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The effect of the epiphytes of *Chaetomorpha crassa* on the total chlorophyll-a and growth of *Gracilaria verrucosa*

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Abstract. Gracilaria belongs to the class of red algae (Rhodophyta), it has many sub-types with different morphology and anatomy. The epiphytes that often attack Gracilaria are *Chaetomorpha crassa* epiphytes; they trap Gracilaria and have a negative effect such as nutrient competition, inhibiting the light penetration and reducing the growth rate. The purpose of this study was to determine the effect of *Chaetomorpha crassa* epiphytes on the amount of chlorophyll-a and the growth of *Gracilaria verrucosa*. The treatment used was an epiphyte mass of *Chaetomorpha crassa* by as much as 0% (control), 25%, 50%, 75% and 100% of the mass of *Gracilaria verrucosa*. The analysis of the data used Analysis of Variants (ANOVA) and continued with further tests using Duncan's multiple range test. The results showed that *Chaetomorpha crassa* had no effect on the amount of chlorophyll-a, but that it affected the absolute weight growth and daily growth rate. The amount of chlorophyll-a, absolute weight growth, daily growth rate, and best long growth were obtained in treatment A (control). The amount of chlorophyll-a, absolute weight growth, and daily growth rate was 0.3110 µg / ml, 0.93 gram, and 1.68% per day respectively.

1. Introduction

The seaweed resources in Indonesia have a large potential, making it easier to increase production. At present, the business of developing seaweed is very rapid due to the demand of both domestic and international markets. Seaweed that has been widely cultivated include *Gracilaria* sp. and *Euchema* sp. [1].

The success of seaweed cultivation is influenced by physical, chemical and biological factors. The factors that influence seaweed cultivation include temperature, brightness, current, waves, pH,

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salinity, ammonia, phosphate [2], predators, symbionic organisms both beneficial and detrimental [3], seed quality and the substrate [4]. Predators such as carnivorous animals and harmful symbiotic organisms such as epiphytes interfere with the growth of seaweed [3]. The epiphytes that often attack Gracilaria include *Chaetomorpha crassa* [5]. The spread of epiphytes in ponds is influenced by environmental factors, namely currents and waves [6].

The number of thallus attacked by *Chaetomorpha crassa* increased during maintenance, which was 40.19 - 57.71% on the 30th day and the epiphytes increased to 90.24 - 95.24% after 60 days. This results in losses due to decreased seaweed growth and production [7]. High epiphytic biomass causes competition for nutrients and blocks the penetration of sunlight. Nutrient competition between epiphytes and Gracilaria causes the nutrients absorbed by Gracilaria to form chlorophyll sub-optimally. Epiphytes can cause a decrease in the amount of chlorophyll for photosynthesis [8]. Based on the description above, it is necessary to do research on the impact of *Chaetomorpha crassa* epiphytes on the amount of chlorophyll-a and the growth of *Gracilaria verrucosa*. The purpose of this study was to determine the effect of Chaetomorpha *crassa* epiphytes on the amount of chlorophyll-a and the growth of *Gracilaria verrucosa*.

2. Material and methods

2.1. Tools preparation

The research phase began with the preparation of tools for the maintenance of *Gracilaria verrucosa* seaweed. The maintenance was carried out in 20 x 20 x 25 cm aquariums. The final container preparation stage was focused on filling them with water and checking the aeration system. The water required for the maintenance of the seaweed was as high as 10 cm from the bottom of the aquarium, or about 8 liters of water [9].

2.2. Selection of seaweed seeds

Gracilaria verrucosa and *Chaetomorpha crassa* seaweed seeds from Kalialo Village, Jabon District, Sidoarjo Regency were the seeds chosen. The selection of the *Gracilaria verrucosa* test was prioritized uniformly, chosen from the number and the size of the thallus [10]. The young reddish thallus was measured from the tip to 20 cm toward the base of the thallus [11].

2.3. Planting method

The thallus to be planted had a uniform size and number of thallus, and was free from other plants or dirt. The thallus was cut to 5 cm long and weighed approximately 1 gram. The thallus was bound using rope on the rock as a substrate. After being measured, the thallus was inserted into a maintenance aquarium equipped with aeration [9].

Chaetomorpha crassa was obtained from Jabon District, Sidoarjo Regency. The attachments of *Chaetomorpha crassa* were 25%, 50%, 75%, and 100% of the mass of *Gracilaria verrucosa* respectively. This was planted by wrapping it onto the *Gracilaria verrucosa* in order to cover the surface of the thallus. This stage was carried out after the acclimatization stage of *Gracilaria verrucosa*.

2.4. Calculation of amount chlorophyll-a

The tool used to calculate the amount of chlorophyll-a was a spectrophotometer. The procedure for measuring the amount of chlorophyll was carried out at the beginning and end of the study. The seaweed was dried for one week at a temperature of 20 - 25 °C. The seaweed pigments were obtained for extraction by using 1 gram of dried seaweed with 5 ml of 80% acetone in a test tube incubated at a temperature of 20 - 25 °C for 24 hours. The acetone and seaweed immersion solution was the sample used to calculate the amount of chlorophyll. The chlorophyll extract was filtered using a Buchner filter. The extract was then centrifuged at a speed of 5000 rpm for 5 minutes. The supernatant from the extract was used to measure the chlorophyll. The distilled water was inserted into the cuvette as a blank, before being placed into the spectrophotometer and calibrated. The sample solution was

inserted into the cuvette and placed on the spectrophotometer. Furthermore, by pressing the wavelength value of 663 nm and 647 nm on the spectrometer, the absorbance value was determined [12].

3. Results and discussion

3.1. Amount of chlorophyll in Gracilaria verrucosa

The average amount of chlorophyll-a found in the initial and final extraction of *Gracilaria verrucosa* can be seen in Table 1.

Table 1. Average amount of chlorophyll-a at the beginning and end of the study.

Treatment	Amount of Chlorophyll-a Initial (µg/ml) ± SD	Amount of chlorophyll-a (µg/ml) ± SD
A	0.0305 ± 0.0000	0.03110^{a} ± 0.0008
B		0.03097^{a} ± 0.0006
C		0.03096^{a} ± 0.0021
D		0.03096^{a} ± 0.0009
E		0.03093^{a} ± 0.0002

The results obtained showed there to be no significant differences (F count < F table) in each treatment A, B, C, D, and E. This can be seen from the same superscript letter notations for all of the treatments. The ANOVA test results were then followed by Duncan's multiple distance test which showed that the *Chaetomorpha crassa* had no effect on the amount of chlorophyll-a *Gracilaria verrucosa*. The highest chlorophyll-a value was obtained in treatment A (control), which was 0.03110 µg / ml and there was no significant differences with treatment B (0.03097 µg / ml), C (0.03096 µg / ml), D (0.0396 µg / ml) and E (0.0393 µg / ml). The increase in the amount of chlorophyll-a is suspected because the levels of nitrate and phosphate in the waters, serving as nutrients for chlorophyll formation, were well fulfilled. There was thus no competition for nutrients between *Gracilaria verrucosa* and *Chaetomorpha crassa*. Plants absorb nitrate as a source of nitrogen for the constituents of proteins, chlorophyll, and nucleic acids [13].

3.2. Growth of Gracilaria verrucosa

Gracilaria verrucosa's growth measurement consisted of calculating the absolute weight growth and daily growth rate. The measurement of heavy growth was carried out every seven days by measuring the weight and length of the seaweed. The absolute growth rate can be seen in Table 2.

Table 2. The average absolute weight growth of *Gracilaria verrucosa*.

Treatment	Absolute weight growth average (gram) ± SD
A	0.93^{a} ± 0.21
B	0.54^{b} ± 0.13
C	0.48^{b} ± 0.09
D	0.33^{bc} ± 0.75
E	0.24^{c} ± 0.69

The results of the analysis of variance (ANOVA) at the 95% confidence interval showed that there were very significant differences between treatments (F count > F Table 0.01) and this continued to show in Duncan's multiple distance test. This shows that the presence of *Chaetomorpha crassa* influences the growth of *Gracilaria verrucosa*. This is because any chlorophyll-a formed cannot be used for photosynthesis properly because the light received by *Gracilaria verrucosa* is blocked by the *Chaetomorpha crassa*. Chlorophyll-a plays a role in photosynthesis by absorbing and converting light energy into chemical energy. Photosynthesis is the process of changing inorganic compounds (CO_2

and H_2O) into organic compounds (carbohydrates and O_2) with the help of sunlight. The received solar energy is used to form ATP and NADPH, which are used for growth [14].

The calculation of the daily growth rate was also done in order to determine the effect of *Chaetomorpha crassa* on growth. The average daily growth rate can be seen in Table 3.

Table 3. Daily average growth rate of *Gracilaria verrucosa*.

Treatment	Average Daily Growth Rate (% per day) ± SD week			
	1	2	3	4
A	2.5^{a} ± 1.41	1.43^{a} ± 1.35	1.93^{a} ± 0.59	1.68^{a} ± 0.32
B	1.4^{ab} ± 0.93	0.87^{a} ± 0.50	1.16^{b} ± 0.28	1.04^{b} ± 0.21
C	0.78^{b} ± 0.34	0.83^{a} ± 0.48	1.14^{bc} ± 0.25	0.98^{b} ± 020
D	0.71^{b} ± 0.45	0.50^{a} ± 0.13	0.70^{bc} ± 0.19	0.71^{bc} ± 0.15
E	0.50^{b} ± 0.58	0.53^{a} ± 0.72	0.61^{c} ± 0.18	0.52^{c} ± 0.14

Based on different superscript letter notations in the same column, this shows that the results of the daily growth rate calculations using the ANOVA test in the first week showed that there were significant differences between treatments (F count > F table 0.05). The same superscript letter notation in the second week column shows that there was no significant difference in the daily growth rate in the second week (F count < F table 0.05). The daily growth rate in the third and fourth week showed that there was a very significant difference (F count > F table 0.01) % per day and that the lowest value obtained in treatment E was 0.52% per day. This is due to the light received by *Gracilaria verrucosa* being obstructed by *Chaetomorpha crassa*, so then the formed chlorophyll-a cannot be utilized for photosynthetic activity. Epiphytes will block the penetration of sunlight so then the photosynthetic activity will be inhibited [8]. Seaweed will decrease the daily growth due to *Chaetomorpha crassa*, which causes damage to the thallus which means that the photosynthetic energy formed is not used for growth [7].

4. Conclusion

The research obtained results that allowed us to conclude that the epiphytic mass of *Chaetomorpha crassa* did not affect the amount of chlorophyll-a of *Gracilaria verrucosa*. The epiphytic mass of *Chaetomorpha crassa* affected the absolute weight growth and daily growth rate of *Gracilaria verrucosa*.

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