JPPIPA 9(5) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Growth Analysis of *Caulerpa lentillifera* Cultivated at Laboratory Scale with Different Light Intensities

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Received: November 15, 2023
Revised: April 10, 2024
Accepted: May 25, 2024
Published: May 31, 2024

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DOI: 10.29303/jppipa.v10i5.6126

Abstract: In Indonesia, *Caulerpa* sp. is often utilized as an edible food ingredient by eating it raw as a vegetable salad or as a vegetable. Different light intensities greatly affect the growth of sea grapes (*Caulerpa lentillifera*). However, this type of seaweed is still few who can cultivate it. Therefore, the purpose of the study was to analyze the growth performance of *Caulerpa lentillifera* seedlings at the cultivation stage in the laboratory with different light intensities. The research was conducted using the RAL method with 5 treatments and 4 replications. Light intensity (1,000, 2,000, 3,000, 4,000, 5,000 Lux) was applied during cultivation. The specific growth rate ranged from 0.0065%/day - 0.0061%/day. The number of ramuli of *Caulerpa lentillifera* ranged from 651 grains - 723 grains. The number of tallus *Caulerpa lentillifera* during 45 days of maintenance with various treatments ranged from 28 branches - 32 branches. The conclusion of this research is that light intensity significantly affects the growth of *Caulerpa lentillifera.*

Keywords: *Caulerpa Lentillifera*; Growth; Light intensity

Introduction

Caulerpa is a genus of marine algae from the Caulerpaceae family and includes species from the Chlorophyceae class (green algae) and includes species from the Chlorophyceae class (green algae). Sea grapes have thallus forming roots, each stolon and ramuli. Ramuli have formed rounds docked regularly covering branching along ± 5cm (de Gaillande et al., 2017; Zubia et al., 2020). According to Sunaryo et al. (2015) this plant lives attached to the bottom substrate of waters such as coral fragments, sand and mud.

Caulerpa sp. in Indonesia is often used as a food ingredient by eating it raw as fresh vegetables or as vegetables (Antara et al., 2022; Apriliyanti et al., 2021; Novianti et al., 2015; Rahayu et al., 2019). Sea grapes (*Caulerpa* sp.) in Indonesia are still few who can cultivate them and the market stock comes from natural collection. The availability of sea grapes in nature is increasingly limited so that cultivation techniques are needed to increase the amount of seaweed production so that demand can be met sustainably.

In this research using different light intensity, this is because different light intensity is very influential on the growth of sea grapes (*Caulerpa lentillifera*). This is closely related to the increase in photosynthesis process will cause metabolic processes that stimulate seaweed to absorb more nutrients that will support its growth (Cai et al., 2021; Lee & Kang, 2020; Zou & Gao, 2014). Light intensity is a strong light emitted by a light source in a particular direction (Rosnawati et al., 2022; Terada, 2021).

Caulerpa lentillifera production is still relatively low. This is because the production of *Caulerpa* sp. still relies on the results of nature so it is very dependent on the season. One of the sea grape cultivation techniques that can be done is by environmental engineering. Laboratory-scale cultivation is one of the cultivation efforts that can be done to improve the cultivation of good quality *Caulerpa* sp. Therefore, the purpose of this

How to Cite:
Apriliyanti, F. J., Risjani, Y., Hertika, A. M. S., & Paricahya, A. F. (2024). Growth Analysis of Caulerpa lentillifera Cultivated at Laboratory Scale with Different Light Intensities. *Jurnal Penelitian Pendidikan IPA*, *10*(5), 2346–2353. https://doi.org/10.29303/jppipa.v10i5.6126

study was to analyze the growth performance of *Caulerpa lentillifera* seedlings at the laboratory cultivation stage using different light intensities.

Method

Research location

This research was conducted in August - September 2023. *Caulerpa lentillifera* cultivation was carried out at Central Algae, 9th Floor, Faculty of Fisheries and Marine Science, Universitas Brawijaya.

Research design

The method used in this research is an experimental method using RAL consisting of 5 treatments with different light intensities. Each treatment was repeated 4 times so that 20 experimental units were obtained. The weight of seedlings of each treatment was weighed at 50 gr/unit with media or cultivation containers filled with seawater 2.5 liters/unit. This study was conducted for 45 days. Using different light intensities of 1000 Lux (A), 2000 Lux (B), 3000 Lux (C), 4000 Lux (D), and 5000 Lux (E) arranged on the culture rack (Figure 1).

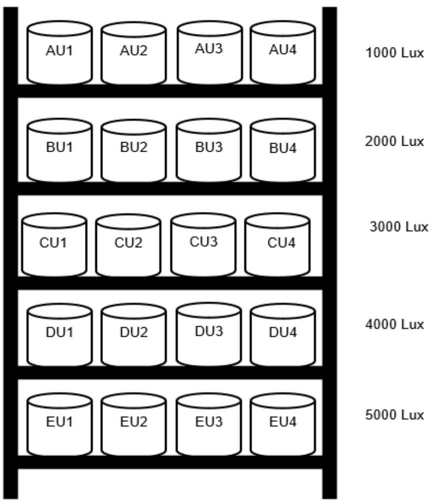

Figure 1. Layout of cultivation units

Preparation of cultivation media

This research begins with the preparation and sterilization of containers and cultivation tools. Then prepared a 5 liter jar, coral, aerator hose and aerator stone then cleaned using chlorine, washed with soap and rinsed thoroughly. Then autoclaved (ozone) for 15 minutes. *Caulerpa lentillifera* culture containers in laboratory-scale cultivation activities are chemically sterilized using chlorine. Chlorine was weighed as much as 20 grams and then dissolved with 1 liter of water. Then chlorine was given at a dose of 1 ml/L into seawater, so that the chlorine needed in this study was 50 ml given in 50 liters of seawater, after which it was allowed to stand for 24 hours. Before the seawater is used for *Caulerpa lentillifera* culture, the seawater is given a Na-thiosulfate solution. Na-thiosulfate was weighed as much as 20 grams and dissolved in 1 liter of water. Na-thiosulfate is given at a dose of 1 mL/L into seawater which serves to neutralize the chlorine still contained in the seawater and allowed to stand for 5 to 10 minutes (Buwono, 2018). Then the seawater is filtered using a filter cloth into the flask and then autoclaved for 30 minutes. After that, seawater that has finished in the autoclave is lowered in temperature first before being put into the cultivation container. After cooling, the sterile seawater was poured into a 5 liter jar as much as 2.5 liters of seawater.

Seed selection

After taking seedlings in nature. *Caulerpa lentillifera* seedlings taken from nature or its natural habitat attached to corals are selected to obtain healthy *Caulerpa lentillifera* seedlings. After that, the seeds are selected by paying attention to the characteristics of healthy *Caulerpa lentillifera* such as, the talus is not easily broken, not opaque, has an intact talus, there is no more slimy and there are no white spots on sea grapes (Syamsuddin et al., 2019; Windarto et al., 2023).

Seedling acclimatization

In this research, acclimatization was carried out on *Caulerpa lentillifera* seedlings which aims to make it easier for *Caulerpa lentillifera* to adapt to a new environment. Acclimatization can increase the survival rate and growth rate of seaweed. Acclimatization in this study was carried out for 7 days. Acclimatization was stopped when the roots of *Caulerpa lentillifera* seedlings have been attached to the cultivation media (coral/rock) and increased growth in the seaweed. According to Ayunindya et al. (2021) states that good acclimatization for seaweed can be done for 7 days.

Specific growth rate

Specific growth rate is the daily growth rate, the percentage of weight gain per day. Absolute weight growth was calculated on days 0, 9, 18, 27, 36 and 45. According to Retnani & Nurlita (2013), specific growth can be measured using the specific weight growth Formula 1:

$$SGR = \frac{(Ln\,Wt - Ln\,Wo)}{t} x\ 100\% \quad (1)$$

Notes:
SGR = Specific daily growth (%/day)
Wt = Average weight of fish at the end of the study (g)
Wo = Average weight of fish at the beginning of the study (g)

t = Time (length of rearing)

Calculation of the number of spheres

Counting the number of spheres on the thalus with a predetermined time on day 0, 9, 18, 27, 36 and 45. Counted all the spheres on the tallus of *Caulerpa lentillifera.*

Calculation of the number of tallus branching

Counting the number of branches with a predetermined time on day 0, 9, 18, 27, 36 and 45. Calculated the number of all tallus/branches on *Caulerpa lentillifera*.

Result and Discussion

Acclimatization

Acclimatization is an important process in cultivation that aims to adapt macroalgae to a new environment before being permanently planted or cultivated. In this study, acclimatization was carried out for 7 days. In *Caulerpa lentillifera*, the acclimatization process aims to familiarize plants with the environmental conditions they will face, such as water temperature, light intensity, salinity, and pH. According to Yengkhom et al. (2019) the acclimatization process in *Caulerpa* sp. aims to familiarize or adjust *Caulerpa* sp. to new environmental conditions such as light intensity, temperature, salinity, pH and DO.

Table 1. Acclimatization of *Caulerpa lentillifera* seedlings.

Day	Roots on substrate
Day-1	Not attached
Day-2	Not attached
Day-3	Some have attached
Day-4	Some have attached
Day-5	Some have attached
Day-6	Already attached to the substrate
Day-7	Already attached to the substrate

In this study it can be seen that the 3rd day caulerpa can already attach to the substrate, namely coral rock. This is characterized by the attachment of *Caulerpa lentillifera* roots to the substrate so that it can be stated that *Caulerpa lentillifera* has been able to adjust to the laboratory. On the 6th and 7th day, *Caulerpa lentillifera* has perfectly attached to the substrate which is characterized by the attachment of roots to the substrate, growth, and the addition of spheres. *Caulerpa lentillifera* usually requires stable temperature and light intensity during acclimatization. According to Roth-Schulze et al. (2018) the acclimatization process involves the gradual adjustment of water temperature from the initial environment in which the plant was purchased or found towards the desired conditions for cultivation. According to Valentine et al. (2021) when environmental conditions are not good, the results of seaweed cultivated or produced can experience damage to the thallus with white spots on the thallus (ice-ice) and there are parts of the thallus that peel off.

Appropriate light intensity is important for the growth of *Caulerpa lentillifera*. According to Stuthmann et al. (2021) the acclimatization process involves adjusting to the light intensity that will be encountered at their cultivation site. In addition, water salinity is an important factor in *Caulerpa lentillifera* growth. According to Bambaranda et al. (2019) the acclimatization process can help plants adjust to changes in salinity that may occur when transferred to a new environment. Water pH can also affect the growth of *Caulerpa lentillifera*. According to Taise et al. (2023) the acclimatization process can help *Caulerpa lentillifera* to adjust to changes in pH in water that can occur in a new environment.

Specific growth rate

The results showed that the specific growth rate during the 45-day rearing period with different light intensity treatments ranged from 0.0061%/day - 0.0065%/day.

The highest specific growth rate of *Caulerpa lentillifera* was found in treatment C which used 3000 Lux light intensity with a specific growth rate value of 0.0065%/day. Then followed by treatment E with light intensity of 5000 Lux with a value of 0.0061%/day, treatment D (light intensity 4000 Lux) with the value obtained is 0.0060%/day, treatment B (light intensity 2000 Lux) with a value of 0.0042%/day, and in treatment A (light intensity 1000 Lux) with the results of the value obtained is 0.0037%/day.

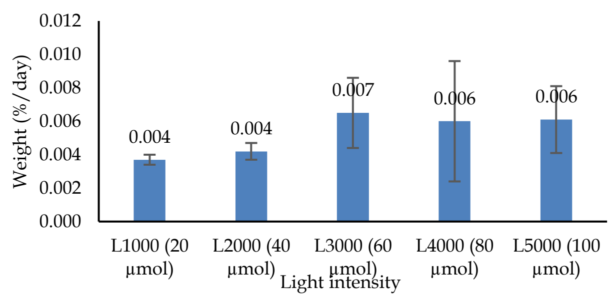

Figure 2. Specific growth rate of *Caulerpa lentillifera* with light intensity

Based on the results of One-Way ANNOVA analysis using SPSS, it was found that the cultivation of *Caulerpa lentillifera* using treatments with different light intensities had a significant effect ($P < 0.05$) on the specific growth rate of Caulerpa lentillifera.

According to Conceicao et al. (2020) showed that the specific growth rate or specific grouth rate (SGR) of seaweed (*C. rasemosa*) maintained for 45 days showed differences between each treatment. Light intensity has a very significant effect on the specific growth of seaweed (*C. rasemosa*). Adequacy of sunlight intensity received by seaweed determines the speed of seaweed to meet the needs of nutrients such as carbon (C), nitrogen (N) and phosphorus (P) for growth and development. Sitorus et al. (2020) light intensity gives a real influence on the growth of sea grapes. The optimal light intensity for the growth of sea grapes (*Caulerpa lentillifera*) between 2500 Lux to 3000 Lux.

According to Jia et al. (2019) algae such as *Caulerpa lentillifera* have high light requirements to grow well. If the light intensity is sufficient, there can be an increase in the specific growth rate because the plant can absorb more energy for metabolic processes. In addition, high light intensity can stimulate the vertical growth of *Caulerpa lentillifera* talus, allowing the plant to reach a greater height quickly. According to Bartlett & Elmer (2021) low light intensity can also cause morphological changes such as the size of the tallus on *Caulerpa* not increasing in size.

Number of ramuli

The results of the research conducted showed that the number of spheres (ramuli) of *Caulerpa lentillifera* during the 45-day rearing period with different light intensity treatments ranged from 651 grains - 723 grains.

The results of One-Way ANNOVA analysis using SPSS showed that the cultivation of *Caulerpa lentillifera* using treatments with different light intensities had a non-significant effect ($P < 0.05$) on the number of spheres (ramuli) of *Caulerpa lentillifera*.

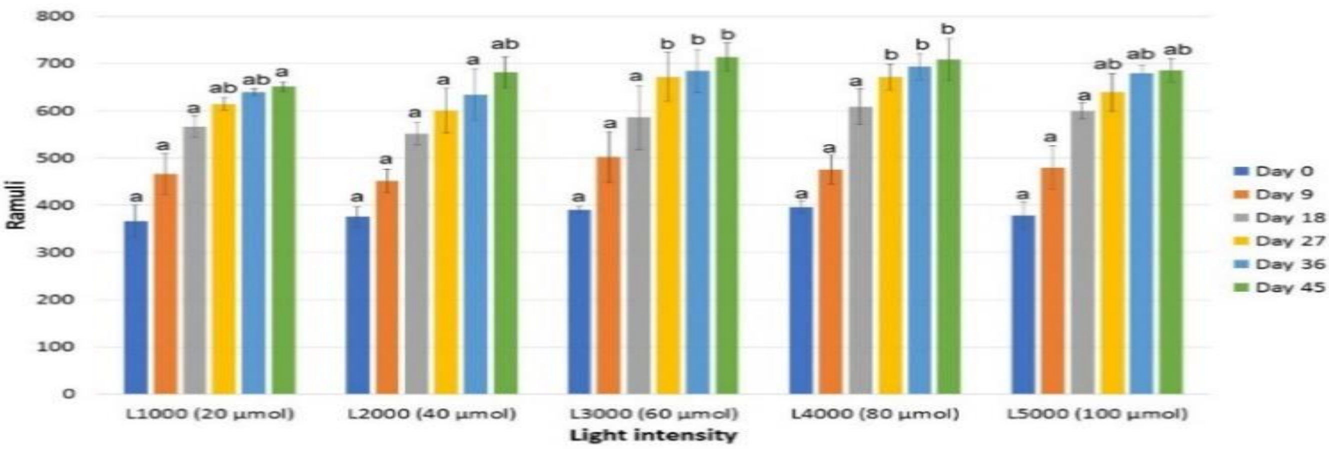

Figure 3. Average number of ramuli of *Caulerpa lentillifera* cultivated with different light intensity (N=4).

Ramuli is a part of *Caulerpa lentillifera* that is round and serves to capture nutrients contained in the surrounding environment. According to Renoncourt & Meinesz (2022) ramuli can help *Caulerpa lentillifera* to capture more sunlight and nutrients from the surrounding environment. The number, length and ramuli on *Caulerpa* can differ between species and environmental factors. The results showed that light intensity, temperature, water current, cultivation method, and density of sea grapes can affect the number of ramuli of sea grapes. In treatment C, the light intensity of 3000 Lux produced the highest number of spheres (ramuli) of *Caulerpa lentillifera* with the number of spheres obtained ranging from 723 grains. This is reinforced by the statement (Anh et al., 2020) emphasizing that technical factors such as initial stocking density, water movement, temperature, culture methods used, nutrient supply in seawater can affect the growth rate, shape, production and texture of ramuli.

Light is the main source of energy for photosynthesis in algae, which is the process by which algae produce their own food. The more light provided, the more energy the algae can use for photosynthesis (Ukabi et al., 2013). This can result in better growth and potentially more ramuli. In the study Gao et al. (2019) new ramuli could be found on both new erect talus and fragments of early erect talus. The number of new ramuli was significantly greater at 25°C than at 15 and 20°C.

The negative impact of low light intensity is that growth is inhibited or prolonged. Low light intensity can inhibit the rate of photosynthesis, which in turn can slow the growth of *Caulerpa lentillifera* ramuli. Photosynthesis is the process by which algae use light to convert carbon dioxide into carbohydrates, which are the main source of energy for growth (Xiao et al., 2019). According to (Balanquit et al., 2015) low light intensity can affect the reduction of photosynthetic pigment production, namely chlorophyll pigment. According to Yıldız & Tiryaki (2017) chlorophyll is very important in absorbing light and converting it into energy that can be used by algae. Lack of chlorophyll can lead to low photosynthetic efficiency and can reduce the production of chlorophyll.

Number of Tallus Branching

The results of this study showed that the number of tallus *Caulerpa lentillifera* during the 45-day maintenance period with various treatments of different light intensities ranged from 28 branches - 32 branches. Based on the results of One-Way ANNOVA analysis, it was found that the cultivation of *Caulerpa lentillifera* using different light intensity treatments showed a significant effect ($P < 0.05$) on the number of tallus on *Caulerpa lentillifera.*

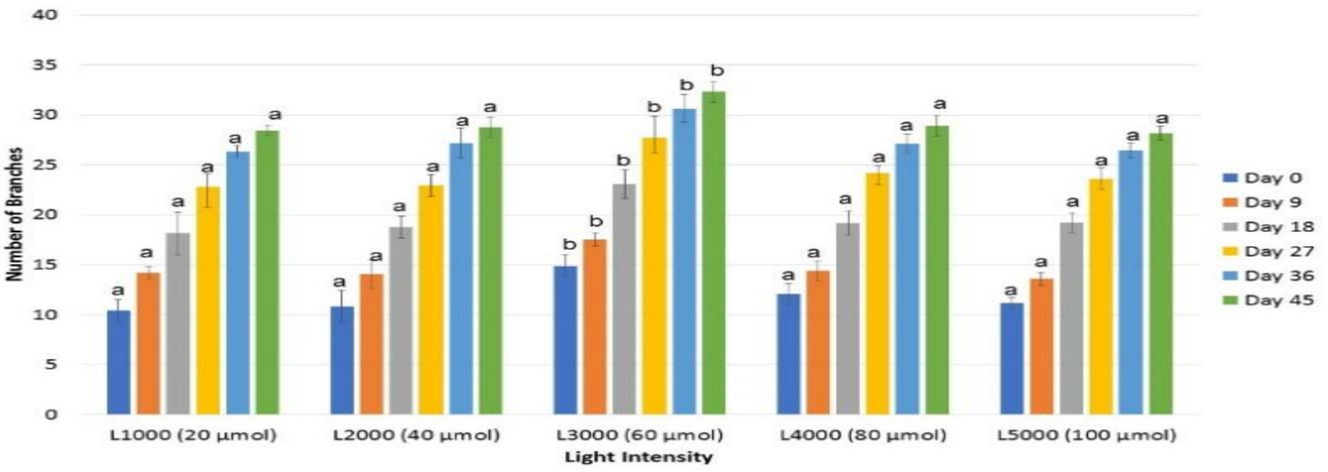

Figure 4. Number of *Caulerpa lentillifera* Tallus Branches per Day with Different Light Intensities during 45 days of Cultivation.

The results showed that different light intensities affect the number of tallus. The highest number of tallus of *Caulerpa lentillifera* was found in treatment C (3000 Lux) which used light intensity of 3000 Lux with the number of tallus 32 branches. The effect of light intensity on the number of tallus in *C. lentillifera* can be caused because algae require a high enough light intensity to perform photosynthesis well. Optimal light intensity will allow *C. lentillifera* to produce food to grow well. According to Shindo et al. (2022); Terada (2021) if the light intensity is too low, photosynthesis will be disrupted and the growth of *C. lentillifera* will be inhibited. If light conditions are too low *C. lentillifera* may grow with fewer tallus. However, if the light intensity is too high *C. lentillifera* will experience stress and potentially damage due to excessive light intensity and can affect the number of tallus of *C. lentillifera*. This is reinforced by the opinion Fakhrulddin et al. (2021) that light intensity in water can affect the growth of *Caulerpa lentillifera*. the deeper the water surface, the less light penetration. Physicochemical factors such as light intensity and salinity can affect the growth of *Caulerpa lentillifera* species.

According to Windarto et al. (2021) *Caulerpa* species have certain temperature preferences for optimal growth. The availability of nutrients, such as nitrogen and phosphorus, can affect growth and branching in algae. Lack or excess of certain nutrients can affect talus structure. Stress factors in the culture environment, such as changes in salinity, pollution, or rapid changes in temperature, can affect the growth and branching of the talus in *Caulerpa*. According to Ly et al. (2021) plants can respond to stress by changing their growth patterns. Plant growth stage and age can affect talus branching. According to Park et al. (2022) there are several stages of growth, plants may focus more on vertical growth than lateral branching.

Low light intensity usually has a significant impact on seaweed thallus branching. When light intensity is reduced, seaweeds tend to experience slower growth because their photosynthesis process is disrupted. Photosynthesis in seaweed requires light as one of the main factors to produce the energy needed for growth. According to Aqmal et al. (2022) seaweed growth is strongly influenced by the amount of light intensity that enters the waters and is received by seaweed.

In low light conditions, seaweed may also experience changes in morphology or physical structure. In low light *Caulerpa lentillifera* can become longer or find ways to reach brighter light sources. This can have an impact on the branching pattern of the *Caulerpa lentillifera* thallus. According to Kang et al. (2020) the growth of *Caulerpa* tallus branching can become unbalanced due to light intensity that is too high or too low.

Conclusion

The conclusion of this research is that light intensity significantly affects the specific growth rate, the number of ramuli and the number of tallus branching *Caulerpa lentillifera.* 3000 Lux light intensity treatment gives a specific growth rate of 0.0065%/day, the number of ramuli is 723 grains and the number of tallus branching is 32 branches.

Acknowledgments
The author would like to thank friends who have supported and helped this research.

Author Contributions
This authors in this research are divided into executor and advisor.

Funding
This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

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