

## **Analysis of Carotenoid Content in Sea Grape (*Caulerpa lentilifera*) Cultivated with Low Plant Standing Different in The Basic Standard System**

### **Analisis Kandungan Karotenoid Pada Anggur Laut (*Caulerpa Lentilifera*) Yang Dibudidayakan Dengan Jarak Tanam Yang Berbeda Pada Sistem Patok Dasar**

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#### **ABSTRACT**

Seaweed is one type of fishery commodity that is widely used both as a raw material for food, industry, medicine, and cosmetics. This causes the economic value of seaweed to help the community's economy and can increase the country's foreign exchange. Seaweed is one of the largest carotenoid producers because green seaweed generally contains chlorophyll a and b compounds and carotene compounds which can function as antioxidants. Factors that affect the content of carotenoids is the spacing of seaweed seeds. The purpose of this study was to determine the effect of spacing in the basic stake system on the carotenoid content of *Caulerpa lentilifera* and to determine the ideal spacing that gives optimal effect on the carotenoid content of *Caulerpa lentilifera*. This study used an experimental method with a completely randomized design (CRD) pattern with 6 treatments and 3 replications with treatments namely Control (25 cm spacing), P1 (20 cm spacing), P2 (30 cm spacing), P3 (20 cm spacing). plant 35 cm), P4 (planting distance 40 cm), P5 (planting distance 45 cm). The data obtained was then analyzed using variance (ANOVA) with a 95% confidence level and to find out the best growth a further test was carried out using the BNT test. The results showed that the spacing applied to the basic stake system had no effect on the carotenoid content of *Caulerpa lentilifera*, but had an effect on the absolute growth and specific growth rates. Spacing of 30 cm is the best distance for the growth of *Caulerpa lentilifera* with an absolute growth of 128.917 g and a specific growth rate of 4.965%, but the difference in spacing has no effect on the carotenoid content. The conclusion of this study was that the difference in plant spacing of the basic stake system had no effect on the carotenoid content of *C. lentilifera*, but had an effect on the absolute growth and specific growth rate. There was no ideal spacing to increase carotenoid content in *C. lentilifera*, because each treatment tended to have relatively the same carotenoid content.

#### **ABSTRAK**

Rumput laut merupakan salah satu jenis komoditas perikanan yang banyak dimanfaatkan baik sebagai bahan baku makanan, industri, obat-obatan, dan kosmetik. Hal ini menyebabkan nilai ekonomi rumput laut dapat membantu perekonomian masyarakat dan dapat menambah devisa negara. Rumput laut merupakan salah satu penghasil karotenoid terbesar karena rumput laut hijau secara umum mengandung senyawa klorofil a dan b serta senyawa karoten yang dapat berfungsi sebagai antioksidan. Faktor yang mempengaruhi kandungan karotenoid yaitu jarak tanam bibit rumput laut. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh jarak

tanam pada sistem patok dasar terhadap kandungan karotenoid *Caulerpa lentilifera* serta mengetahui jarak tanam ideal yang memberikan pengaruh optimal pada kandungan karotenoid *Caulerpa lentilifera*. Penelitian ini menggunakan metode eksperimental dengan pola Rancangan Acak Lengkap (RAL) dengan 6 perlakuan dan 3 kali ulangan dengan perlakuan yaitu Kontrol (jarak tanam 25 cm), P1 (jarak tanam 20 cm), P2 (jarak tanam 30 cm), P3 (jarak tanam 35 cm), P4 (jarak tanam 40 cm), P5 (jarak tanam 45 cm). Data yang didapatkan kemudian dianalisis menggunakan sidik ragam (ANOVA) dengan taraf kepercayaan 95% dan untuk mengetahui pertumbuhan terbaik dilakukan uji lanjut menggunakan uji BNT. Hasil penelitian menunjukkan bahwa jarak tanam yang diterapkan pada sistem patok dasar tidak memberikan pengaruh terhadap kandungan karotenoid pada *Caulerpa lentilifera*, namun berpengaruh terhadap pertumbuhan mutlak dan laju pertumbuhan spesifik. Jarak tanam 30 cm merupakan jarak terbaik untuk pertumbuhan *Caulerpa lentilifera* dengan pertumbuhan mutlak 128,917 g dan laju pertumbuhan spesifik 4,965%, namun perbedaan jarak tanam tidak berpengaruh terhadap kandungan karotenoid. Kesimpulan dari penelitian ini adalah perbedaan jarak tanam sistem patok dasar tidak memberikan pengaruh terhadap kandungan karotenoid pada *C. lentilifera*, namun berpengaruh terhadap pertumbuhan mutlak dan laju pertumbuhan spesifik. Tidak ditemukan jarak tanam ideal untuk meningkatkan kandungan karotenoid pada *C. lentilifera*, karena setiap perlakuan cenderung memiliki kandungan karotenoid yang relatif sama.

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**Kata Kunci** *Jarak tanam, karotenoid, Caulerpa lentilifera, pertumbuhan*

**Keywords** *Spacing, carotenoids, Caulerpa lentilifera, growth*

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## INTRODUCTION

Seaweed is a type of fishery commodity that is widely used as raw material for food, industry, medicines and cosmetics. This causes the economic value of seaweed to help the community's economy and increase the country's foreign exchange (Widyasari, 2016). Seaweed is easy to cultivate in Indonesia because it has a climate and geography that suits the biological needs and growth of seaweed. In Indonesia, the land area for seaweed cultivation has reached 769,452 ha, while land use for seaweed cultivation is only around 50% of the existing potential area (Kemendag, 2013).

Seaweed is one of the largest producers of carotenoids because green seaweed generally contains chlorophyll a and b compounds as well as carotene compounds which can function as antioxidants (Tamat et al., 2007 in Darmawati *et al.*, 2016). Carotenoids found in plants are companion pigments to chlorophyll and function to absorb light energy in the photosynthesis process (Maleta, 2018). The function of carotenoids can help reduce the formation of free radicals which can be detrimental to health, influence the regulation of cell growth and modulate gene expression and the body's immune response.

Green algae, which can be in the form of seaweed, is one of the main producers of carotenoids in the form of  $\beta$ -carotene, lutein, vi-olaxanthin, antheraxanthin, zeaxanthin and neoxanthin. One type of green seaweed that can be used as a potential carotenoid producer is the sea grape *Caulerpa lentilifera*. *Caulerpa lentilifera* contains chlorophyll a and b, carotene, xanthophyll and lutein which are photosynthetic pigments (Burtin, 2003 in Darmawati *et al.*, 2016). Cyponosanthin is the main carotenoid content in *Caulerpa* seaweed (Fretes et al., 2012). The carotenoid content in *Caulerpa* varies depending on light absorption. This is because *Caulerpa* forms carotenoids through the absorption of light from the photosynthesis process (Ismianti,

2018). Many people use *Caulerpa* for consumption. In Indonesia it is known as Latoh (Java), Bulung Boni (Bali), Lawi-Lawi (Sulawesi).

One factor that influences the carotenoid content is the spacing of seaweed seeds. The spacing of seedlings affects the absorption of nutrients carried by water movement to the seaweed (Desy *et al.*, 2001). This is related to the effective distance for seaweed to absorb nutrients. Narrow planting distances will cause competition between seaweeds in absorbing nutrients. Plant spacing that is too wide will provide space for phytoplankton to grow, resulting in competition in photosynthesis and respiration (Desy *et al.*, 2016)

Research on the effect of planting distance for seaweed *Caulerpa* sp. was carried out by Darmawati *et al.*, (2016), where the highest carotenoid content in seaweed was obtained with a planting distance of 30 cm in the longline system. However, research using a base stake system has never been carried out, therefore it is necessary to carry out this research to determine the carotenoid content in *Caulerpa lentilifera* sea grapes which are cultivated with different planting distances in a base stake system. The aim of this research was to determine the effect of planting distance in the base stake system on the carotenoid content of *Caulerpa lentilifera* sea grapes. To determine the ideal planting distance that provides optimum influence on the carotenoid content of sea grape *Caulerpa lentilifera*. The benefits that are expected to be obtained from this research are that it can provide information about the effect of planting distance in the base stake system on the carotenoid content of *Caulerpa lentilifera* sea grapes and can be an alternative in implementing the cultivation of *Caulerpa lentilifera* sea grapes using the base stake method to obtain optimum carotenoid content.

## METHODS

### Place and Time

This research was carried out for 45 days, starting from January 14 2020 to February 27 2020, at Kertasari Village, Taliwang District, West Sumbawa Regency. Carotenoid calculations were carried out at the Analytical Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University.

### Methods

The method used in this research is an experimental method and the design used is a Completely Randomized Design (CRD). The aspect studied is the effect of different planting distances on seaweed cultivation using a base stake system with a depth ranging from 0.3 m to 0.6 m at lowest tide so as to prevent drying out of the seaweed (Akbar, 2014). There were 6 (six) treatments with 3 (three) repetitions, so that 18 experimental units were obtained. Control: Plant distance 25 cm Treatment 1: Plant distance 20 cm Treatment 2: Plant distance 30 cm Treatment 3: Plant distance 35 cm Treatment 4: Plant distance 40 cm Treatment 5: Plant distance 45 cm.

The placement of each experimental unit was carried out randomly and can be seen in Figure 4. The determination of the planting distance above was carried out based on research conducted by Pong-Masak and Sarira (2018) that a planting distance of 25 cm gave a higher daily growth rate of 1.79 g/ day. Alifatri's (2012) research shows that seaweed seeds planted with a spacing of 40 cm produce an average daily growth rate of 4.07 g/day and an average agar content of 15.71%/day. Meanwhile, research by Darmawati *et al.*, (2016) showed that the highest carotenoid content was obtained at a planting distance of 30 cm at 12.53 mg/g.

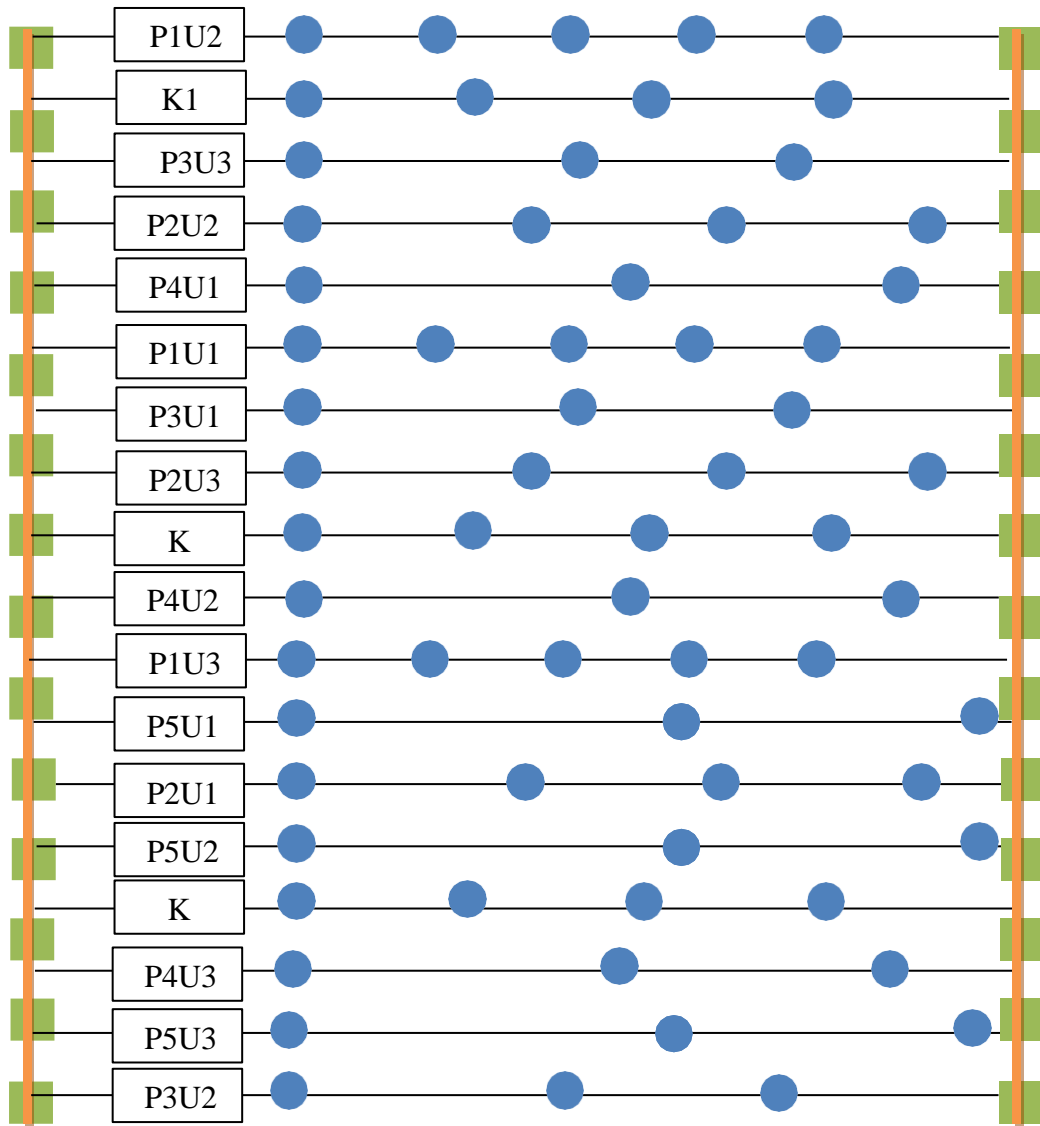


Figure 1. Treatment plan

Information :

- : Main rope
- : Wooden peg
- : Seaweed
- : Ris rope



**Prosedurs**

**Cultivation Preparation Stage**

This preparation includes preparing tools and materials. Preparation of tools in the form of 4 mm PE rope as a 250 cm long ris rope. The distance between seaweed seeds depends on the treatment used, namely 20 cm, 25 cm, 30 cm, 35 cm, 40 cm and 45 cm. Next, tagging is installed as a sign of the treatment. Tagging K is for a planting distance of 25 cm, tagging P1 is for a planting distance of 20 cm, tagging P2 is for a planting distance of 30 cm, tagging P3 is for a planting distance of 35 cm, and tagging P4 is for a planting distance of 40 cm, and tagging P5 is for a planting distance of 45 cm. Prepare a bondre made of net with a mesh size of 0.5 cm.

The cultivation container was made with dimensions of 450 cm × 250 cm. The container frame is a 10 mm PE rope as the main rope. These ropes are connected by wooden stakes. The wood used is bamboo cut to a size of 1 m. 26 pieces of bamboo were used.

### Preparing *Caulerpa lentilifera* Seeds

The seaweed seeds used in this research came from Kertasari Village, Taliwang District, West Sumbawa Regency. The age of the seedlings is 30 days (Cokrowati, 2016). Each bundle is tied with the same seed, namely 25 g. Then the seeds are wrapped using *bondre* and *tagged*.

### Planting *Caulerpa lentilifera* Seeds

Seedlings are planted at low tide and spread out from east to west so that all rows of seaweed receive optimum sunlight intensity (Rebekka *et al.* 2018). The planting process is carried out by tying seaweed to ropes. Then the seaweed is covered with *bondre* by tying *bondre* to each seaweed. Next, tie the ris rope to the main rope with two ties so that the ris rope is strong against currents and waves. The rope is stretched tightly and must not be slack so that the rope does not move easily due to current movements.

### Maintenance of *Caulerpa lentilifera*

During the maintenance period, seaweed will be covered in a lot of dirt carried by the current. Therefore, it is necessary to control every seven days. This control can be done by shaking the seaweed so that the dirt falls off. Control is carried out in the afternoon or morning when the water recedes.

### Harvesting

The seaweed harvested is seaweed that is 45 days old. Harvesting is carried out at low tide and is carried out directly at the cultivation location. Harvesting is done by removing the seaweed from the ris rope carefully so that the seaweed *thallus* does not break.

The seaweed that has been harvested is then put into a thermos. Seaweed should not be stacked with excessive weight because it can break the *thallus*. The seaweed is then put into a thermos to avoid high temperatures, free from dirt and avoid mixing with rainwater or fresh water.

## RESULT AND DISCUSSION

### Absolute Growth

The absolute growth parameter observed in this study was the weight growth of *Caulerpa lentilifera*. The results of weight measurements during the maintenance period can be seen in Figure 2.

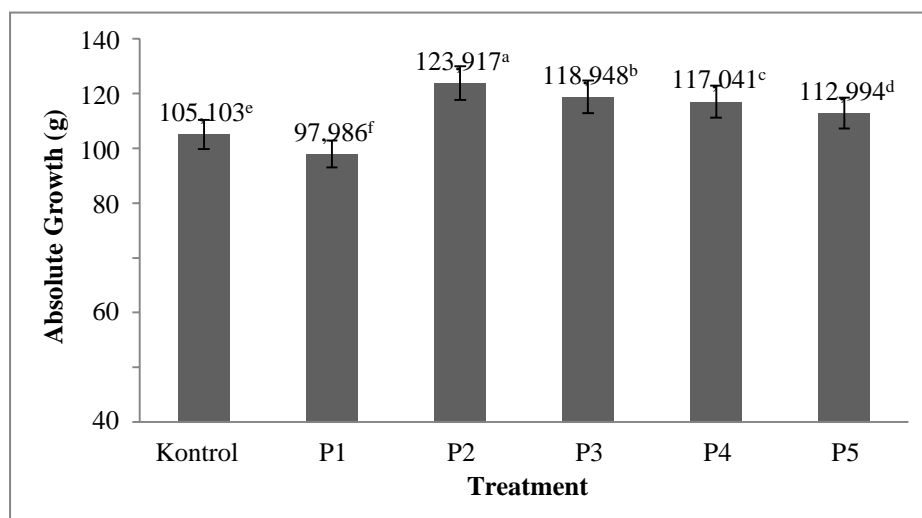


Figure 2. Absolute growth of RL in Control treatments (planting distance 25 cm), P1 (planting distance 20 cm), P2 (planting distance 30 cm), P3 (planting distance 35 cm), P4 (planting distance 45 cm), P5 (planting distance 45 cm).

The absolute growth of *C. lentilifera* in each treatment was sequential from the highest, namely P2, 123,917 g, followed by P3, 118,948 g, then P4, 117,041 g, and P5 showed growth of 112,994 g, while the control was 105,103 g and the lowest growth was P1, 97,986 g. Absolute growth data showed significantly different results ( $p < 0.05$ ). The results of further tests using the Duncan test (Appendix 4.) showed that the highest absolute growth was in P2, significantly different from the control treatment, P1, P3, P4, and P5.

### Specific Growth Rate

The specific growth rate of *C. lentilifera* is related to weight growth during the cultivation period. The growth results of *C. lentilifera* can be seen in Figure 3.

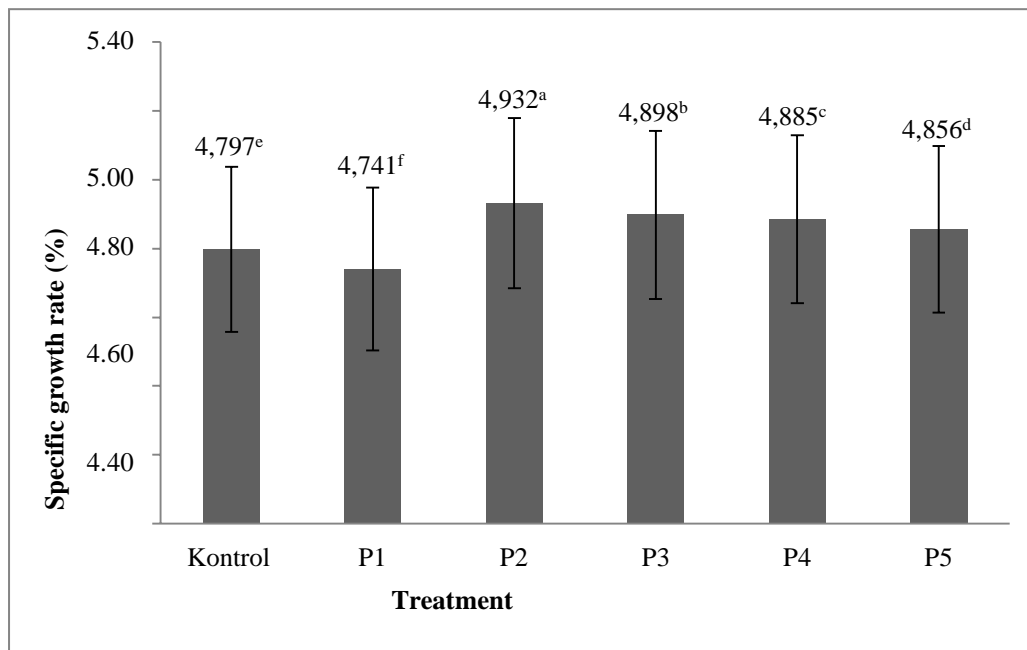


Figure 3. Specific growth rate

Information: Kontrol = plant distance 25 cm, P1 = plant distance 20 cm, P2 = plant distance 30 cm, P3 = plant distance 35 cm, P4 = plant distance 45 cm, P5 = plant distance 45 cm.

Based on the observation results, the specific growth rate values of *C. lentilifera* were in order from the highest in each treatment, namely P2 of 4.932%, P3 of 4.898%, P4 of 4.885%, P5 of 4.856%, kontrol of 4.797%, and P1 of 4.741%. Specific growth rates showed significantly different values ( $p < 0.05$ ). Further tests using the Duncan test (Appendix 4.) showed that the highest specific growth rate was found in P2 which was significantly different from the control, P1, P3, P4, and P5.

### Karotenoid

The carotenoid content in *C. lentilifera* can be measured using a spectrophotometer. The results of observations of the carotenoid content in *C. lentilifera* can be seen in Figure 4.

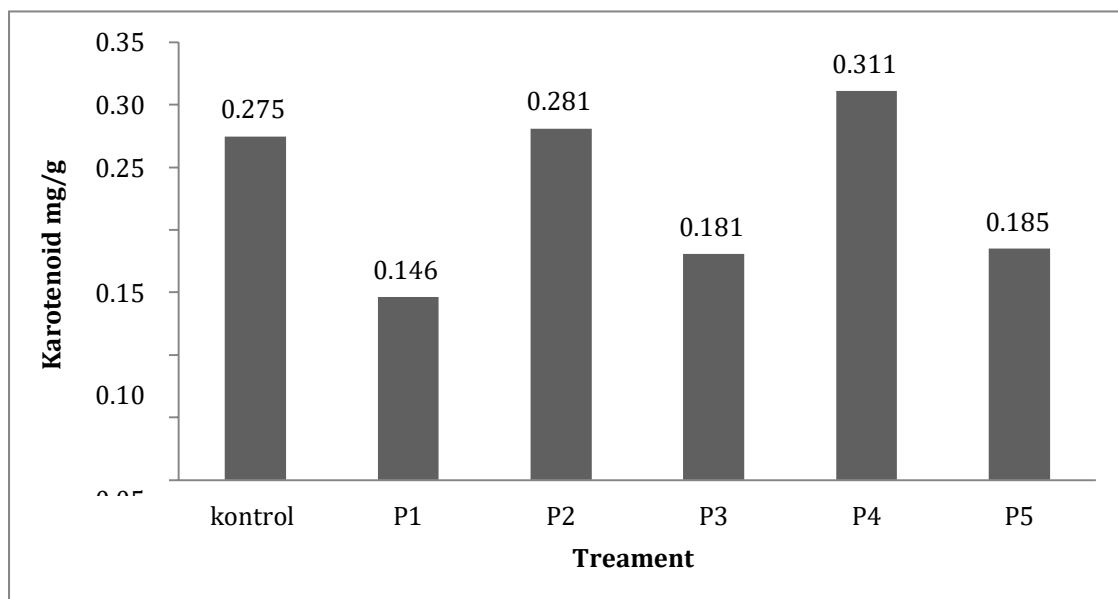


Figure 4. Carotenoid Content. Note: Control = plant distance 25 cm, P1 = plant distance 20 cm, P2 = plant distance 30 cm, P3 = plant distance 35 cm, P4 = plant distance 45 cm, P5 = plant distance 45 cm.

The relationship value between carotenoid content and planting distance is weakly correlated (Appendix 5.) which means that planting distance is not related to carotenoid content in *C. lentilifera*. Observation of the carotenoid content in *C. lentilifera* showed sequential results for each treatment, namely control at 0.275 mg/g, P1 at 0.146 mg/g, P2 at 0.281 mg/g, P3 at 0.181 mg/g, P4 at 0.311 mg/g, and P5 of 0.185 mg/g.

### Water Quality

*C. lentilifera* cultivation activities are also influenced by the water quality in the waters. Water quality values must comply with standards for the life of *C. lentilifera*. The water quality values during cultivation activities are in Table 3.

Table 3. Results of Water Quality Parameter Measurements During Research

Parameter	Value Range	Referensi
DO (mg/l)	5-7,12	5-7,24 (Jaelani, 2015)
Salinity (ppt)	31-36	30-37 (Aslan, 1991 <i>dalam</i> Widowati <i>et al</i> , 2015)
pH	7,7-8,1	6-9 (Zatnika, 2009 <i>dalam</i> Alifatri, 2012)
Temperature (°C)	27-31	25-31 (Piazzi <i>et al.</i> , 2002 <i>dalam</i> Iskandar <i>et al.</i> , 2015)
Current (m/s)	0,085-0,116	0,15-0,50 (Akbar, 2014)
Depth (cm)	40-113	>30 cm (Akbar, 2014)
Brightness (m)	0,36-0,61	0,6-0,8 (Sediadi, 2000 <i>dalam</i> Iskandar <i>et al.</i> , 2015)

### Discussion

#### Absolute Growth and Specific Growth Rate

Absolute growth and specific growth rate have the same measurement parameter, namely the weight of the thallus of *C. lentilifera*. Absolute growth is used to measure the growth of *C. lentilifera* during cultivation activities, while the specific growth rate is calculated based on

growth and length of cultivation time. The results of the ANOVA test showed that the absolute growth and specific growth rates for each treatment gave significantly different results.

The highest absolute growth and specific growth values were found in P2 (planting distance 30 cm), namely 123.917 g and 4.932%. At a planting distance of 30 cm, it is thought that the distribution of nutrients is quite good, this influences seaweed to grow optimally through the photosynthesis process. According to Darmawati (2015), a planting distance of 30 cm reduces competition between seaweeds in absorbing nutrients. According to Azizah et al. (2018) each thallus will get the same opportunity for photosynthesis by applying appropriate plant spacing.

Plant spacing that is too wide causes the space between seaweed to become larger, resulting in more space for the existence of phytoplankton. According to Desy et al. (2016) competition between seaweed and phytoplankton occurs in the processes of respiration and photosynthesis through the diffusion of O<sub>2</sub> and CO<sub>2</sub> in the waters. This can disrupt the photosynthesis process in seaweed, causing disruption to growth.

Treatment (P1) showed the lowest growth, thought to be because during cultivation activities *C. lentilifera* continued to grow and resulted in the space between *C. lentilifera* becoming narrower. The narrower distance is thought to cause a decrease in metabolism in seaweed due to higher competition between *C. lentilifera* thallus for nutrient absorption. Based on Widiastuti's (2011) statement in Azizah (2018), seaweed that continues to grow causes the space between seaweed to become narrower, making it less optimal for nutrient absorption. Pong-Masak and Sarira (2018) added that planting distance also affects the photosynthesis process because narrow planting distance will cause the thallus to be covered by dirt. Desy et al. (2016) also added that plant spacing that is too narrow will disrupt the diffusion process for seaweed and will affect the photosynthesis process.

## **Karotenoid**

The results of the regression analysis for carotenoid content (Appendix 5.) show that the relationship between planting distance and carotenoid content in *C. lentilifera* is weakly correlated, namely 2%. The carotenoid content in all treatments ranged from 0.146 mg/g to 0.311 mg/g. According to Ismianti (2018) carotenoid content can be influenced by various physical, chemical, gene factors and accuracy in laboratory testing. The weak relationship between carotenoid content and planting distance in this study is thought to be because nutrient absorption at each planting distance does not affect the difference in nutrients absorbed to improve seaweed quality. According to Darmawati et al., (2016) planting distance is related to water currents that carry nutrients, where nutrients are an important factor supporting photosynthesis for the growth and quality of seaweed, including carotenoid content. Nutrients in these waters are carried by currents and then enter the seaweed through a diffusion process. Darmawati (2017) stated that nutrients are needed to support growth and cell division to improve the quality of seaweed in the form of nitrogen (N), phosphorus (P), and carbon (C). Pong-Masak & Sarira (2018) added that nitrogen plays a role in stimulating thallus growth while phosphorus is a supporting factor for the photosynthesis process so that it can stimulate growth and improve the quality of seaweed. According to Thirumaran & Anantharaman (2009) in Herliany et al. (2016) the growth and quality of seaweed is the result of interactions between sunlight, temperature, nutrients and water movement.

Absorption of nutrients in waters will help the photosynthesis process. Photosynthesis is also influenced by the intensity of sunlight absorbed by seaweed. The intensity of sunlight affects the structure of enzymes that play a role in carotenoid biosynthesis. This is as stated by Johnson & An (1991); Albrecht & Sandman (1994) in Hasidah et al. (2017) one of the factors that influences carotenoid biosynthesis is light intensity which can increase carotenoid hydroxylase (CH) and phytoen synthase (PSY) mRNA levels. Madja (1997) in Ismianti (2018) added that chlorophyll biosynthesis is carried out by certain genes in the chromosomes where the photosynthesis process occurs. These genes become enzymes that will play a role in the tetrapyrrole biosynthesis pathway as a structural center and chlorophyll in the formation of thallus and stems from seaweed..



The weak correlation between plant spacing and carotenoid content is also thought to be due to the depth applied in the base stake method. According to Runtuboy & Abadi (2018), the water depth in the long line and floating raft methods can be adjusted, while in the base stake method it cannot be adjusted according to the desired depth because the cultivation construction is placed at the bottom of the water. According to Darmawati (2017), the long line and floating raft methods move according to the applied depth so that the seaweed depth remains below the water surface as desired. In research conducted by Darmawati et al. (2016) and Darmawati (2017) found that the highest carotenoid content was at a planting distance of 30 cm and a depth of 50 cm below the surface of the water. The depth of the water affects the intensity of sunlight that enters the water and will influence the photosynthesis process that occurs, while the distance between plants affects the absorption of nutrients in the water. The carotenoid content in seaweed is not only influenced by planting distance but also by water depth. According to Bischof et al. (2006) in Darmawati (2017) the carotenoid content is influenced by the response of seaweed to the environment as a form of carotenoid photoprotection where this is influenced by the depth at which it grows.

The relationship between the growth of *C. lentilifera* and the carotenoid content of *C. lentilifera* in this study was weakly correlated, namely 21%. Based on research by Ismianti (2018), there is no relationship between carotenoid content and seaweed growth. This is because the carotenoid content is influenced by genetics and light absorption on carotenoid production.

### **Water Quality**

Water quality parameters also influence seaweed growth. Dissolved oxygen in waters is produced by photosynthesis from aquatic plants including seaweed. Based on the results of measurements during the research ranging from 5-7.12 mg/l, it can be said that DO is suitable for seaweed growth. According to Jaelani (2015) the range of dissolved oxygen in waters suitable for seaweed growth is 5-7.12 mg/l. The pH value range in this study was 7.7-8.1 which can be said to be suitable for seaweed growth. Alifatri (2012) stated that an acidity level of less than 6.5 greatly affects the growth rate and will stop the reproductive process. The salinity in this study shows values in the normal range, namely 31-36 ppt. Aslan (1991) suggested that a salinity value of 30-37 ppt is suitable for seaweed cultivation. If the salinity value is less than the optimal limit, it will affect the growth of seaweed. According to Darmawati (2017), inappropriate salinity values will affect the process of changing osmotic pressure in cells. Different concentrations of solutions inside and outside the cell cause the Golgi body to work harder to adjust to the changes that occur, thus requiring greater energy that should be used for growth.

The temperature values in this study are included in the category suitable for seaweed growth, namely around 27-31°C. Based on the statement of Piazzini et al. 2002 in Iskandar et al. 2015, a good temperature range to support seaweed cultivation is 25-31 °C. According to Akbar (2014) temperature has an influence on the physiological processes of seaweed. The brightness in this study shows a value range of 0.36-0.61 m and a depth value of 40-113 cm. According to Sediadi (2000) in Iskandar et al. (2015) the appropriate brightness for the growth of *C. lentilifera* is 60-80 cm. According to Akbar (2014), a good depth for seaweed cultivation is >30 cm. This brightness and depth can still be said to be good for the growth of *C. lentilifera* because sunlight can enter the water. According to Iskandar et al. (2015) sunlight entering the waters is used for photosynthesis by seaweed. Current measurements still show a good range of values for seaweed life, namely between 0.085-0.116 m/s. According to Akbar (2014), the appropriate current for seaweed cultivation is 0.15-0.50 m/s, this current plays a role in absorbing nutrients brought by water movement. Appropriate water movement causes the talus to be washed of adhering dirt and obtains an adequate supply of nutrients for growth. The depth of the water for cultivation will influence the cultivation method applied. The depth for cultivation using the basic stake method is a minimum of 30 cm at the lowest tide. This aims to ensure that the seaweed does not dry out when the water recedes.

## CONCLUSION

### Conclusion

Based on the results of this research, it can be concluded that:

1. The difference in plant spacing applied in the base stake system does not have an influence on the carotenoid content of *C. lentilifera*, but does influence absolute growth and specific growth rate.
2. No ideal planting distance was found to increase carotenoid content in *C. lentilifera*, because each treatment tended to have relatively the same carotenoid content.

### Suggestion

The less than optimal carotenoid content in *C. lentilifera* is thought to be due to the inappropriate intensity of sunlight entering the water due to the water depth that was applied in this study could not be controlled. It would be advisable to conduct research on the effect of depth on the carotenoid content of *C. lentilifera*.

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