Effect of different N:P ratios application on the cultivation media for the growth and fiber content of Caulerpa racemosa reared in tarpaulin ponds

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Effect of different N:P ratios application on the cultivation media for the growth and fiber content of *Caulerpa racemosa* reared in tarpaulin ponds

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Abstract. *Caulerpa racemosa* or commonly known as sea grape contains higher protein than other seaweed. In addition to protein, sea grape (*C. racemosa*) also contains fat and fiber that are useful for health, especially to facilitate digestion. Environmental factors such as water temperature, salinity, light intensity, and water nutrients can affect the nutritional content of seaweed like fat, fibers, and protein. Besides influence the nutrient content in seaweed, water nutrients also affected seaweed's growth. This research aims to find out NO_3-N and PO_4-P concentrations for sea grape (*C. racemosa*) culture to optimized the growth and nutritional value, especially fiber content. This research was conducted in June-July 2019 at Balai Besar Perikanan Budidaya Air Payau (BBPBAP), Jepara. Sea grape (*C. racemosa*) used for this study was obtained from Jepara, Central Java. The N:P treatments used were 5N:1P, 6N:1P, 7N:1P, and 8N:1P. The parameters that were measured were growth, specific growth rate (SGR), nitrate absorption, fiber content, and water quality. The results showed that the N:P ratio for optimizing sea grape (*C. racemosa*) growth was 6N:1P (3.35±0.002% d^{-1}), whereas the best N:P ratio for sea grape fiber (*C. racemosa*) was 7N:1P (4.05%).
Key Words: seagrapes, nutrients, fiber, nitrogen, phosporus.

Introduction. Seaweed of the genus *Caulerpa* is a widespread seaweed and is commonly found in the intertidal and subtidal zones (Ukabi et al 2013). Distribution of *Caulerpa racemosa* seaweed includes tropical and subtropical regions, such as the Philippines, Vietnam, Singapore, Malaysia, Thailand, Taiwan, China, Indonesia, and western Pacific waters (Ridhowati & Asnani 2016). *C. racemosa* or commonly known as seagrape or sea grape, is a green alga that grows on rocky substrates that are in the intertidal area of water (Bhuiyan et al 2016).

C. racemosa contains relatively higher protein than some other types of seaweed. In addition to protein, *C. racemosa* also contains fats and fibers, which are useful in health aspects, especially to facilitate digestion. Furthermore, Bhuiyan et al (2016) reported that *C. racemosa* contains 10-47% protein; it also contains higher fat and fiber when compared to seaweed that is commonly consumed by humans, such as the genus *Gracilaria*.

Several things influence the growth and nutrient content of *C. racemosa* that is cultured. One of them is a nutrient found in the waters where *C. racemosa* is cultured. The nutrients needed for *C. racemosa* for the growth process and also to increase its nutritional content are Nitrogen (N) and Phosphorus (P) (Guo et al 2014). The content of N and P in each water is different. If the N and P content in the waters to be used for *C. racemosa* cultivation is not sufficient, then the N and P in these waters should be added. Addition of N and P can use Na_2NO_3 as a source of N and KH_2PO_4 as a source of P in the addition of nutrients during cultured. The purpose of this study was to determine the effect and administration of the best N:P ratio on maintenance media on the growth and fiber content of *C. racemosa* in tarpaulin ponds.

Material and Method

Seaweed material. *C. racemosa* used for this study came from Jepara waters, Central Java, which was purchased from fishers. *C. racemosa* used for research was first adapted in a tarpaulin pond for two weeks. Total *C. racemosa* used was 4,000 g. Then, the adapted *C. racemosa* was divided into 16 maintenance containers with a weight of *C. racemosa,* each of 250 g.

Research methods and treatments. The method used in this study is an experimental method with a completely randomized design (CRD) using four treatments and four repetitions. The research treatment used is a modification of the research of Guo et al (2014) namely:

A = NO_3-N concentration of 0.5 mmol L^{-1} and PO_4-P concentration of 0.1 mmol L^{-1} (N:P ratio = 5:1);
B = NO_3-N concentration of 0.6 mmol L^{-1} and PO_4-P concentration of 0.1 mmol L^{-1} (N:P ratio = 6:1);
C = NO_3-N concentration of 0.7 mmol L^{-1} and PO_4-P concentration of 0.1 mmol L^{-1} (N:P ratio = 7:1);
D = NO_3-N concentration 0.8 mmol L^{-1} and PO_4-P concentration 0.1 mmol L^{-1} (N:P ratio = 8:1).

Research procedure. The study began with the preparation of containers in the form of a tarpaulin pool (diameter:height = 2:1 m), totaling 16 units. The container was filled with seawater with 34 ppt salinity as high as 0.8 m (water volume 2,512 L). Seawater was sourced from BBPBAP, Jepara, Central Java. Each container was equipped with woven bamboo with a size of 0.5 x 0.5 m, which functions as a *C. racemosa* attachment media. Besides, the container was also equipped with a hose and aeration stone, which works as a mixer, so that the nutrients provided in the pond could be homogeneous. The layout of the placement of the container used was obtained by random placement. The arrangement of the containers can be seen in Figure 1.

C2	D2	A1	B1	C1	D1	A4	B4
B2	A2	D3	C3	B3	A3	C4	D4

Figure 1. The layout of the containers.

The next step was weighing $NaNO_3$ and KH_2PO_4, which would be used for treatments in the study. The next step was weighing $NaNO_3$ and KH_2PO_4 according to each treatment in this study. For treatments A (5:1), B (6:1), C (7:1), and D (8:1), the $NaNO_3$ used was 0.0425; 0.0510; 0.0595; and 0.068 g L^{-1}, respectively. While KH_2PO_4 used in each treatment was the same, i.e. 0.0136 g L^{-1}.

The step taken after weighing $NaNO_3$ and KH_2PO_4 was weighing the initial weight of *C. racemosa*, that will be used in the study, using a digital scale. *C. racemosa* used in each treatment was 250 g. *C. racemosa* that has been weighed was then placed on an attaching medium in the form of woven bamboo measuring 0.5x0.5 m by clamping *C. racemosa* on bamboo woven using bamboo sticks.

Containers containing seawater and *C. racemosa* were then given $NaNO_3$ and KH_2PO_4 following the specified treatment. The administration of $NaNO_3$ and KH_2PO_4 was done by first diluting $NaNO_3$ and KH_2PO_4 in a measuring cup using aqua dest. The ratio of aqua dest used as a diluent was 1 mL of aqua dest equal to 1 L of seawater in the container. Diluted $NaNO_3$ and KH_2PO_4 were then put into containers. The culture of *C. racemosa* was done for 30 days during June-July 2019. During the culture period, water quality data was collected daily, at 08.00 and 16.00, which included water temperature, salinity, dissolved oxygen (DO), and pH. Meanwhile, growth data and the fiber content of *C. racemosa*, as well as water nitrate concentrations, were taken at the beginning and at the end of the study.

Water quality on each variable was measured using different instruments. The DO, temperature and pH were measured using a water quality checker (YSI environmental 550A), salinity using a refractometer (Atago S/Mill-E). All of them were measured in situ. On the other hand, the water nitrate concentration was measured using a spectrophotometer (Optima SP-3000 plus), which was conducted in the Laboratory of Fisheries Resource Management at Universitas Diponegoro. To find out the content of *C. racemosa* fiber, the test was carried out at the Institute for Agricultural Technology Assessment, Ungaran, Central Java. *C. racemosa* samples, which would be tested for fiber content, were first dried for three days.

Absolute growth calculation was done using the following formula (Togatorop et al 2017):

$$G = Wt - Wo$$

where: G = absolute growth test (g);
Wo = weight at the beginning of culture (g);
Wt = weight at the end of culture (g).

Specific growth rate (SGR) values can be calculated based on the following formula (Syahlun et al 2013):

$$SGR = \frac{Ln\ Wt - Ln\ Wo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
Wt = weight at the end of culture (g);
Wo = weight at the beginning of culture (g);
t = length of culture (days).

Calculation of nitrate absorption rate was done by the equation (Skriptsova & Miroshnikova 2011):

$$M = \frac{(Co - Ct) \times V}{W \times t}$$

where: M = nitrate absorption rate (mg g *C. racemosa*$^{-1}$ day^{-1});
Co = initial nitrate concentration (mg L^{-1});
Ct = final nitrate concentration (mg L^{-1});
V = container volume (L);
W = growth of seaweed weights (g);
t = duration of absorption (days).

Data analysis. Data obtained during the study were then analyzed using Analysis of Variance (ANOVA) and was continued using the Duncan test to find out the significant differences between treatments.

Results. The weight of *C. racemosa* at the beginning of each treatment (D-0) was 250 g. The highest growth weight of *C. racemosa* was at treatment B (6:1), where at the end of the treatment (D-30) the weight was 684 g, in other words, experiencing growth weight of 434.00±49.3 g. Meanwhile, the lowest was in treatment D (8:1), which was ±462.75 g or experiencing weight growth of 212.75±39.8 g. The complete results are presented in Figure 2.

Specific Growth Rate (SGR). As similar to growth, the highest SGR value of *C. racemosa* was in treatment B (6:1), which was 3.35±0.002% d^{-1}. While the lowest was in treatment D (8:1), namely 2.04±0.003% (Figure 3).

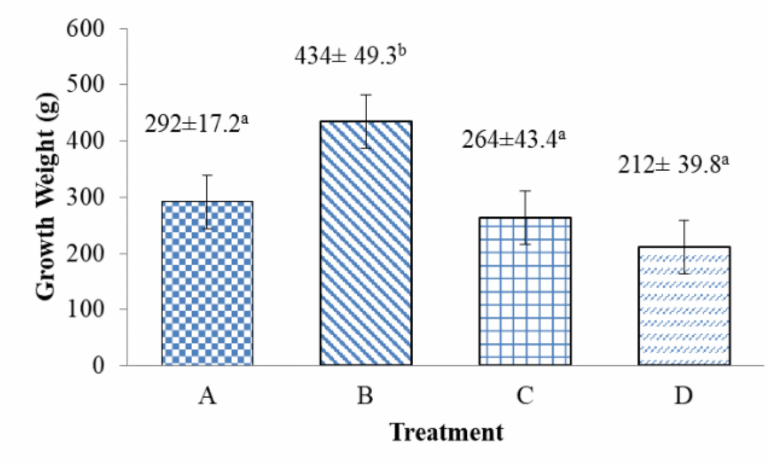

Figure 2. Growth weight of *C. racemosa* on the different N/P ratio.

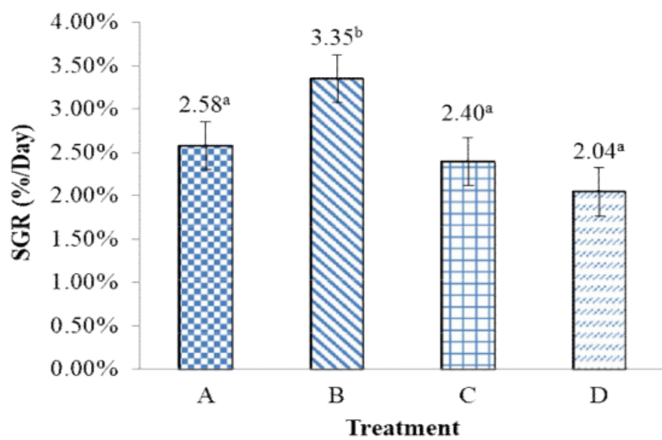

Figure 3. Specific growth rate of *C. racemosa* on the different N/P ratio.

Fiber content. The *C. racemosa* fiber content at the beginning of each treatment (D-0) was 3.02%. After a 30-day culture period (D-30), the highest and lowest fiber content were obtained in treatments C (7:1) and A (5:1), namely 4.05% and 2.96%, respectively. Data on *C. racemosa* fiber content are presented in Table 1.

Table 1

The fiber contents of *C. racemosa* on the different N/P ratios (30 days)

Day	*Fiber contents (%)*			
	A (5:1)	*B (6:1)*	*C (7:1)*	*D (8:1)*
0	3.02	3.02	3.02	3.02
30	2.96	3.63	4.05	3.88

Nitrate absorption. The highest absorption rate of nitrate by *C. racemosa* was in treatment B (6:1). The nitrate content in treatment B (6:1) at D-0 was 2.366 mg L^{-1}, and at D-30 was 1.728 mg L^{-1}. This showed that the absorption of nitrate in treatment B was 0.078 mg g *C. racemosa*$^{-1}$ d^{-1}. While the intake of nitrate in treatment A (5:1) was 0.040

mg g *C. racemosa*$^{-1}$ d^{-1}. On the other hand, in treatment C (7:1) and treatment D (8:1), it turned out that *C. racemosa* could not use the nitrate administration in the culture ponds. The results of the absorption rate of nitrate by *C. racemosa* are presented in Table 2.

Table 2

Nitrate absorption of *C. racemosa* on the different N/P ratios

No.	*Treatments*	*Nitrate (mg L^{-1})*		*Nitrate absorption rate (mg g C. racemosa^{-1} day^{-1})*
		D-0	*D-30*	
1.	A (5:1)	1.228	0.969	0.040
2.	B (6:1)	2.366	1.728	0.078
3.	C (7:1)	2.417	4.486	-
4.	D (8:1)	4.297	4.659	-

Water quality. Water quality values that include temperature, pH, and DO in all *C. racemosa* culture ponds were still considered suitable for cultivation activities. As for salinity, it was slightly above the good value for *C. racemosa* cultivation (Table 3).

Table 3

Water quality during the research

Treatments	*Temperature (°C)*	*Salinity (ppt)*	*pH*	*DO (mg.L^{-1})*
A (5:1)	26-28	34-42	8.2-8.4	3.15-3.91
B (6:1)	26-28	34-42	8.3-8.7	4.92-5.44
C (7:1)	26-28	34-42	8.2-8.5	3.01-3.17
D (8:1)	26-28	34-42	8.2-8.5	3.07-3.18
References	25-31**	30-40*	8.5-9*	3-8***

*Horstmann (1983); **Piazzi et al (2001); *** Wantasen & Tamrin (2012).

Discussion. The results of this study indicate that each treatment was experienced an increase in weight from the initial weight. The increase in weight was due to the administration of nutrients in the form of Na_2NO_3 as a source of N and KH_2PO_4 as source of P carried out in each treatment. Carpenter & Capone (1983) mentioned that N and P are two essential nutrients needed for seaweed growth. Although the administration of nutrients, both N and P, have a good effect on the growth of *C. racemosa*. Still, the administration of both nutrients must be considered in quantity, so that optimal growth can be maintained. According to Yin et al (2007), inadequate or excessive nutrition is not suitable for seaweed because it can inhibit growth.

Different ratios of nitrogen and phosphorus (N:P) can influence the growth of *C. racemosa* weights. This can be seen from the increasing weight in each treatment. The highest increase in *C. racemosa* weight during 30 days of cultured was in treatment B (6N:1P) with 434±49.35 g, while the lowest was in treatment D (8N:1P), which was 212.75±39.89 g. The results obtained are following the study of Deraxbudsarakom et al (2003), who performed on *C. lentilifera*. The best results obtained in treatment B were thought to be because NO_3-N concentrations of 0.6 mmol L^{-1} were the threshold for nutrient assimilation and absorption, due to the complex enzyme behavior for nitrogen nitrate conversion and reduction of ammonium nitrogen in algae (Hockin et al 2012).

The function of N in *C. racemosa* is to stimulate growth so that growth can be optimal. Besides, N is an element used by *C. racemosa* in the process of photosynthesis. N is a macro element that is useful for stimulating the growth of a plant so that it can develop rapidly (Kushartono et al 2009). The deficiency of N will inhibit the growth of seaweed because it is an element used in the process of photosynthesis.

The N element is absorbed by plants in the form of NO_3 or ammonium nitrate ions (NH_4) (Follet et al 1987; Follet 1989; Follet & Walker 1989; Follet et al 1991). N is very instrumental in the formation of photosynthetic devices, namely chlorophyll and RuBP carboxylase enzymes, which function in the fixation of CO_2, to be further reduced to sugar. A high photosynthesis rate enables the accumulation of dry plant biomass (Lakitan 2007). In plant nutrition, N is an essential element and is closely related to root development and maintaining respiration (Raun & Johnson 1999). N deficiency can cause a reduction in the number and length of roots so that the weight will be reduced. In addition to influencing root development, the element N that is absorbed also affects the chlorophyll content and green color of the leaves. According to Faozi & Wijonarko (2010), N fertilization increases the chlorophyll content and leaf greenness.

The element phosphorus (P) in *C. racemosa* functions as a source of energy needed for metabolic processes. P is required by *C. racemosa* to convert the N into an energy source used in the photosynthesis process. According to Kuhl (1974), P is an essential element for all aspects of life, especially in the transformation of metabolic energy. Besides being an element needed to convert the N into an energy source, P in *C. racemosa* can be a limiting factor for the process of photosynthesis. This is the reason why they need for P in *C. racemosa* is smaller when compared to the need for the N element. Phosphate is a form of P that is beneficial to plants (Waite 1984).

The highest specific growth rate during culture also obtained from treatment B (6N:1P) with a value of 3.35% d^{-1}, while the lowest value was from treatment D, i.e., 2.58% d^{-1}. The SGR results in this study were 0.02% lower when compared with studies conducted by Guo et al (2014), where the growth rate for *C. lenitilifera* during their research was 3.37% d^{-1}. These different results are thought to be due to environmental conditions, such as temperature, salinity, nutrient content, and light intensity, in the two studies also differed (Scrosati 2001).

The results showed that the highest value of fiber content was in treatment C (7N:1P) with a value of 4.05%. This value is higher when compared to studies on other *Caulerpa* species, namely *C. lentilifera*, which was cultured in ponds in National Penghu University of Science and Technology (NPUST), Taiwan (Nguyen et al 2011), and *C. lentilifera* cultured in ponds in Amphor Banlam, Petchburi province (Ratana-arporn & Chirapart 2006). Based on Nguyen et al (2011), the fiber content of *C. lentilifera* in their study was 2.97%. Meanwhile, Ratana-arporn & Chirapart (2006) reported that the fiber content in *C. lentilifera* from their research was 3.17%.

However, the fiber value of the treatment C (7N:1P) in this study is still far lower when compared to the *C. racemosa* fiber content taken directly from nature. As example, the fiber content found in *C. racemosa* as a result of sampling in the St. Isles. Martin, Bangladesh (Bhuiyan et al 2016). The fiber content of *C. racemosa* in their study was 11.51%. This difference might be because the environmental conditions in each study were also different. Furthermore, Bhuiyan et al (2016) stated that several factors affect the nutrient content of seaweed, namely, water temperature, salinity, lighting, and nutrients absorbed by itself.

The difference in fiber content in each treatment indicated that differences in the administration of nutrients, in the form of N and P, could affect the fiber content in *C. racemosa*. This can be seen from the fiber content of treatments A (5:1), B (6:1), C (7:1), and D (8:1), which varies, which is 2.96; 3.63; 4.05 and 3.88%, respectively. These results indicate that treatment C with the administration of 0.7 mmol L^{-1} Na_2NO_3 and 0.1 mmol L^{-1} KH_2PO_4 was the optimal administration limit, which is suitable for increasing the *C. racemosa* fiber content. According to Bhuiyan et al (2016), environmental conditions such as salinity temperature and suitable nutrition will produce *C. racemosa,* which has a higher nutrient content when compared to *C. racemosa* originating from an environment of which conditions are not suitable.

Nitrate absorption in each treatment showed a difference in value. The rate of nitrate absorption is directly proportional to the growth rate of *C. racemosa*'s weight. If there is an increase in the intake of nitrates, the growth rate of *C. racemosa*'s weight will also increase. This is shown in the treatment of B (6:P), where the nitrate absorption rate and growth rate of *C. racemosa* are the highest. According to Gordillo et al (2002), the rate of absorption of nitrate and phosphate is corresponding and has a positive correlation with an increase in growth rate.

Nitrate absorption in treatment A (5:1) was 0.04 mg g^{-1} *C. racemosa* day^{-1}. Whereas in treatment C (7:1) and treatment D (8:1), the administration of nitrates in maintenance ponds cannot be utilized by *C. racemosa*. This is evidenced by the results of measurements of nitrate content in treatment C (7:1) and treatment D (8:1). At the beginning of maintenance (H-0), the nitrate content in treatment C (7:1) and treatment D (8:1) were 2.417 and 4.297 mg L^{-1}, respectively, and at the end of maintenance (H-30), it was 4.486 and 4.659, respectively. This phenomenon shows that the administration of NO_3 0.6 mmol.L^{-1} is the threshold of *C. racemosa* to assimilate and absorb nutrients (Hockin et al 2012).

Water quality is one of the parameters that can affect the growth of *C. racemosa*. Therefore, regular water quality monitoring is needed. Measuring water quality in this study was done twice a day in the morning and afternoon. During the study, DO values ranged from 3.01 to 5.44 mg.L^{-1}. This result indicates that DO levels in the container had met the eligibility for culturing *C. racemosa*. According to Wantasen & Tamrin (2012), DO levels that suitable for *C. racemosa* culture are 3-8 mg L^{-1}. The pH value obtained during the study ranged from 8.2 to 8.8. The results showed that the dissolved pH levels in the container also met the feasibility of culturing *C. racemosa*. The proper pH value for *C. racemosa* culture is 8.5-9.0 (Horstmann 1983). Water temperature ranged from 26 to 28°C, indicating that the water temperature in the container was proper for the culture of *C. racemosa*. Piazzi et al (2001) reported that *C. racemosa* could live in a temperature range between 25 and 31°C. During the study, salinity obtained was between 30-42 ppt. These values were slightly higher than the tolerance limit for culturing *C. racemosa* optimally. According to Horstmann (1983), the salinity required for *C. racemosa* to be able to do photosynthesis optimally is 30-40 ppt. However, even though salinity at the time of the study was higher than the optimum value, the *C. racemosa* studied was still able to survive and also grow. This is because *C. racemosa* can survive at salinity up to 50 ppt (Guo et al 2014).

Conclusions. Based on research that has been done, it can be concluded that the difference in the N:P ratio can affect the growth and fiber content of *C. racemosa*. The optimal N:P ratio for *C. racemosa* growth was 6:1 (3.35±0.002% d^{-1}), while for the N:P, the optimal ratio for increasing the fiber content of *C. racemosa*) was 7:1 (4.05%).

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