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The Application of Different Fertilizers and Depths in The Rearing of Seaweed Caulerpa racemosa Using a Concrete Tank

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Abstract: This study used a concrete tank to analyze the interaction between different fertilizers and depths in *C. racemose* seaweed rearing. This study was experimental, using a factorial Completely Randomized Design (CRD) consisting of 2 factors, namely fertilizer (P) and depth (K). The fertilizers consisted of NPK (P1) and sap (P2), and the depths (K) consisted of 5 cm (K1), 10 cm (K2), and 15 cm (K3). Each treatment was repeated 3 times, so we had 18 combined treatments. The study was done at the Cultivation Laboratory of the National Innovation Research Agency (BRIN), Lombok, Indonesia. Research procedures included preparing material and tools, rearing *C. racemosa*, and harvesting *C. racemosa*. Research parameters included final weight, final length, specific decline rate, and survival rate. Data were analyzed using ANOVA. The results showed that the interaction of different fertilizers and depths affected the final weight, specific decline rate, and survival rate of *C. racemosa*. Meanwhile, the final length of *C. racemosa* was only influenced by different fertilizers. The best treatment happened in the interaction of NPK fertilizer and a depth of 15 cm, which gave a survival rate of 89%, a final weight of 88.66 g, and a specific decline rate of -10.67%/day

Keywords: C. racemosa; fertilizer; growth; NPK; palm sap

Introduction

Caulerpa racemosa, also known as sea grapes, is a species of green seaweed (*Chlorophyta*) commonly found in tidal areas or clam areas free from tidal pools. This green algae is capable of quickly adapting to its environment and is known to have a high survival rate. The availability of *C. racemosa* sea grapes still depends on natural products due to no cultivation techniques capable of continuously producing this sea grape. Several essential factors in cultivating *C. racemosa* are seed quality, environmental factors, pests, methods used, and nutrient availability (Pradhika & Sedjati, 2019).

The use of various fertilizers is one of the methods to guarantee the availability of nutrients in seaweed cultivation. There are two types of fertilizers namely organic fertilizers and inorganic/chemical fertilizers. Factory-made chemical fertilizers are often used as the main choice because they are considered easy, practical, and have a fast effect (Hapsari et al., 2023).

NPK is one kind of fertilizer suitable for *C. racemosa* cultivation. NPK fertilizer can increase the growth of young shoots and plant resistance to diseases. NPK compound fertilizer contains macronutrients, namely nitrogen, phosphorus, and potassium (Yoskader et al., 2023). The N (Nitrogen) element is an energy supplier in photosynthesis. The P (phosphorus) element is vital in cell division; insufficient P will hamper seaweed growth. Meanwhile, the K (potassium) element functions as a stabilizer for the respiration process, enhances thallus growth, and plays a role in the photosynthesis process of seaweed (Kushartono et al., 2009).

Apart from chemical fertilizers, natural (organic) fertilizers can also be used in laboratory-scale seaweed cultivation. One of the organic fertilizers used is sap or palm juice from palm trees. Organic fertilizer generally comes from animals or plants with high nitrogen levels

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(N); this fertilizer can be solid or liquid. Liquid organic fertilizer has a better content than solid organic fertilizer; liquid organic fertilizer is more efficient and effective in stimulating plant growth (Sundari et al., 2014). Palm juice has a sucrose content of 13.9-14.9%, ash content of 0.04%, and fat content of 0.02% (Ismail et al., 2020). Palm sap contains nutrients in the form of N, P, and K; these elements influence plant growth. Therefore, applying palm sap as an organic fertilizer to seaweed will help to increase available nutrients in the form of phosphorus and nitrogen (Lukman, 2018). The main problem in cultivating sea grapes is that data on appropriate cultivation techniques, and optimal fertilizer use are unavailable. Thus, it is necessary to research to analyze the use of different types of fertilizer and depths in *C. racemosa* seaweed cultivation using concrete tanks.

Method

This study took place from October to December 2022 at the Cultivation Laboratory of the National Innovation Research Agency (*Badan Riset Inovasi Nasional* - BRIN) of the Science Complex of Lombok (Kurnaen Sumadiharga) in Pemenang District, Lombok Utara Regency, West Nusa Tenggara, Indonesia. The flow of this research is as Figure 1.

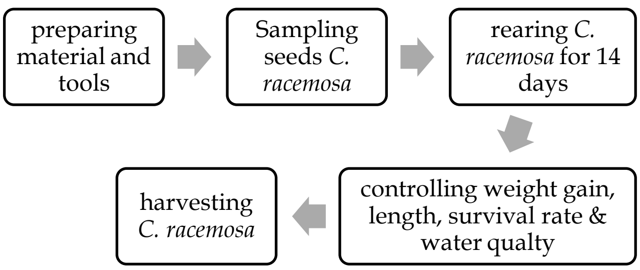

Figure 1. Flow of this research

Research Materials

The research materials included seawater, palm sap, *C. racemosa*, NPK fertilizer, and Vitamin B1, and samples of water, sediment, and shell. Other materials were chemical reagents for analyzing heavy metals, dissolved oxygen, organic matter, texture, distilled water, plastic bags, labels, and filter paper. Equipment used in this research included stationery, concrete tanks, scissors, a camera, NO_2 Profi Test, NO_3 Profi Test, a ruler, a pH meter, scales, and a water quality checker.

Research Design

This study was experimental, using a factorial Completely Randomized Design (CRD) consisting of 2 factors, namely fertilizer (P) and depth (K). The fertilizers consisted of 2 levels of NPK (P1) and sap (P2), and the depths (K) consisted of 3 levels of 5 cm (K1), 10 cm (K2), and 15 cm (K3). Each treatment was repeated 3 times, so we had 2x3, or 6, combined treatments.

Preparation of the Medium

The cultivation container was a concrete tank measuring 70 x 300 x 80 cm with a volume of 5000 L. Meanwhile, the seaweed cultivation media was a basket or a net bag. The concrete tank was cleaned using a brush before use. The net bags were cleaned, and the top was closed using a *waring*[1]. The bottom of the net bags was tied using a rope so that the bags did not easily float to the surface. The bottom rope was tied according to the treatment, namely K1 = 5 cm depth, K2 = 10 cm depth, and K3 = 15 cm depth. Meanwhile, the bag was made of a cube-shaped *tento,* and its outside was lined with *waring* measuring 25 cm, 30 cm, and 35 cm.

Preparation of the Fertilizers

The fertilizers used were NPK and palm sap. The NPK fertilizer, brand Green Tonik, was obtained from a specialist plant fertilizer shop. Meanwhile, the palm sap was obtained from the area around the research location. The fertilizer dose was 3 ml for the NPK fertilizer and palm sap. Before using the two fertilizers, 1 ml of vitamin B1 was mixed as a vitamin enhancer for seaweed growth. Next, each fertilizer was mixed in sterile sea water with a 3 ml:1 ml:2000 ml ratio for fertilizer, vitamins, and seawater.

Preparation and Rearing of Seaweed Seedlings

We obtained the seedlings from Ujang Village of Lombok Timur Regency. The seedlings were fresh, clean, and free from other types of seaweed. The seedlings were taken directly from their original habitat. The seaweed was transported from its habitat to the study site using a dry system by placing seaweed in sacks to avoid evaporation. Seedlings were acclimatized by selecting ones in a dark green color, not soft when handled, and the thallus did not break off easily. Acclimatization was carried out for 30 minutes before spreading into the concrete tank. The seedlings were weighed 100 g for each treatment in bag and basket media. Rearing was carried out for 14 days by controlling growth, such as weight gain, length, and survival rate. Furthermore, seaweed control was carried out to prevent pests. Apart from that, healthy seaweed was separated from damaged seaweed. The water was also changed once a week carefully to avoid wasted seaweed.

Research Parameter

Final Length and Weight

The final length and weight were measured at the end of rearing on day 14.

Specific Decline Rate

The specific decline rate is calculated using the following formula (Gultom et al., 2019):

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

In which:

SGR = Specific Growth Rate (%/day)
Wo = Initial weight (g)
Wt = Final weight (g)
t = Rearing time (days)

Survival Rate

Sea grapes *Caulerpa racemosa* is a species of green seaweed (*Chlorophyta*) commonly found in tidal areas or clam areas free from tidal pools. This green algae can quickly adapt to its environment and has a high survival rate (Pradhika et al., 2019). The survival rate of *C. racemosa* is obtained by calculating its final weight (Fernando et al., 2021).

$$\mathrm{SR} = \frac{\mathrm{Nt}}{\mathrm{N0}}\mathrm{x}100\% \quad (2)$$

In which:

SR = Survival rate (%)
Nt = Final weight at the end of rearing (g)
No = Final weight at the beginning of rearing (g)

C/N Ratio

C and N are the primary macronutrients bacteria need for cell metabolism, producing compounds essential for bacterial growth. C element has a significant role in forming cells of bacteria. Meanwhile, N plays a role as a constituent of nucleic acids, amino acids, and various enzymes (Oktaviana et al., 2014).

Water Quality Parameters

Water quality was measured every day in the morning so that the results of the measurements remained stable. Water quality measurements included temperature, pH, DO, salinity, nitrate, and nitrite. Temperature, pH, salinity, and DO were measured using a Water Quality Checker. Meanwhile, nitrate was measured using the NO_3 profi test and nitrite using the NO_2 profi test.

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) employing SPSS to evaluate the effect of each treatment. Since the analysis resulted in a significant difference, a histogram was created using Microsoft Excel. Water quality was analyzed descriptively.

Result and Discussion

Results

Final Weight and Final Length

The average length of *C. racemosa* at the end of rearing ranged from 3.4 cm - 12 cm. ANOVA results showed that the single treatment of different fertilizers significantly affected the final length of *C. racemosa*. In contrast, the single treatment of depth and the interaction treatment of different fertilizer types and depths did not significantly affect the final length. The NPK fertilizer (P1) treatment gave an average final length of 9.56 cm, and the palm sap fertilizer (P2) gave an average final length of 4.66 cm (Figure 2.).

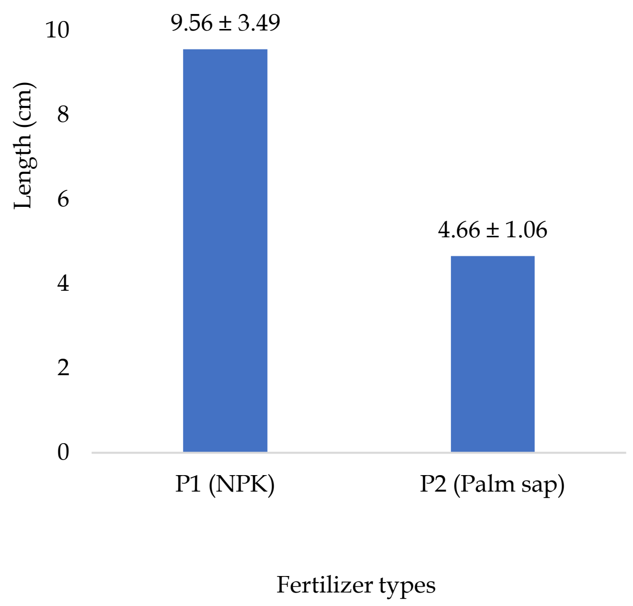

Figure 2. Final Length of *C. racemosa* on Treatments using Different Fertilizers

Meanwhile, the average weight of *C. racemosa* at the end of rearing ranged from 25.3 g – 88.7 g. The histogram shows that at the end of rearing, there was an increase in the weight of *C. racemosa* in line with the increasing depth of NPK fertilizer application. On the contrary, there was a decrease in the weight of *C. racemosa* in line with the increasing depth of palm sap application (Figure 3).

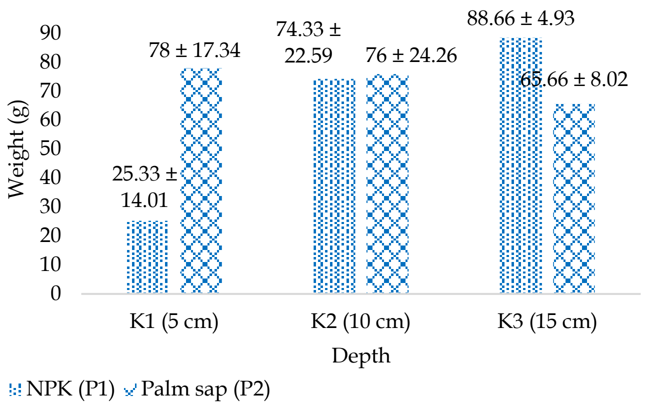

Figure 3. Final Weight of *C. racemosa* on Interaction Effects of Different Fertilizer Types and Depths

ANOVA results showed that the interaction of different depths and fertilizer types significantly influenced the final weight of *C. racemosa*. In contrast, the single treatment of depth and the single treatment of different types of fertilizer did not significantly influence the final weight. The interaction of NPK fertilizer treatment with a depth of 15 cm (P1K3) gave the highest final weight of *C. racemosa* at 88.66 g. In comparison, the interaction of NPK fertilizer treatment with a depth of 5 cm (P1K1) gave the lowest final weight of *C. racemosa* at 25.33 g.

Specific Decline Rate

Findings showed that the average specific reduction rate of *C. racemosa* at the end of rearing ranged from -10.6 – (-0.8)%/day. The histogram shows that at the end of rearing, the specific decline rate decreased with the increasing depth of NPK fertilizer application. In contrast, the decline rate increased with the increasing depth of palm sap application (Figure 4).

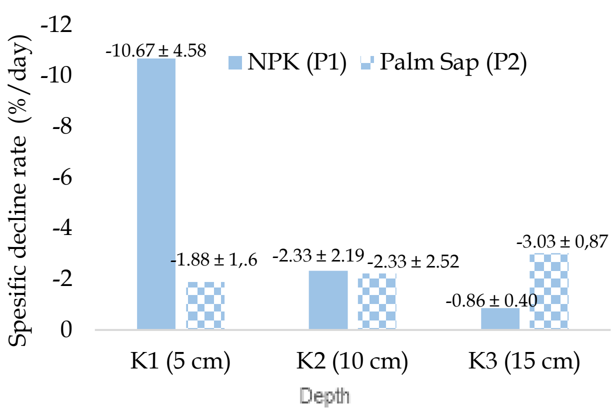

Figure 4. Specific Decline Rate of *C. racemosa* on Interaction Effects of Different Fertilizer Types and Depths

ANOVA showed that the interaction effects of different fertilizers and depths significantly affected the specific decline rate of *C. racemosa*. In contrast, the single-depth treatment and the different fertilizer treatments did not significantly affect the specific decline rate. The interaction of NPK fertilizer treatment with a depth of 5 cm (P1K1) gave the highest specific decline rate at -10.67%/day. In comparison, the interaction of NPK fertilizer treatment with a depth of 15 cm (P1K3) gave the lowest specific decline rate at -0.86%/day.

Survival Rate

Findings showed that the average survival rate of *C. racemosa* at the end of rearing ranged from 25% - 89%. The histogram shows that the survival rate of *C. racemosa* increases with the increasing depth of NPK fertilizer application; conversely, there is a decrease in the weight of *C. racemosa* along with the increasing depth of palm sap application (Figure 5).

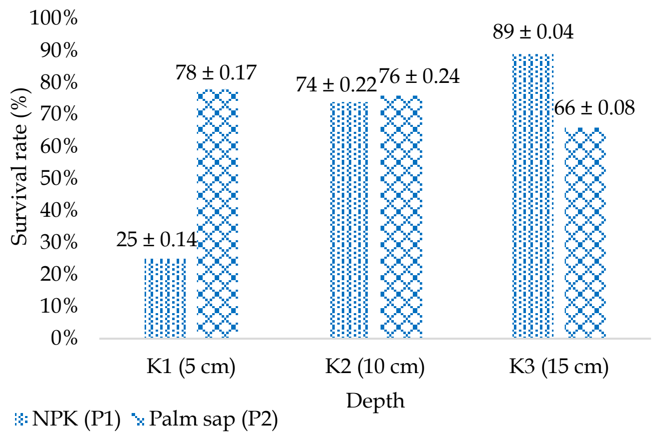

Figure 5. Survival Rate of *C. racemosa* on Interaction Effects of Different Fertilizer Types and Depths

ANOVA showed that the interaction effects of different depths and fertilizers significantly affected the survival rate of *C. racemosa*. In contrast, the single-depth treatment and single-fertilizer treatment did not significantly affect the survival rate (Appendix 5). The interaction of NPK fertilizer treatment with a depth of 15 cm (P1K3) gave the highest survival rate at 89%. In comparison, the interaction of NPK fertilizer treatment with a depth of 5 cm (P1K1) gave the lowest survival rate at 25%.

Water Quality Parameter

Water quality measurements showed that water qualities such as temperature, salinity, pH, DO, and nitrite were optimal for seaweed cultivation. At the same time, nitrate levels exceeded the optimal seaweed cultivation condition (Table 1). The C/N Ratio results are presented in Table 2.

Table 1. Water Quality Parameters

Water Quality Parameter	Fertilizer Types		SNI
	NPK	Palm Sap	
Temperature (°C)	27.5-28.7	27.5-28.62	25-31 (Iskandar et al., 2015)
Salinity (ppt)	25.4-29.99	25.47-27.24	25-30 (Ardiansyah et al., 2020)
pH	8.6-8.15	8.33-8.15	6.6-9 (Ardiansyah et al., 2020)
DO (mg/l)	4.16-10.5	4.32-7.86	>3.5 (Irfan et al., 2021)
Nitrate (mg/l)	5-50	5-100	<0.006 (Mudeng & Ngangi, 2014)
Nitrite (mg/l)	0	0	0-50 (Mudeng & Ngangi, 2014)

Table 2. C/N Ratio Measurements

Parameter	Unit	Measurement Results	Method
N-Total	%	0.01	Kjeldahl
C-Organic	%	0.11	Walkey & Black
C/N Ratio		11.00	
P Total	ppm	0.25	Morgan Wolf
K Total	m.e/liter	10.13	Morgan Wolf

Discussion

Findings showed that the final length of *C. racemosa* was only influenced by the single treatment using different fertilizers, showing that NPK fertilizer was better at maintaining the final length of *C. racemosa* than palm sap (Figure 1). The better final length of *C. racemosa* in the NPK fertilizer treatment might have been because the nutrient content in NPK fertilizer could still be absorbed well through the diffusion process in the thallus walls of *C. racemosa* compared to palm sap water. Lush et al. (2021), state that NPK fertilizer contains N, P, and K that help the growth of seaweed. The nutrients N, P and K are macro nutrients for plants (Raksun et al., 2019). According to Harrison and Hurd (2002), the growth and development of seaweed require sufficient lights and nutrients such as nitrate and phosphate. Nitrogen and Phosphorus is a mineral nutrient that limits plant growth due to its large availability in media (Harpole et al., 2011).

Other elements cannot replace the benefits of nitrate and phosphate for seaweed growth. This is due to the role of nitrate as a constituent of protein and phosphate as an energy provider. NPK content in fertilizer complies with SNI (Indonesian National Standards), namely N content of 0.04%, P content ± 0.30%, and K content > 0.10%.

Palm sap is a natural ingredient containing hormones that can act as a Plant Growth Regulator (PGR). Wibawa et al. (2013), state that natural ingredients contain Plant Growth Regulators (PGRs), a type of nutrient that may inhibit growth if it is too little, but at a certain low amount will encourage growth; on the other hand, if the amount is too high, it will inhibit growth and can poison or even kill the plant itself. However, this study showed that giving palm sap resulted in a lower final length of *C. racemosa* than giving NPK. This could be due to the lower amount of N, P, and K in palm sap than NPK fertilizer. (Lukman, 2018) states that palm sap contains N, P, and K, which can affect plant growth. Palm sap only has an N-total of 0.13%, a P-total of 0.002%, and a K-total of 0.002%. (Jaelani et al., 2021), show that NPK fertilizer brings good results compared to using other types of fertilizer on the growth of young thallus in seaweed. (Yudasmara, 2014) adds that regular application of NPK fertilizer will provide optimal growth results.

In addition, the final weight of *C. racemosa* was influenced by the interaction of different fertilizers and depths (Figure 2), where the final weight of *C. racemosa* was better as the depth of NPK fertilizer application increased. Vice versa, the final weight of *C. racemosa* decreased in line with the decreasing depth at which sap was applied. This streng thens the assumption that applying NPK fertilizer results in a better final weight than palm sap. Several previous research results show that adding N and P elements greatly influences the growth of seaweed. (Cyntya et al., 2018) report that adding N and P elements with a ratio of 6:3 can increase the absolute growth of *Gracilaria sp.* seaweed of 8.28 g and a specific growth rate of 0.28%/day for 28 days. Zainuddin and Nofianti (2022), add that nitrogen and phosphate greatly influence the survival of seaweed.

The N content functions in the photosynthesis process, and P functions in the formation of meristem tissue. Apart from that, N influences the amount of protein in algae, and P influences the total protein in seaweed. Nitrate and phosphate are needed as basic constituents of protein and chlorophyll formation in photosynthesis (Lumbessy, 2019). The protein has potential to activate the enzymes in the plant body; it will change the substrate into new products as the result of duplication or multiplication of cells (Lea and Azevedo, 2006). Therefore, lack of N and P will cause a lack of photosynthetic pigments and cellular proteins because algae absorb and utilize these pigments for proteins and photosynthesis. Thus, N and P elements are very influential in cultivating *C. racemosa*, so controlling their availability in the rearing media is necessary.

The final weight of *C. racemosa* was better when using NPK fertilizer and increasing the depth of cultivation; NPK fertilizer treatment could maintain a better final weight when applied at a depth of 15 cm. This 15 cm depth is considered the optimum distance for *C. racemosa* to conduct photosynthesis. (Serdiati & Widiastuti, 2010)mention that different depths in seaweed cultivation affect the photosynthesis process of the seaweed—if the seaweed has sufficient distance from the water surface, the seaweed will utilize sunlight for the photosynthesis process and nutrients. In other words, different depths will lead to different production due to the ability of seaweed to obtain sunlight for photosynthesis.

The better final weight of *C. racemosa* in the interaction effect of NPK fertilizer and 15 cm depth was also supported by the better survival rate and specific decline rate of that interaction effect (Figures 3 and 4). (Yudiastuti et al., 2018)state that one factor influencing the survival rate of seaweed is the intensity of light for the growth of the seaweed thallus. Overall, applying NPK fertilizer with a planting depth of 15 cm could provide better growth and survival in *C. racemosa,* even though there was a specific growth reduction process at the end of the rearing period. This may happen because this research is still at the domestication stage to rear *C. racemosa* from its natural habitat to a controlled environment or cultivation container. Another factor that is thought to cause a decrease in growth is the high nitrate content in the rearing water, around 5-100 mg/l. Meanwhile, according to (Cahyanurani & Rifkiyatul Ummah, 2020) the appropriate nitrate level for seaweed cultivation is 0.9-3.5 mg/l.

This high nitrate content is thought to be due to the inability of *C. racemosa* to tolerate the dose and application time of fertilizers. Atmanisa (2020), explain that nitrate is a limiting factor for seaweed. Nitrate requirements below 0.1 mg/l or above 45 mg/l will be toxic for seaweed growth, resulting in algae blooms. This aligns with Astuti et al. (2021), stating that a good fertilizer dose for cultivating *C. racemosa* is 40 mg/l. (Umasugi & Polanunu, 2019) also show that cultivating *Eucheuma cottonii* using liquid fertilizer above 350 cc causes a decrease in weight because the seaweed absorbs too many nutrients, which can lead to a saturation effect, which will result in the death of some cells and finally reduce the final weight of the seaweed. Sutejo (2002) also emphasizes that plants need 16 essential nutrients (macro and micro) during growth. If one of the nutrients is unavailable or excessive, plant growth, development, and productivity will be hampered. Meanwhile, high nitrate levels will impact humans and cause diseases, such as hematological and neurological diseases (Ardhaneswari & Wispriyono, 2022).

Meanwhile, the water quality parameters during the research, including temperature, salinity, pH, and dissolved oxygen (DO), were still optimal for the growth of *C. racemosa*. Temperature influences photosynthesis and indirectly affects the solubility of oxygen used for respiration by marine organisms; even though temperature is not deadly, it can inhibit seaweed growth. Temperature levels during the NPK application could be said to be quite fluctuating. The same thing happened with the palm sap application, but temperature fluctuations were less volatile than in the NPK application. However, these fluctuations were still within the normal range, 27°C-28°C. Iskandar et al. (2015), state that the normal temperature for *Caulerpa sp.* is between 25°C-31°C. Temperature can be influenced by meteorological conditions, one of which is the intensity of solar radiation.

Salinity can influence the osmoregulation process in seaweed. The salinity level during NPK application decreased from Day 1 to Day 3, but after that, the salinity fluctuated quite normally and was considered stable. Meanwhile, when using palm sap, there was a decrease in salinity on Day 3, but there was an increase in salinity from Day 3 until the end of the rearing period. However, the salinity levels during rearing were normal, around 26-28 ppt. Ardiansyah et al. (2020), state that salinity has a vital role in seaweed cultivation because if more fresh water enters the seaweed cultivation tank, the quality of the seaweed will get worse and can cause seaweed death. The salinity level suitable for the growth of *Caulerpa sp*. is in the range of 20-50 ppt.

Acidity (pH) is an environmental parameter that greatly influences water organisms. The pH level during NPK application increased on Day 3, but then it returned to a relatively stable range. In contrast to NPK fertilizer, the pH levels during the application of palm sap were not so different with each observation. The pH results were declared normal because they were still in the range of 8.12-8.4, as (Khatimah et al., 2016) mention that the optimal pH value for cultivating *Caulerpa sp.* ranges from 5-8. However, in their study, Ardiansyah et al. (2020), found that the optimal pH level for cultivating *Caulerpa sp.* was 6.6-9.

Dissolved oxygen (DO) levels are needed by seaweed to support growth. The DO level during NPK application was highest on Day 8 and significantly increased from Day 1. When using sap water, oxygen levels fluctuated but were not as high as when using NPK fertilizer. DO results could be declared optimal because they were still in the range of 4 mg/l-10 mg/l. Irfan et al. (2021), state that dissolved oxygen is an essential limiting factor in seaweed growth. The optimal DO for seaweed cultivation is around 3.3 mg/l-5.3 mg/l. However, seaweed can grow if DO exceeds 3.5 mg/l.

The C/N ratio in the rearing media was still within the normal range for seaweed growth. Barus *et al.* (2019) confirmed a 10-17% C-organic content in their study; this is considered high because the criteria for organic content sediment are >35% very high, 17-35% medium, 7-17% low, and <3.5% very low. Likewise, a value of 0.61-1.41% was obtained for N-total. This value is classified as high because the criteria for N-total levels are 0.1-0.3% as low, 0.3-0.6% as medium, 0.6-1.0 as high, and > 1.0 as very high. Oktaviana et al. (2014), state that the C/N ratio level functions to increase bacteria; a low C/N ratio level will inhibit the growth of the bacteria. C and N are the main macronutrients that bacteria need to carry out cell metabolism, producing compounds essential for bacterial growth. C has a major role in forming bacterial cells. Meanwhile, N plays a role as a constituent of nucleic acids, amino acids, and various enzymes. When N was scarce, microbial development slowed down, which led to a gradual breakdown of the available C (Yanqoritha, 2023).

Nitrite levels in all treatments showed the same results at 0 mg/l. This result was optimal for seaweed rearing. This means no waste or pollution entering the cultivation tank. Mudeng & Ngangi, (2014)mention that nitrite levels in waters are 0.001 mg/l, at least not more than 0.06 mg/l; nitrite levels exceeding 0.05 mg/l will be toxic to aquatic organisms.

Conclusion

The interaction of different fertilizers and depths affected the final weight, specific decline rate, and survival rate of *C. racemosa* seaweed. Meanwhile, the final length of *C. racemosa* was only influenced by different fertilizers. The best treatment happened in the interaction of NPK fertilizer and a depth of 15 cm, which gave a survival rate of 89%, a final weight of 88.66 g, and a specific decline rate of -10.67%/day

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Author Contributions
Conceptualization, M. A. A. and S.Y.L. ; methodology, S.Y.L. and M.A.A. ; software, M. A. D..and R.K.; validation, S.Y.L, M.A.A., M.A.D. and R.K.; formal analysis, S.Y.L.; investigation, M.A.A. ; resources, M.A.D.; data curation, M.A.A and R.K.; writing—original draft preparation, S.Y.L. and M.A.D; writing—review and editing, S.Y.L, M.A.A. and A.M.; visualization, R.K..; supervision, R.K..; project administration, M.A.D. .; funding acquisition, M.A.A.

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Conflicts of Interest
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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