available cultivation land and the increasingly intensive use of tiger shrimp cultivation system. One of the characteristics of intensive cultivation is by feeding in the form of artificial feed with doses tailored to cultivated organisms. It aims to supply feed needs so that the growth of organisms becomes optimal. On the other hand, this artificial feeding has the effect of lowering water quality due to the increase in the remaining inedible feed and the excretion of metabolic results from cultivated organisms. Poor water quality conditions resulted in stressful shrimp and decreased immune against disease (Rahmaningsih, 2012). Therefore, a system is required to achieve the goal of optimizing fishery production by minimizing the negative impact of water quality reduction.

Integrated Multi Trophic Aquaculture (IMTA) in the form of multi species cultivation is one of the cultivation systems that offer solutions to minimize waste from cultivation released into the water, where waste from organisms at higher tropic levels is used as input for organisms at lower tropic levels. Based on previous research (Largo et al., 2016; Neori et al., 2014), various organisms used namely seaweed (*Gracilaria verucosa*), Green mussel (*Perna viridis*), Tilapia (*Oreochromis niloticus*) as a companion organism that has a role to improve the water quality of cultivation media in the target organism of tiger shrimp cul tivation (*Penaeus monodon*). Seaweed can be used as an organism cultivated with tiger shrimp, as well as serves to improve the quality of the environment for its growth (Jones et al., 2001). Seaweed has the potential to lower nitrogen when cultivated with shrimp and in fish ponds (Hernandez et al., 2005) and is also used to lower the content of ammonium produced from high density fish cultivation and feeding (Murachman, 20010). Although seaweed can effectively lower residual levels of the remaining tiger shrimp metabolism, the excessive use of seaweed density can have a negative impact on the organism, including oxygen competition at night and a decrease in nutrients that are important for phytoplankton constituents of the cultivation media. Therefore, optimal density observation of both companion organisms (gracilaria seaweed) and target organisms (tiger shrimp) cultivated became the main objectives for this research. It is expected that the best seaweed density as a companion organism can support the optimal growth of tiger shrimp as the target organism of cultivation.

Seaweed *Gracilaria* sp. is an euryhalin seaweed type, which is able to live in water with salinity of 15 – 40 ppt. Therefore, *Gracilaria sp.* can be cultivated in coastal areas or ponds (Hasan et al, 2015). According to Sakdiah (2009), that the utilization of seaweed (*Gracilaria sp.*) to improve the quality of the water depends on the determination of density. This way plants can grow without lacking in nutrients due to the high density, and the competition of space can be decreased to avoid growth difficulty. In principle every living organism can be cultivated. However, each organism has different environmental quality criteria to be able to grow optimum (Srisunot and Babel, 2015; Christensen et al., 2003) This research aims to analyze the role of seaweed (*Gracilaria sp.*) to reduce organic waste and find the optimal density of seaweed (*Gracilaria sp.*) as a companion organism cultivated with tiger shrimp, to create an ecosystem balance. Growth performance and survival are observed to assess the success of cultivation.

RESEARCH METHODS

The research employed an experimental approach and was conducted in Tambakbulusan Village, Kab. Demak, Central Java Province (Figure. 1) from March to July 2018.

Figure 1. Research Station in Tambak Bulusan Village, Demak Regency, Central Java.

Tiger shrimp (*Penaeus monodon*) stadia post larvae 30, measuring 0.11 – 0.14 grams/indv, weight 0.05 – 0.20 grams/indv (Fig.2a) were cultivated with density of 80 ind/m^2. Seaweed originated from Brebes region, was selected by choosing seeds that had large, lush and clear brown thallus (Figure.2b)

The container used in this study was using a fibre tub measuring 1x1x1 m long, 7 cm substrate was added to create the pond mesocosm. Fibre tubs are placed in semi-outdoor locations with water sources from canals 200 m from the site. Shrimp feed is given twice times a day, every morning and evening (08.00 and 16.00 WIB) as much as 5% of the biomass weight on a fix feeding rate.

Daily measurement of water quality (DO, pH, salinity, temperature) using DO meter (YSI Professional Plus Multiparameter Water Quality), pH meter (Combo pH & ORP) and refractometer (Atago Pocket Refractometer), Spectrophotometer with Optima-Japan brand used to measure nitrate, nitrite and ammonia content in water.

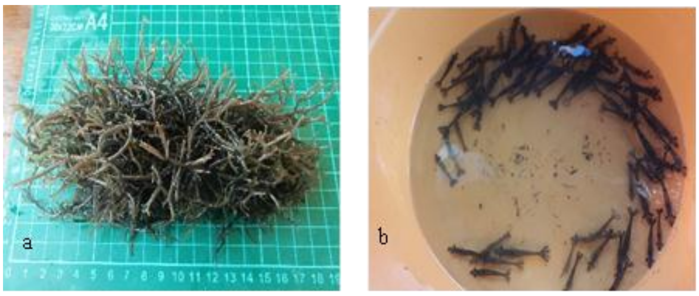

Figure 2. Seaweed (a) cultivated Together with tiger shrimp (b)

Research Design

The treatment in this study was a difference in seaweed density (grams) which are 50 (RL50), 100(RL100), 150(RL150), 200(RL200) and control. Each clump of seaweed weighs 25 grams, is tied using a rope and hung on a bamboo blade on top of the tub. This seaweed is cultivated in conjunction with 80 tiger shrimp. The research design uses a complete randomized design (RAL) with 5 treatments and 4 replays.

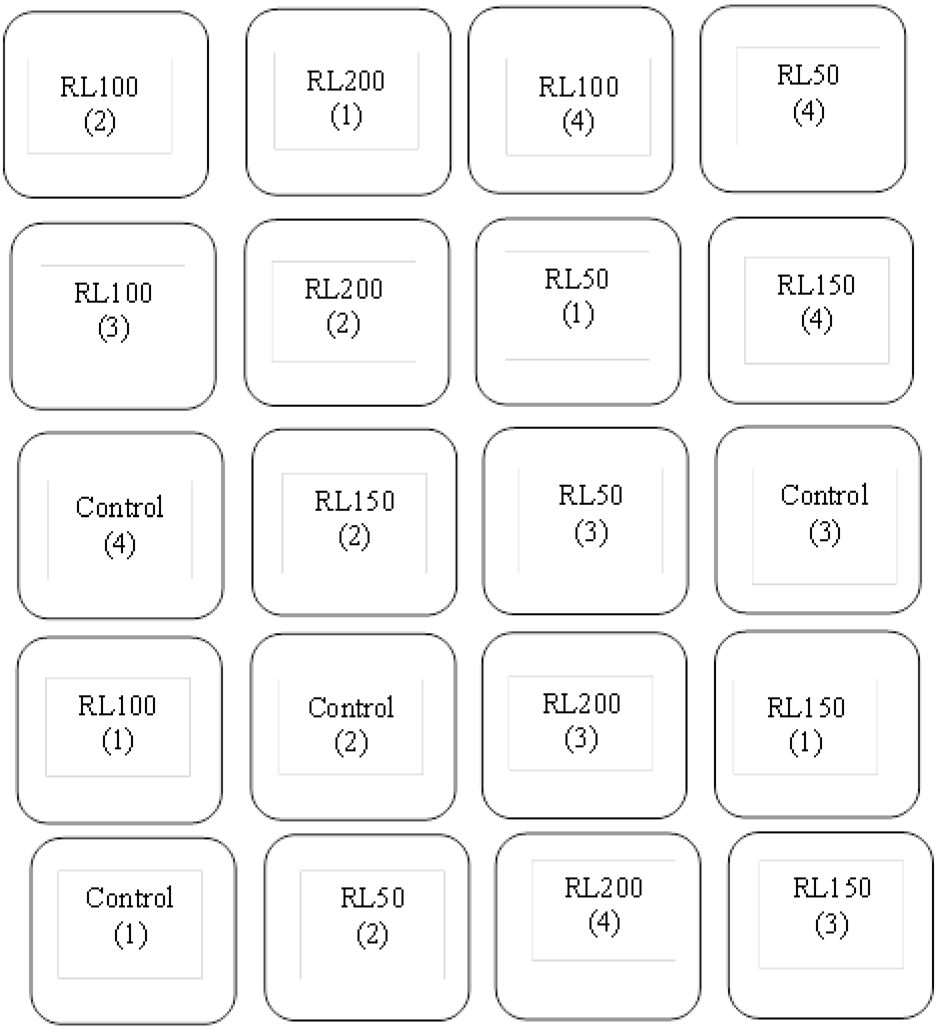

Figure 3. Completely Randomized Design for Four densities of *G. verucosa* as co-culture with shrimp. Data Collection and Analysis

Data taken include Specific Growth Rate (SGR) and survival rate (SR) of tiger shrimp, and seaweed, successively according to Busacker et al.(1990), presented in equations (1) and (2)

SGR (% /day) = Ln Wt- Ln Wo / t*100 .... (1)

SR= Nt /No ×100%... (2)

Where SR is the survival rate (%); Nt is the number of shrimp at the end of experiment (number of individual); No is the number of fish at the beginning of experiment (number of individual)

SGR is the relative growth rate (%/day); Wt is the final average body weight of maintenance (g); Wo is the initial average body weight of maintenance (g); t is the length of maintenance (days). The percentage of removal rate that can be calculated using the formula as in the equation (3)

RR = (Ct-Ci) / Ci x 100 %... (3)

Where RR is removal Rate, Ct : final concentration, Ci: Initial concentration

Water quality data in the form of Total Ammonia Nitrogen (TAN), Nitrate (NO_3), Nitrite (NO_2), Dissolved Oxygen (DO), pH, Temperature and salinity. The removal rate of TAN, NO_3 and NO_2 is defined as the difference before and after the cultivation period and is calculated using equations (3) (Srisunot and Babel, 2015).

Water quality analysis in the form of TAN, NO_3 and NO_2 from 300 ml of samples from each tank were analysed weekly; samples covered with ice were labelled and stored in cold boxes to prevent changes during transport and storage. TAN, NO_3 and NO_2 values were measured using spectrophotometer (Optima 3000), as described by the Indonesian National Standardization Agency: 06-6989.30-2005, 6989.99-2011 and 06-6989.9-2004 (SNI, 2004; SNI, 2011). TAN was measured using the Salicylate method and wavelength of 650 nm; NO_3 with Cadmium reduction method and wavelength of 500 nm and NO_2 with Diazotization method and wavelength of 507 nm.

The average values of TAN, NO_3 and NO_2 were calculated weekly in SPSS® during the experimental period. Data homogeneity was tested with the Levene test for one-way ANOVA. A one-way ANOVA is used to verify whether the average of independent variables (seaweed density) is different, and if there is a significant interaction between the two. Furthermore, some post-hoc test comparisons, the Duncan tests were used in case of significant interaction were found and the effects of dependent variables.

RESULTS AND DISCUSSION

Result

Growth of tiger shrimp and seaweed

Integrated cultivation between shrimp and *G. verucosa* with different densities makes a significant difference to the growth of shrimp and seaweed itself ($p<0.05$). There was a three groups according the Duncan test, which RL100 and RL150 were the highest SGR, RL.50 and RL 150 were the second highest, and Control was the lowest. RL100 treatment is recorded as the best SGR treatment among others, which is 6.66%, then RL150 with SGR 5.31% (Figure 2). While the cultivation of shrimp without seaweed in the control pond gives effect at a low SGR value (4.49%). On the other hand, maximum seaweed growth occurs at the lowest density (RL50), reaching 2.66 %/day. This differs noticeable when compared to treatment with densities of 100, 150 and 200 gr/m2, where at the highest density resulted in low SGR *G. verucosa* (1.36%/day).

The survival of t tiger shrimp

The difference in seaweed density showed a significant influence on tiger shrimp SR ($p<0.01$), and according to Duncan analysis divided into four groups (Figure 3.) In tiger shrimp cultivation with seaweed density of 100 gr/m^2, resulted in a value of SR 79%, which is the highest value among other treatments.

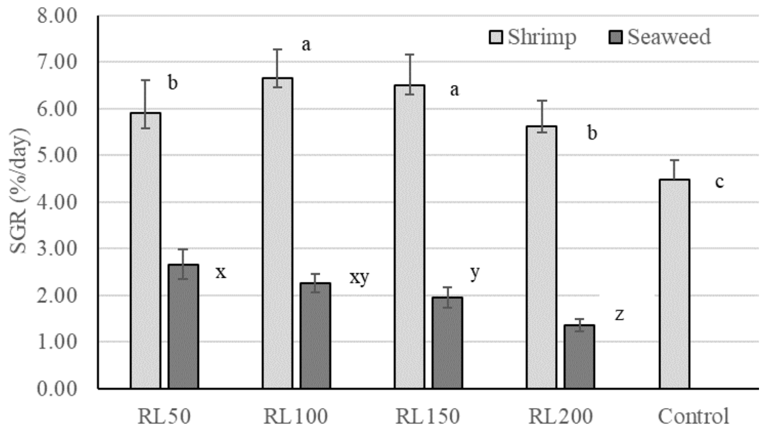

Figure 2. Mean Specific Growth Rate (%/day) of Tiger Shrimp and Seaweed in Integrated Cultivation. Letter Above the Bars Reflect Significance of Differences (Duncan test, $P < 0.05$)

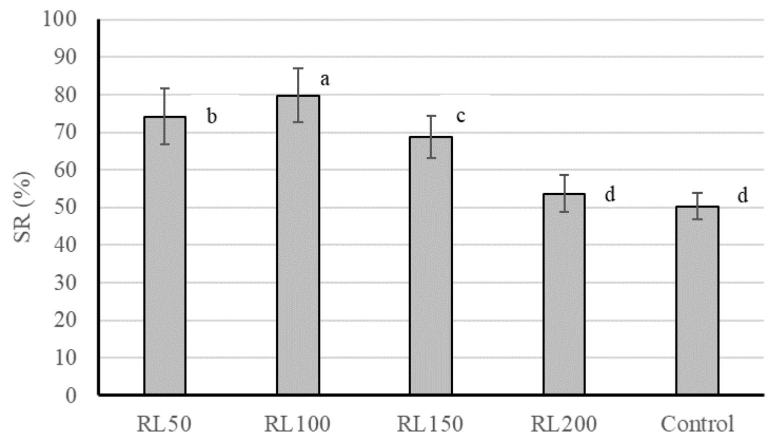

Figure 3. The Mean of Survival Rate of Tiger Shrimp in Cultivation Integrated with Seaweed at Different Density Letter Above the Bars Reflect Significance of Differences (Duncan test, $P < 0.05$)

Water quality

Levene's test shows that the data is homogeneous ($p>0.05$) and comparable. For all three parameters of organic waste concentration: TAN, NO3 and NO2, one-way ANOVA reveals that density has a significant effect. Further analysis shows that the concentration of all parameters is significantly different from that in the control.

Total Ammonia Nitrogen (TAN)

At all densities, the concentration of TAN becomes lower than the control ($p <0.01$). The continued decrease in TAN concentrations at three lower densities showed an increase in high density in the last week of the study (Figure 4). In the last week, there was an increase in TAN concentrations in two high-density treatments, from 0.06 to 0.08 in RL 150 and a drastic increase from 0.13 to 0.18. While at the two lowest densities (RL 50 and RL 100) showed a decrease.

Nitrate

In RL50, RL150 and RL 200 treatment there was a decrease in nitrate concentration in the second and third weeks, however, in the fourth week there was an increase to more than 1.8 mg/L. While in the RL.100 treatment, in the fourth week there was a significant decrease recorded at a concentration of 1.3 mg/L (Figure 5). All treatment using seaweed provides a lower nitrate concentration value compared to control.

Nitrite

The concentration of nitrites showed significance to the highest density of seaweed (RL200), and showed a trend that is almost the same as control (without seaweed treatment). While in RL50 there is an increase in nitrites from 0.004 to 0.008 mg/L over the cultivation period. A constant decrease in nitrites occurred in RL100 treatment, from 0.005 to 0.001. There is a trend of increased nitrite concentrations at the lowest seaweed density (RL50), highest seaweed density (RL200) and control. While in RL100 and RL150, a significant decrease in nitrite concentration occurs ($p<0.001$) (Figure 6).

Removal Rate (RR)

In Figure 7 it is clear that the RL100 treatment has positive RR values for TAN, nitrites and nitrates of 73, 28 and 80%. While the highest and lowest density treatment, it has negative RR values for nitrites (-100% and -260%) this means that the content of nitrites in cultivation media is increasing.

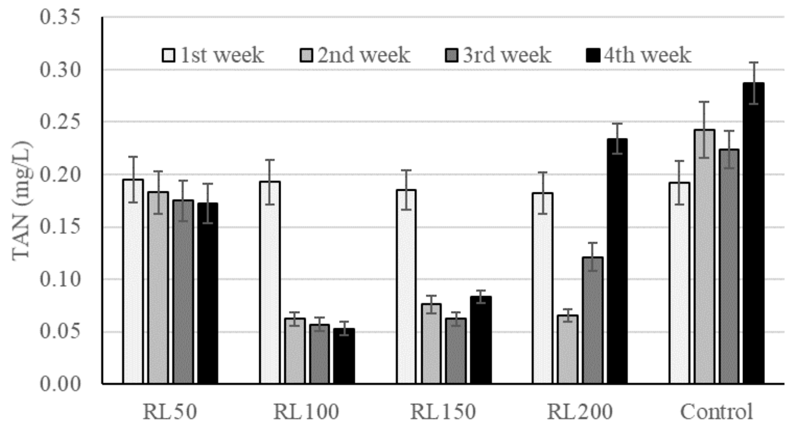

Figure 4. Concentration of TAN (mg/L) in Shrimp Media Cultivated with Various Seaweed Density Compared to Control (Without Seaweed).

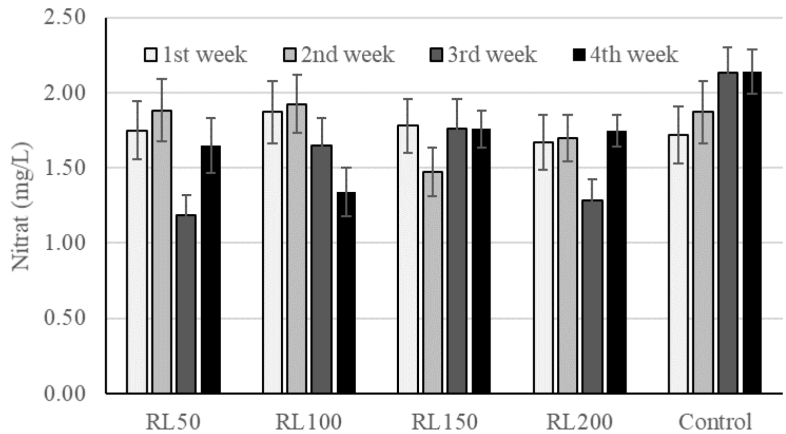

Figure 5. Nitrate Concentration (mg/L) in Shrimp Media Cultivated with Seaweed at Different Density Compared to Control

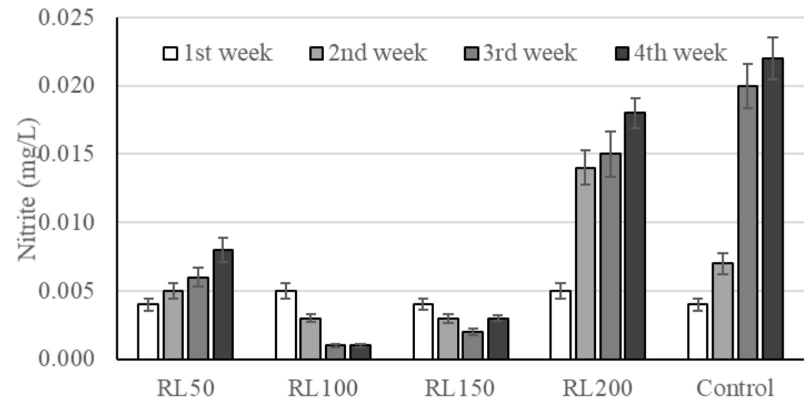

Figure 6. Concentration of Nitrite (mg/L) in Shrimp Media Cultivated with Different Densities of Seaweed Compared to Control

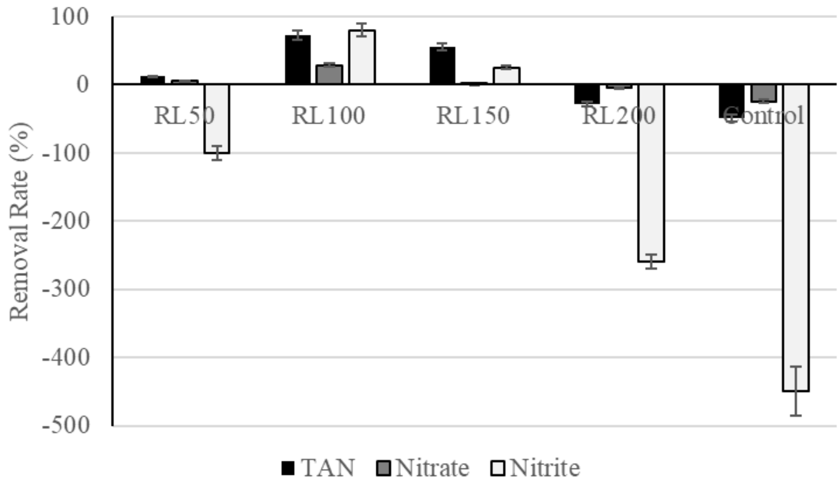

Figure 7. Removal Rate of TAN, Nitrates and Nitrites in Shrimp Media Cultivated with Different Densities of Seaweed Compared to Control.

Table 1. Water Quality Parameters in Tiger Shrimp Media (*Penaeus monodon*)

Variable	Treatment					Feasibility
	RL50	RL100	RL150	RL200	Control	
Dissolved Oxygen (mg/l)	3.71-6.44	3.42-7.63	3.11-6.29	3.05-5.43	3.32-7.74	>4[a]
Temperature (^{0}c)	27.3-31.3	27.0-31.5	27.3-31.5	27.7-31.8	27.4-31.5	29-32[a]
pH	7.9-8.96	7.88-8.92	7.99-8.90	7.22-7.65	7.18-7.57	7.5-8.5[a]
Salinity (ppt)	26-46	24-46	29-45	28-43	25-44	10-35[b]

Notes: a = (KKP,2016) b = (Amri, 2003)

Discussion

The results showed that the optimal density of seaweed to support the productivity of tiger shrimp with a density of 80 individuals /m^2 is 100 gr/m^2. Based on the data obtained from the observations, in the integration of seaweed with such density, SR, SGR, TAN reduction, Nitrites and Nitrates are at the most effective level compared to other densities. Removal Rate *G. verucosa* from TAN (73%) higher than reported by Carton-Kawagoshi. (2014) for *Gracilaria* sp. cultivated in waterways out of fish cultivation (45%), while Removal Rate NO_3 has a smaller rate compared to this study (50%).

Based on the results of the study, the density of seaweed effective to support the growth of tiger shrimp is 100 gr / m^2, as well as the impact on the removal rate of contaminants. If referring to the function of seaweed as a remediator, the higher the density of seaweed will increase the removal rate of contaminants (Oberholster et al., 2019). The removal rate of high contaminants at a density of 100 gr /m^2 indicates the role of seaweed in absorbing nutrient organic. At a density of 100 gr/m^2, the interaction between the plankton and seaweed communities is at the most optimal level of synergy. At a density of 50 gr/m^2, the removal rate of contaminants is relatively low because the function of phytoremediation is dominated by plankton. Low seaweed density causes the contribution of phyto-remediation given is also low, (Pagand et al., 2000; Bhatt et al., 2014). At this density, it is possible that the ability of seaweed is too low to absorb nitrogen due to the supply of nitrogen obtained from feed and shrimp manure that increases over the length of the cultivation time (Msuya and Neori, 2002; Nelson et al.,2001. In the highest density, DO levels reach a low level of 3 ppm, reducing the activity of Nitrosomonas and Nitrobacter bacteria, a paired reaction that requires oxygen to decompose ammonium through nitrites in nitrates in a two-step process (Watten and Sirbrell, 2006). Low DO levels may be the cause of high nitrite concentrations at high seaweed densities.

One of the factors that affects seaweed growth is the intraspecific competition in seaweed. Intraspecific competition is a competition that occurs between similar organisms in a population. Intraspecific competition increases as the density of a species increases (Cronin et al., 2012) and decreases as species diversity increases (Benard and Middlemis Maher, 2011). The intraspecific competition in seaweed is demonstrated by the SGR which tends to decrease as the density of seaweed increases. The decrease in the rate of growth due to intraspecific competition is due to a decrease in access to resources in each individual (Boulay et al., 2010). The density of seaweed at the surface of the water will significantly affect the penetration rate of sunlight. This refers to Kang et al. (2019) research that proved an increased of algae biomass can decrease the transmission of sun rays in the water.

Furthermore, in RL200 treatment and control, there is a low pH with a value of less than 7.5 and a high concentration of nitrites (0.02 $mg.L^{-1}$), both values beyond the water feasibility threshold for tiger shrimp (Permen KKP, 2016; Neori, 2014). This is in line with Boyd (2015) that the limited nitrification of excreted ammonia-nitrogen and decay by microorganisms led to a decrease in pH levels. The breakdown of organic matter increases oxygen consumption affecting the nutrient cycle due to nitrogen depletion (Christensen et al., 2003; Carlsson et al., 2010; Vaquer-Sunyer and Duarte., 2008). At night, DO becomes low (2,6-2,9 $mg.L^{-1}$) in the highest density of *Gracilaria* sp (RL200), which inhibit nitrification in treatment, as mentioned above. For lower densities of *G. verucosa,* TAN and nitrite levels remain in a good range for cultivation.

Shrimp survival rates range from 50 to 79%, in the same range (50-62%) with reporting by Thakur and Lin (2003). The highest SR observed at medium seaweed density RL100 (79.75%) and achieved in the best water quality conditions. The lowest shrimp SR (53.57 and 50.25%) was found at the highest seaweed density (RL200) and control. This is due to the value of nitrite water quality, DO and pH which is at the normal range for the life of tiger shrimp (KKP, 2013). The result of measuring other water quality parameters, like temperature and salinity, is in decent conditions for the cultivation of Tiger shrimp.

CONCLUSION

Density *G. verucosa* 100 g/m^2 is the recommended density for integrated cultivation along with tiger shrimp, at a density of 80 $indiv/m^2$. This is evidenced by shrimp SGR and SR values reaching 6.6 %/day and 79.75%. The role of seaweed in improving water quality is seen in the decrease in TAN, NO_3 and NO_2 values by 73, 28 and 80%, respectively. On the other hand, in treatment with a high density of seaweed, the value of such parameters is precisely increased.

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