GROWTH OF SEAWEED *Gracilaria verrucosa* CULTURED ON DIFFERENT INITIAL WEIGHT WITH LONGLINE METHODS IN KARIMUNJAWA WATERS

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ABSTRACT

One factor that can optimize seaweed production is determining the initial weight of planting. However, planting seaweed in open waters is very vulnerable to aggregation from herbivorous fish. This study aims to determine the best initial weight for the growth of *G. verrucosa*, which is cultivated in net cage by the longline method. Net cages, made of nylon, are applied as protection for *G. verrucosa* from aggregation of herbivorous fish. This research was conducted in Karimunjawa waters, Jepara Regency, Central Java, for 42 days. The experimental design used was Randomized Block Design (RBD), with three treatments and four replications each. The treatment was different initial planting weights of 25, 50, and 75 g. The observed variables included culture techniques, relative growth rates, specific growth rates, and water quality. The analysis showed that the different initial weight was very significant ($P < 0.01$) on the growth of *G. verrucosa*. Treatment with an initial weight of 25 g gave the best relative growth rate (2.07±0.25%.d^{-1}), and the best specific growth rate (1.48±0.13%.d^{-1}). Furthermore, this study was able to prove that the use of a net planting cage on a seaweed hanger can avoid aggregation of herbivorous fish, which is indicated by the growth of *G. verrucosa*.

KEY WORDS: *Gracilaria verrucosa*, Karimunjawa, mariculture, red seaweed, *Siganus* sp

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INTRODUCTION

Seaweed culture plays an essential role in increasing the capacity of Indonesian fisheries production. Seaweed is included in the fisheries revitalization program, which is expected to have a significant contribution in improving community welfare. Seaweed is one of the marine commodities that have quite high economic value. Indonesia has an area of 1,110,900 ha for seaweed culture, but the development so far has only utilized 222,180 ha (20% of the potential area) (Ditjenkanbud 2008). Likewise, with *Gracilaria*, the production still relies on the results of culture on ponds, where the production is categorized as low because it always depends on the season and the period of the pond production itself. Thus, to increase production, a culture of *G. verrucosa* in open marine waters is considered very necessary.

Karimunjawa waters are one of the marine waters that has been developed as an area for seaweed culture. However, seaweed production in Karimunjawa waters is currently unable to meet the demand for seaweed production for Jepara district. To achieve maximum production, several important factors need to be considered. One of the factors that can affect the quantity of seaweed production is a determination on the initial weight of planting seedlings. The difference in biomass is very influential in the growth of seaweed. This is closely related to competition of individual seaweed in getting nutrients as its food. The balance between the number of nutrients and seaweed density needs to be taken into account so that seaweed can grow without nutrient deficiencies. Balance in nutrient absorption per body kg per hour in seaweed planting with low stocking densities showed better results than high stocking densities (Sakdiah 2009).

Moreover, the factor that still often fails seaweed cultivation in Karimunjawa waters is the presence of herbivorous coral fish aggregation. This is a serious threat to seaweed that is traditionally cultured without a cage as protection in the open waters. The mechanism of seaweed predation by herbivorous fish such as *Siganus* sp. has become an obstacle among seaweed farmers, but efforts to overcome the problem are still happening slowly (Framegari et al. 2012).

This study aims to determine the impact of determining the initial weight of planting on the relative growth rate and specific growth rate of *G. verrucosa*. The effect of the use of planting cage on the aggregation of *G. verrucosa* was also evaluated. This research was carried out in Karimunjawa Waters, Jepara Regency, Central Java (Fig. 1), with 42 days of data collection, from May to July 2018.

MATERIALS AND METHODS

The test material used in this study was *G. verrucosa* from Jepara Waters, Central Java. The cage used in this study was a 50 mm mesh net of nylon. The net was used as a cage for hangers for every spot of seaweed on each line. There were 12 seaweed lines which were divided into three different weights. The net used to accommodate each seaweed hanger was intended as a protector so that the seaweed thallus was not easily broken and lost, as well as a form of protection from the aggregation of herbivorous fish that can attack seaweed (Framegari *et al*. 2012; Nadlir *et al*. 2019).

Seaweed hangers were carried out with a depth of 35 cm from the surface of the water. Determination of the depth of seaweed hanger was based on the height of tides during day and night. This is because the tidal differences during the day and night were very significant. Therefore, the depth of seaweed hanger was adjusted, so that during the daytime, when seawater was rising, seaweed still obtained sufficient light penetration. While at night, when sea water receded, seaweed hangers remained in water's body (Fig. 2).

The spacing between the hangers used in this study was 25 cm. Cleaning seaweed cage was carried out every three days by considering the reliability of seaweed morphology. If the net was cleaned too often, then the risk in handling that caused the new thallus broken accidentally would be higher.

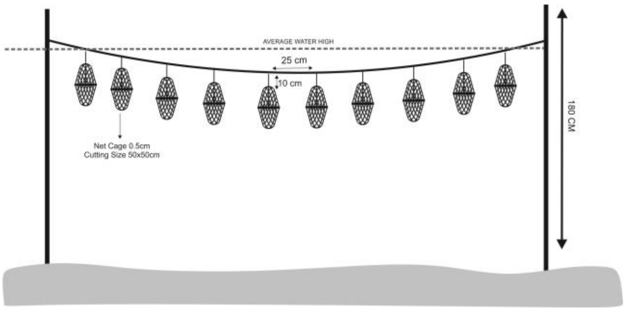

Fig 2. Planting of *G. verrucosa* in net cage by the longline method

The method used in this study was experimental in the field. The experimental design used was Randomized Block Design (RBD), by applying three different treatments at the initial planting weight, namely A: 25 g, B: 50 g, and C: 75 g. Determining the initial weight of planting was based on previous studies (Hasan *et al.,* 2015). Each treatment had four replications. The variables measured included the value of the Relative Growth Rate (RGR) and Specific Growth Rate (SGR) of *G. verrucosa*, also water quality.

1. Relative Growth Rate (RGR, %.d^{-1})

According to Effendie (1997), RGR could be calculated using the formula:

$$RGR = \{(W_t - W_0) / (W_0 \times t)\} \times 100\%$$

where:
RGR = Relative Growth Rate (%)
W_t = Final weight of *G. verrucosa* (g)
W_0 = Initial weight of *G. verrucosa* (g)
t = culture duration (days)

2. Specific Growth Rate (SGR, %.d^{-1})

Also according to Effendie (1997), SGR was calculated using the following formula;

$$SGR = \{(\ln W_t - \ln W_0) / t\} \times 100\%$$

where:
SGR = Specific growth rate (%.d^{-1})
W_t = Final weight of *G. verrucosa* (g)
W_0 = Initial weight of *G. verrucosa* (g)
t = culture duration (days)

3. Water quality parameters

Measurement of water quality, which included temperature (°C), pH, salinity (ppt), and dissolved oxygen (mg.L^{-1}), was carried out every day in the morning using Water Quality Checker. Measuring the level of clarity (cm) and depth of water (cm) was using Secchi disk, while waters current (cm.s^{-1}) was used the current meter. Measurement of free carbon dioxide (mg.L^{-1}), nitrite (mg.L^{-1}), and phosphate (mg.L^{-1}) content was carried out at the beginning and end of the study by conducting tests in Laboratory of Physics and Chemistry, BBPBAP, Jepara.

4. Data analysis

The RGR and SGR data obtained were then analysed using analysis of variance (ANOVA) with a 95% confidence interval to see the effect of treatment. Before analysing the variance, the normality test, homogeneity test and additive test were first performed to ensure the data was range normally, homogeneous, and additive in nature. Data were analysed by variance (F test) at 95% confidence level. If the analysis of variance obtained significantly different ($P < 0.05$), then the Duncan's multiple region test was performed to determine differences between treatments.

RESULT AND DISCUSSION

The *G. verrucosa* seedlings in treatment A with an initial weight of 25 g had higher RGR than that in treatment B (50 g) and treatment C (75 g). Based on data analysis, it was known that the difference in RGR values among of three treatments were different significantly (Table 1).

Table 1. Data of Relative Growth Rate (RGR) and Specific Growth Rate (SGR) of *G. verrucosa*

Variable	Treatment		
	A	B	C
RGR (mean±SD, %.d^{-1})	2.07±0.25^{a}	0.81±0.13^{b}	0.31±0.06^{c}
SGR (mean±SD, %.d^{-1})	1.48±0.13^{a}	0.69±0.10^{b}	0.29±0.06^{c}

Note: Mean values with different superscript letter in the same row show a significant difference ($P < 0.05$).

Table 2. Data of Water Quality Parameter

Parameter	Unit	Value			Feasibility
		Initial Day	Final Day	Average	
Salinity	‰	30	31	30.86	15-35^{a}
Temperature	°C	28	30	29.67	17-30^{a}
pH	-	8	8	8.17	6.0-9.0^{b}
Clarity	cm	139	150	148.67	>150^{c}
Depth	cm	139	150	148.67	220-1,000^{d}
Current	cm.s^{-1}	26	34	29.07	10-100^{e}
DO	mg.L^{-1}	6.2	6.4	6.39	5.0-6.2^{f}
CO_2	mg.L^{-1}	4.8	4.62	4.63	>2^{g}
Nitrate	mg.L^{-1}	nd*	nd*	-	0.02-0.04^{h}
Phosphate	mg.L^{-1}	0.11	0.07	0.09	0.02–1.00^{i}

Note:
*nd=not detected
a) Choi et al. 2006; b) Zatnika 2009; c) Puja et al. 2001; d) Utojo et al. 2007; e) Wood 1987; f) Tarigan and Sumadhirga 1989; g) Ariyati et al. 2007. h) Efendi (2003), i) Sulistijo and Atmaja (1996)

Fig 1. Research location of *G. verrucosa* planting in net cage by longline method

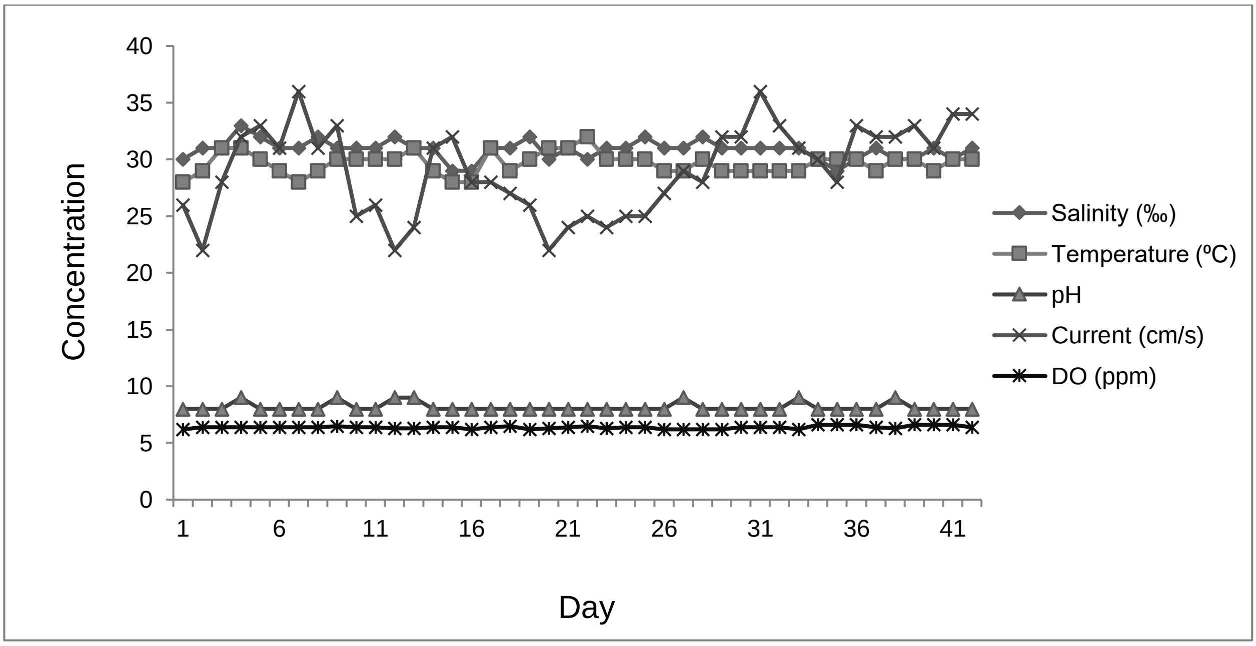

Fig 3. Water quality dynamic of *G. Verrucosa's media* during study

The RGR value of *G. verrucosa* cultured for 42 days ranged from 0.25 - 2.30%.d^{-1}, with mean average of A (2.07%.d^{-1}), B (0.81%.d^{-1}), and C (0.31%.d^{-1}). The highest average RGR was treatment A.

The *G. verrucosa* in treatment A with an initial weight of 25 g also had a higher SGR than treatment B (50 g) and treatment C (75 g) (Table 1). The SGR value showed that growth at A with initial weight of 25 g had better growth compared to treatments B and C. The highest SGR was found in treatment A which had an average of 1.48%.d^{-1}, while the lowest rate at treatment C which had an average of 0.29%.d^{-1}.

The measured water quality data consisting of physical and chemical parameters during the study are presented in Table 2. Most of water quality aspects were relatively stable during study (Fig. 3) and were still within the range of tolerance values.

Culture Method of *G. verrucosa*

The use of net cage in each seaweed hanger cultured was able to minimize herbivore fish aggregation and thallus breakage due to water currents. This is because the net was able to protect *G. verrucosa* optimally. Seaweed 'bags' are containers or places that facilitate seaweed to be cultured through methods that have been developed (Cahyadi 2013). The use of bags in seaweed cultivation can overcome the aggregation of herbivorous fish against thallus and keep seaweed thallus from being cut off suddenly due to marine physics factors, and furthermore, the volume of seaweed production can be well controlled.

Aggregation attacks from herbivorous fish were known to occur only in parts of *G. verrucosa* that growth outside the net. This was due to the nature of the seaweed that develops by lengthening the thallus, thus allowing the thallus to come out of the container and can be eaten by herbivorous fish such as *Siganus* sp.

In this study, the longline method was used with a spacing of 25 cm between hangers of seaweed in each line, and the depth of planting was 35 cm. This depth is also consistent with previous studies with a range of 30-45 cm (Syahputra 2005; Thamrin 2011; Pongaarang *et al.* 2013).

Spacing and depth of planting are related to the absorption of light penetration and the level of cleanliness of the seaweed thallus, thus affecting the health and photosynthesis of seaweed. Spacing is related to land area unity. Plant spacing is used in addition to influencing the movement of water movement will also avoid the accumulation of dirt on the thallus, which will help the air so that the photosynthesis needed for seaweed growth can take place, and prevent large fluctuations in salinity and water temperature.

The difference in spacing is very influential on the growth of seaweed. This is closely related to the competition of individual seaweed in getting nutrients as its food. Soegiarto *et al.* (1989) mentioned that seedling spacing is one of the technical factors that influence seaweed growth because it is closely related to nutrient absorption. Furthermore, it reported that the higher the planting area, the smaller the number of planting points, so that there is a lot of unused space, hence production is not optimal.

Seaweeds maintenance techniques are needed to support the excellent growth of seaweeds in one planting period. Efforts to maintain seaweed in this research were by carrying out cleaning of seaweed hangers once every 3 days and checking pegs, buoys, and seaweed ties every day. Cleaning was done to clean seaweed cages from various kinds of animals and plant attachments such as shells and moss that can cover the net. Dirt attached can hide the penetration of light entering the seaweed hanger, as well as inhibit the process of recirculation of water in the cage. This is following the study of Asaf *et al.* (2014) where dirt attached to algae can disrupt the metabolic process, so that the growth rate decreases. Some plant or other algae attachments such as Ulva, Hypnea, Chaetomorpha, and Enteromorpha often entwine plants and cultivation constructions so that they can cause damage. Therefore, the things that need to be considered during maintenance of seaweed are cleaning the net from dirts that cover it and cleaning seaweed from the plants that wrap it.

Relative Growth Rate (RGR) of *G. verrucosa*

The RGR in each treatment A, B, and C were 2.07, 0.81, and 0.31%.d^{-1}, respectively. The RGR value of treatment A was better than in treatment B and C. It is assumed that the lower the initial weight, the better the growth will be, whereas if the initial weight is high, the growth will be less good. The results showed that the treatment with the least weight of seaweed (25 g) had the highest weight gain compared to the other two treatments. The low initial weight of seaweed at the beginning of planting, causing the absorption of nutrients in the metabolic process of seaweed can work very optimally. This is in accordance with study of Choi *et al*. (2006); Sakdiah (2009); Mustafa *et al.* (2009); and Cirik *et al.* (2010) who also conducted studies on *G. verrucosa*, and they found that the fulfilment of nutrients greatly affects the growth of seaweed. A balance between the amount of nutrients and plant density is needed so that plants can grow without nutrient deficiencies. Planting seaweed with low stocking density has a better balance than high stocking density in nutrient absorption per body kg per hour.

The availability of nutrients such as nitrates and phosphates in the culture location also played an important role in the relative growth rate of seaweed. Nitrates in the waters are macronutrients that can be absorbed by seaweed, as well as controlling primary productivity. Nitrate-nitrogen levels in natural waters were almost never exceeded 0.1 mg.L^{-1}, but if nitrate levels reach 0.2 mg.L^{-1} will lead to eutrophication which further stimulates the growth of algae and aquatic plants rapidly (Effendie 2003). Phosphate in water is also one of the nutrients that is important for seaweed growth. Phosphate content affects the level of water fertility. According to Masyahoro and Mappiratu (2010), the main nutrient content that is needed in seaweed, such as nitrate and phosphate, is very influential in its reproductive stage. In their study, nitrate and phosphate content in seaweed ranged between 0.60-0.65 and 0.44-0.45 mg.L^{-1}, respectively, during the culture period.

Moss and dirt in the form of sediment from the substrate water could cover the surrounding nets so that the supply of nutrients for seaweed was not optimal. The growth of moss often results in the less optimal exchange of nutrients for seaweed and impacts on growth rates. Increasing the daily growth rate of seaweed has increased with increasing exchange and frequency of nutrients in culture media (Failu *et al.* 2016).

The RGR values of *G. verrucosa* in this study are still lower than those in study from Cirik *et al*. (2010). In their study, the values ranged from 4.03±1.63 to 1.21±0.34%.d^{-1}. This is possible because their study was carried out in a closed system and in a greenhouse with a controlled environment. The RGR of this study is also lower when compared to research from Mustafa *et al.* (2009), which obtained RGR values of 1.52 and 3.63%.d^{-1}. They conduct their studies in ponds. Both studies resulted in better RGR values because they were able to minimize interference from herbivorous fish or other nuisances that can potentially harm *G verrucosa*.

On the other hand, the RGR in this study is comparable to the study of Choi *et al.* (2006). They conducted a study of the growth of *G. verrucosa* in open water. The RGR values in their study were (-) 0.71±0.07 to 3.13±0.63%.d^{-1}. Some of their RGR results showed negative growth because *G. verrucose* growth was disrupted by the presence of h epiphytes, which occurred at days 21 to 55.

Specific Growth Rate (SGR) of *G. verrucosa*

The SGR of seaweed illustrates the ability of seaweed to grow daily. Based on the results of *G. verrucosa* seaweed planting, it is known that SGR of treatment A was 1.48%.d^{-1}, B was 0.69%.d^{-1} and C was 0.29%.d^{-1}. The SGR at treatment A was the highest compared to other treatments. Less optimal growth can be caused by high seaweed planting density, so it did not have sufficient growth space. High density can affect the growth of seaweed so that seaweed is difficult to absorb nutrients as food intake. Nutrients that are around the waters of the study site were more sufficient for growth at 25 g of seed weight compared with 50 g and 75 g of seed weight. In the initial seed weight treatment 25 g was more tenuous so that the nutritional needs were evenly distributed or the competition in obtaining growth was not too tight. High stocking density, causing space to be narrow as a result seaweed is difficult to develop. Insan *et al.* (2013) also mentioned that size of the growth is due to the use of cultivation systems related to growing space, and the absorption of sunlight as a regulator of the process of photosynthesis.

Seaweed is a chlorophyll plant that requires sunlight for its growth, so the determination of the depth of seaweed cultivation is one of the success factors in seaweed production. Seaweed hangers that are too deep can result in seaweed not receiving light penetration properly. This allows the SGR of *G. verrucosa to* be less than optimal. The amount of intensity of sunlight entering the waters is influenced by the brightness of the sea water. In order for sunlight requirements to be available in optimal amounts, the depth level of seaweed cultivation must be set appropriately. According to Susilowati *et al.* (2012), the low growth rate of seaweed with increasing depth due to low oxygen circulation. Photosynthesis will increase in line with an increase in light intensity at a certain optimum value. The intensity of light is also directly related to primary productivity at a certain limit.

Basically, water currents also influence the amount of dirt that is attached to the maintenance container. In the monitoring activities of *G. verrucosa* seaweed, many kinds of dirt are found in the seaweed container, so that it covers the net. This can prevent recirculation of water in the container. Therefore, it requires an ideal movement of water that can clean the layer of dirt on the net. The business of *G. verrucosa* seaweed cultivation often fails due to lack of attention to several factors that can affect the growth rate of seaweed in its cultivation, including the location of cultivation. Putra *et al.* (2011) reported that the movement of water at the cultivation site plays a role in helping plants to become clean from the attachment of mud and biota substrates, carrying nutrient material, and the application of hydraulic factors to stimulate seaweed growth. One important factor regarding the effects of water movement is the immiscible boundary layer. Unmixed boundary layers are caused by friction between the thallus surrounding the waters. The thickness of this layer is inversely proportional to the movement of water and the disruption of the flow which disturbs the boundary of the layer.

Water quality of *G. verrucosa*'s media

Observation and retrieval of water quality data was carried out to determine fluctuations in water quality in the culture environment. The results were analysed descriptively with the ideal suitability of the *G. verrucosa* environment. Data collection on several parameters of water quality such as temperature, salinity, clarity, depth, DO flow, and pH was carried out every day in the morning before noon, while CO_2 was carried out once every 3 days, as well as phosphate and nitrate measurements performed laboratory tests at the beginning and final research. The suitability of water quality parameters in *G. verrucosa* culture is very influential on growth. Radiarta and Erliana (2014) mentioned that some parameters which significantly affect the rate of photosynthesis are temperature, salinity, light intensity, and drought conditions due to tidal influences.

Water salinity affects the osmoregulation process in seaweed. The salinity value obtained in the waters of Karimunjawa had a range of 29-33 ppt. The salinity was still included in the ideal salinity for the cultivation of *G. verrucosa* so that it was able to support the growth and development of seaweed. This is according to a study from Zatnika (2009) who reported that the salinity of waters for the cultivation of *G. verucossa* ranges between 15-44 ppt. Furthermore, in a study conducted by Choi *et al.* (2006), mentioned that the range of good salinity for the cultivation of Gracilaria is 15-35 ppt.

The temperature of the waters in the *G. verrucosa* seaweed planting environment had a ranged of 28-32 °C. Temperature is an environmental factor that is very influential on the metabolic process of seaweed. Temperatures that are too high can slow down the growth process of *G. verrucosa* due to decreased enzyme work and also *G. verrucosa* bleeds rapidly. The temperature ranged that obtained in the study was appropriated. Piazzi *et al.* (2001) mentioned that the optimal temperature range to support seaweed cultivation ranged from 25-31 °C. While the pH value measured every day in this study showed a range of 8.0-9.0. The pH value obtained is still suitable for algal growth. This is in line with the statement of Widowati *et al*. (2015) that the range of acidity levels suitable for seaweed farming activities tends to be alkaline. The appropriate pH value for seaweed cultivation is in the range of 7.0–8.5. Papalia and Arfah (2013) added that the condition of waters that are very acidic or very alkaline will endanger the survival of organisms because it will cause metabolic disorders and respiration. If the pH is very low, it will cause the mobility of various heavy metal compounds that are toxic, which is higher, which certainly will threaten the survival of aquatic organisms. High pH value will cause the balance between ammonium and ammonia in water will be disturbed, where the increase in pH above will increase the concentration of ammonia which is also very toxic to the organism.

Dissolved oxygen (DO) is a basic need for the life of living things in the water. The DO and free carbon dioxide (CO_2) play an important role in the balance of water quality in waters. The values of DO and CO_2 waters had a ranged of 6.2–6.6 and 4.4–4.8, respectively. The contents of DO to support the business activities of seaweed cultivation ranged between 3-8 $mg.L^{-1}$ (Ditjenkanbud, 2008). Furthermore, Ginting *et al*. (2015) reported the range of DO obtained was 4-14 $mg.L^{-1}$ and according to the study of Ariyati *et al*. (2007), CO_2 which is good for seaweed growth is > 2 $mg.L^{-1}$.

Seaweed growth is influenced by nitrate and phosphate content. It seems the Nitrate in the waters was very low (not detected). On the other hand, phosphate contents were 0.071-0.125 $mg.L^{-1}$, which met the supply for seaweed growth. According to Sulistijo and Atmaja (1996), the ideal phosphate content for seaweed growth is in the range of 0.02-0.04 $mg.L^{-1}$. The main nutrient content that is needed by seaweed, such as nitrate and phosphate, is very influential on its reproductive stage. According to Masyahoro and Mappiratu (2010), if the two nutrients are available, the fertility of seaweed increases rapidly. The elements N and P are nutrients that are needed by algae in their growth. The P elements which are few in number and in comparison with the incompatible N elements are often a limiting factor for alga growth.

CONCLUSION

Some conclusions can be drawn from the results of this study. The use of a planting container in the form of a net with a mesh size of 0.5 cm with an area of 50x50 cm in *G. verrucosa*, can handle herbivorous fish aggregation so that seaweed has increased growth. Different initial weights (25, 50, and 75 g) have a very significant effect on the growth of *G. verrucosa* seaweed that was cultivated by the longline method in Karimunjawa waters, Jepara Regency. The *G. verrucosa* seaweed that was cultivated with an initial weight of 25 g had the best RGR value of 2.07%.d^{-1} and the best SGR value of 1.48%.d^{-1}

It is recommended to do *G. verrucosa* seaweed farming activities with an initial weight of planting 25 g. But, further research on the effect of the different extent of the net container as a protection of seaweed on the growth performance of *G.verrucosa* is needed.

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