Effect of inorganic and organic fertilizers on growth, survival rate and chlorophyll-a content of green seaweed *Caulerpa racemosa*

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Abstract. This research aimed to determine the effect of inorganic and organic fertilizers on the growth, survival rate, and chlorophyll-a content of *Caulerpa racemosa*. *C. racemosa* with an initial weight of 9.71±0.4 g was cultured for 28 days in three different culture media which were seawater without fertilizer (control), medium with inorganic hydroponic nutrient fertilizer (HN), and liquid organic fertilizer (LO). The cultured medium had significant differences in growth rate among treatments. The highest specific growth rate (SGR) of *C. racemosa* occurred when cultured in the HN medium with a rate of 2.70±0.02% per day, followed by the LO medium (2.07±0.03% per day) while the lowest SGR was in the control which was only 1.65±0.01% per day. In contrast, the three treatments media did not affect the survival rate of *C. racemosa*. The chlorophyll-a content showed a positive correlation with growth rate, where the highest chlorophyll-a content was observed on the HN medium with a rate of 222.0±4.5 µg per gww), followed by the LO medium (208.6±6.6 µg per gww) and control (121.2±10.2 µg per gww). The HN medium could be used for land-based culturing of *C. racemosa* at the dosage of 1.5% (ml/L).

1 Introduction

Seaweed cultivation is one of the microeconomic endeavors extensively developed by coastal communities as an alternative effort to achieve economic benefits. Due to its high nutritional value, Seaweed is a marine commodity widely utilized by local communities as a food source [1]. According to [2], seaweed contains fiber, vitamins, and minerals. It is also a source of natural antioxidants that are easy to obtain and available in quite abundant quantities. Sea grape (*Caulerpa* sp.) is a type of seaweed that has the potential to be cultivated. Indonesia is the largest producer of this type of seaweed. Seaweed cultivation in Indonesia mostly occurs in Sulawesi, Maluku, West Nusa Tenggara, East Nusa Tenggara, North Kalimantan and Java [3].

Indonesia with a coastline of more than 61,000 km and with approximately 17,000 islands is the second largest producer of seaweed after China. Due to rapid developments in

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aviation logistics and cultural blending in food, sea grapes have gained a large market in China and some Chinese state regions [4]. Indonesia contributes to producing almost 38% of seaweed, which is 11,300,000 tons, while China contributes 47%, which is 14,000,000 tons [3].

Caulerpa racemosa is a green seaweed that can be used as feed. Besides that, *C. racemosa* has many benefits, one of which is for health [5]. Caulerpa is a commodity with economic value and has local and international markets. Caulerpa is used by the community as a vegetable [6]. Japan, Korea, the Philippines and other Southeast Asian countries generally consume it directly when fresh, for salads, sushi and preserved with salt so it doesn't spoil easily [7].

Caulerpa has considerable potential to be cultivated in several regions in Indonesia. Caulerpa cultivation activities are necessary to increase productivity, ensure sustainable stock availability, control quality, and maintain the balance of natural stocks so that extinction does not occur. The cultivation technique carried out so far uses fertilizer because this fertilizer is a source of nutrients for *C. racemosa*, however further studies need to be carried out to be able to evaluate suitable form of fertilizer, according to needs and does not damage the living environment. Apart from that, the cultivation of *C. racemosa* is generally influenced by temperature, salinity, pH, light intensity and nutrients. One of the nutrients that can be used is nitrogen, which comes from fertilizer [8]. This research uses inorganic and organic fertilizers, the benefit of using organic fertilizer is that the use of this fertilizer does not cause residue in the harvest so it does not harm humans and the environment [9], while inorganic fertilizer is generally used for hydroponics. *C. racemosa* needs nutrient content in organic and inorganic fertilizer, and utilization of these fertilizer could be useful to increase *C. racemosa* growth, and farmers expect to benefit from cultivating this *C. racemosa*. This study aims to determine the effect of inorganic and organic fertilizers on the growth and survival of *C. racemosa.*

2 Methods

2.1 Time and location

The research was carried out in June - July 2020. The research location was at the Brackish Water Aquaculture Fisheries Center (BPBAP) in Takalar, South Sulawesi.

2.2 Research design

The seaweed used was the *C. racemosa* type with an initial weight of 9.71±0.4 g wet weight (gww). The test materials used were hydroponic nutrient fertilizer (HN) as an inorganic fertilizer and liquid organic fertilizer (LO) as an organic fertilizer. The culture media were sea water, and sea water added these inorganic and organic fertilizer. *C. racemosa* were cultured in the bottom of a fiber tank with 100 x 60 x 50 cm^3 size containing the culture media and the culture period was 28 days.

This study used a completely randomized design (CRD) method which consisted of 3 treatments, namely the control treatment (K) in the form of seawater culture media without fertilizer, inorganic culture the media treatment in the form of seawater added hydroponic nutrient fertilizer (HN) and organic culture media treatment in the form of seawater added liquid organic fertilizer (LO), and each treatment has 3 replications. Sea water as a source of this experiment was pumped from the sea and filtered by sand and active charcoal. Each treatment was carried out three replicates. The dosage of inorganic fertilizer (hydroponic nutrition) used refers to research by [10], which was 15 ml of nutrient AB mix/L of water. In

contrast, the dose of liquid organic fertilizer was 3.5 ml/L based on the best growth results in culturing of *C. lentillifera* [11].

This research began with culturing samples for the acclimatization process of *C. racemosa* in a new environment for two weeks. Then, selected seedlings were weighed to determine the initial weight of the seaweed. Bassal part of the seedlings were inserted in a sand substrate placed in plastic bucket and then the plastic bucket containing the sedlings were cultured in the rearing container containing culture media of HN and LO fertilizer with the doses as mentioned above, there was no fertilizer was added for control media. The plastic bucket volume containing sand substrate was 300 ml and the rearing container volume was 300 L (100 x 60 x 50 cm^3) filled by 150 L or 25 cm depth of cultured media. There were 5 plastic buckets in rearing container and each plastics bucket containing seeds of 9.71±0.4 g, and there were 3 rearing container each treatment. Culture media were aerated to provide water movement. Sampling of weight carried out every 7 days to determine the growth rate of *C. racemosa*, and the mortality of *C. racemosa* was recorded at the end of culture in order to count survival rate. Water exchange was carried out every 7 days just after doing weight sampling and the fertilizer was added just after water exchange with same dosage.

The data taken in this study were survival rate, weight specific growth rate (SGR), chlorophyll-a content of *C. racemosa* and water quality including temperature, light intensity, salinity, pH, nitrate and phosphate. Measurements of parameters of temperature, light intensity, salinity and pH were carried out in situ every day, while the parameters of chlorophyll-a, phosphate and nitrate were only measured at the beginning and end of the rearing. Nitrate and phosphate content were measured in sea water source in earthen pond reservoir. Survival Rate (SR) was calculated using the formula:

$$\text{SR (\%)} = \frac{Nt}{No} \text{ x } 100 \qquad (1)$$

Where: SR = Survival Rate (%), Nt = Number of alive *C. racemosa* at the end of culturing, N_0 = Number of initial maintenance seeds.

Specific Growth Rate (SGR) was calculated using the formula from [12]:

$$\text{SGR (\%)} = [(\ln Wt - \ln Wo) / (t)] \text{ x } 100 \qquad (2)$$

Where: SGR = Specific Growth Rate (% per day), Wo = initial weight (g), Wt = final weight (g), and t = culture time (t).

Temperature and light intensity were measured using an ONSET hobo data Logger model UA-002-64, and pH salinity were measured using a multiparameter water quality equipment (YSI Pro-1030). Nitrate and phosphate were measured using a spectrometer method.

Measurement of chlorophyll-a refers to [13]. Extracting of seaweed samples (0.14 g per sample) for 24 hours using 5 mL of N, N Dimethylformamide (DMF) solution and storing them in the refrigerator with temperature of 4 ^{o}C and then measuring their absorbance using a spectrophotometer (PEAK C-7000V). Chlorophyll-a content was calculated using the following formula [14]:

$$\text{Chlorophyll-a } (\mu g/mL) = 12.0 \text{ x } (Abs_{663.8} - Abs_{700}) - 3.11 \text{ x } (Abs_{646.8} - Abs_{700}) \qquad (3)$$

Where: Chlorophyll-a = content of chlorophyll-a and Abs = absorbance value on the spectrophotometer.

2.3 Data analysis

The data were analyzed statistically using the one-way ANOVA test and Duncan's test, while water quality parameter data were analyzed descriptively.

3 Results

The weight of *C. racemosa* in all treatments up to the end of the study showed values that continued to increase. The highest weight gain of *C. racemosa* was found to occur in the treatment in which the medium was given hydroponic nutrient fertilizer (HN), followed by the treatment using liquid organic fertilizer (LO), and the lowest was the treatment in the medium without the use of fertilizer (K). The weight gain of *C. racemosa* after being reared for 28 days is shown at Figure 1.

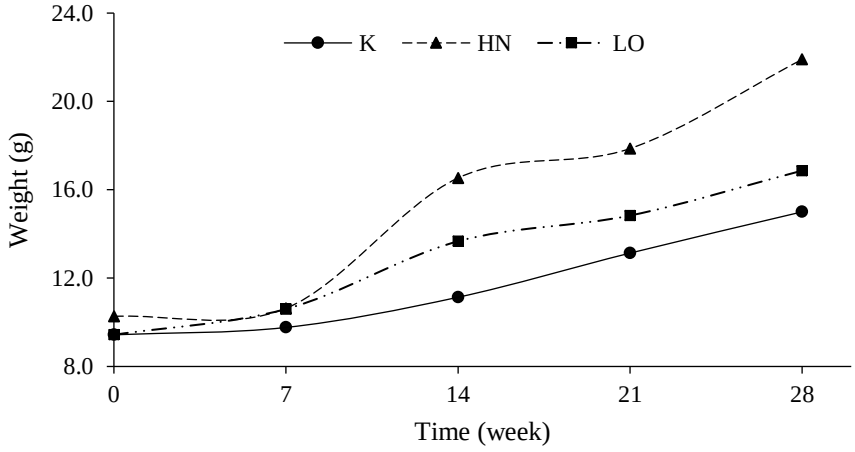

Fig. 1. Weight gain of *Caulerpa racemosa* cultured in medium without fertilizer (K), inorganic hydroponic nutrient (HN) and liquid organic fertilizer (LO) for 28 days.

3.1 Specific growth rate

The specific growth rates in different treatments resulted in the control treatment being significantly different ($P<0.05$) from the HN treatment (15 ml/L hydroponic nutrient fertilizer) and LO treatment (3.5 ml/L liquid organic fertilizer). The highest specific growth rate was in the HN treatment (2.70±0.02% per day), followed by the LO treatment (2.07±0.03% per day), while the lowest growth rate was in the control treatment (1.65±0.01% per day) as presented in Figure 2.

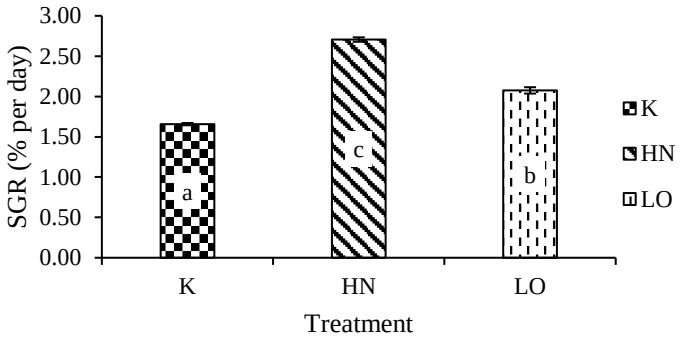

Fig. 2. Specific Growth Rate (% per day) of *C. racemosa* cultured for 28 days. K is Control, HN was Hydroponic Nutrient fertilizer treatment, LO was Liquid Organic fertilizer treatment

3.2 Survival Rate (SR)

The survival rate of *C. racemosa* after culturing for 28 days treated with different treatment doses had no significant effect ($P<0.05$) on the survival rate. The highest survival rate was in the LO treatment (3.5 ml/L liquid organic fertilizer) and the control treatment, namely 100%, while the lowest survival rate was in the HN treatment (15 ml/L hydroponic nutrient fertilizer) at 93.3%. SR of seaweed is presented in Figure 3.

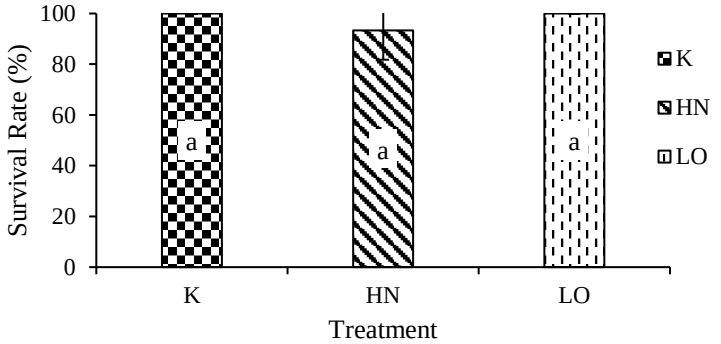

Fig. 3. Survival rate (%) of *C. racemosa* cultured for 28 days at different treatments. K was Control, HN was Hydroponic Nutrient Fertilizer, LO was Liquid Organic Fertilizer

3.3 Chlorophyll-a content

The chlorophyll-a content in average at the beginning of rearing was 136.9±2.5 µg per gww (mean±SD). The initial chlorophyll-a content of *C. racemosa* seaweed was similar because they had not been treated, and after being given different treatments for 28 days the content was changed. Chlorophyll-a content of *C. racemosa* cultured in control (without inorganic and organic fertilizer media) showed decreased value after culturing for 28 days. The highest chlorophyll-a content was in HN treatment (222.0±4.5 µg per gww) as seen in Table 1.

Table 1. Chlorophyll-a content (μg per g_{ww}) of *C. racemosa* at different treatments

Parameter	Treatment		
	Control (mean±SD)	HN (mean±SD)	LO (mean±SD)
Chlorophyll-a initial (μg per g_{ww})	136.9 ± 2.5	136.9 ± 2.5	136.9 ± 2.5
Chlorophyll-a final (μg per g_{ww})	121.2 ± 10.2	222.0 ± 4.5	208.6 ± 6.6

3.4 Water quality

The water quality parameters tested in this study included temperature, salinity, pH, light intensity, nitrate, and phosphate. The results of these measurements can be seen in Table 2. Furthermore, Figure 4 and Figure 5 show fluctuations in temperature and light intensity, respectively.

Table 2. Temperature, light intensity, salinity, pH, nitrate and phosphate during the research period

Parameter	Observed value				Optimal value
	K	HN	LO	Sea water source*	
Temperature (°C)	25.71 – 30.15	25.42 - 30.15	23.39 - 30.36		27 - 32[1)]
Light intensity (lux)**	0 – 4,133.4	0 – 2,326.0	0 – 1,808.3		70 - 5000[2)]
Salinity (ppt)	29.5 – 30.1	29.1 – 30.5	29.6 – 30.8		20 - 50[3)]
pH	7.1 – 7.3	7.2 – 7.5	7.1 – 7.5		6 - 8,5[4)]
Nitrate (mg/L)				0.5876 – 0.1044	0.03-0.10[5)]
Phosphate (mg/L)				0.0055- 0.0078	<0.007[6)]

Note: [1)] [11], [2)] [15]; [3)] [16]; [4)] [17]; [5)] [18]; [6)] [19] *Earthen pond reservoir. ** Culture area was semi-outdoors with roof.

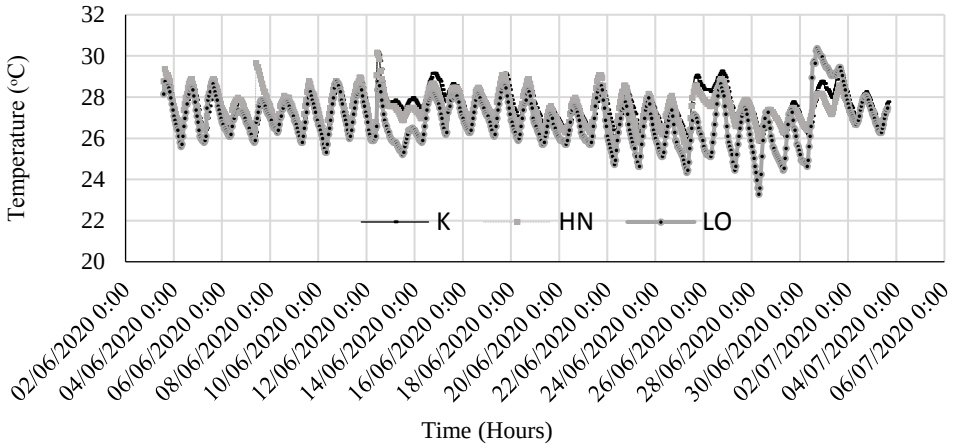

Fig. 4. Temperature (°C) fluctuation recorded every 1 hour along the culture period

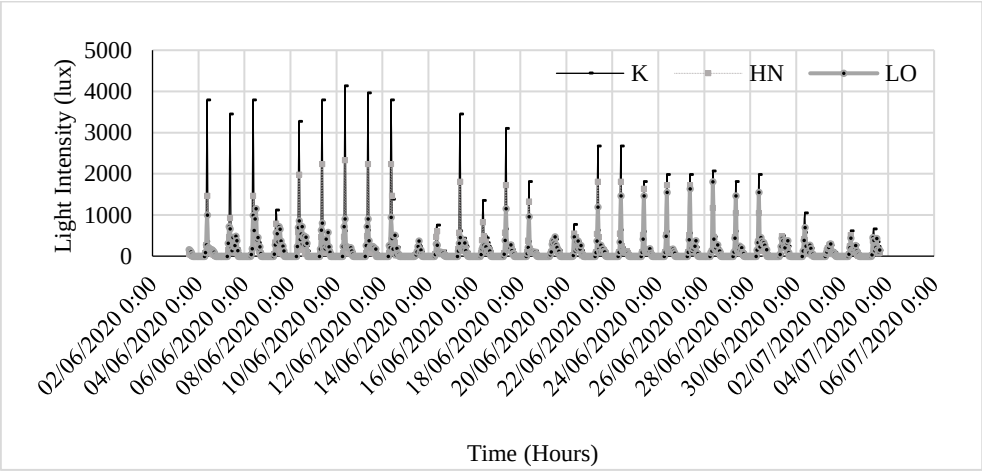

Fig. 5. Light intensity (lux) fluctuation recorded every 1 hour along the culture period

4 Discussion

Based on the existing results, the survival rate of *C. racemosa* with different treatments shows different results. The highest survival rate was in the control treatment and LO treatment (3.5 ml/L liquid organic fertilizer) at 100%, while the lowest was in the HN treatment (15 ml/L hydroponic nutrient fertilizer) at 93.3%. Statistically, the survival rate of *C. racemosa* cultured in the media was not significantly different ($P < 0.05$). Liquid organic fertilizer contains growth regulators such as auxin, gibberellins and cytokinins. The low survival rate in the HN treatment is thought to be due to external factors such as water quality. In line with the opinion of [17], temperature, light and nutrition can influence seaweed's growth and survival rate.

The weight growth of *C. racemosa* seaweed is known from measuring its weight every week. The highest growth was in the HN treatment at 2.70% per day, followed by LO and the lowest in the control at 1.65% per day. It is suspected that hydroponic nutrient fertilizer at a dose of 15ml/L can be well absorbed by *C. racemosa* for their growth. Supported by the opinion of [11] that seaweed requires several macronutrients and micronutrients for its growth. Macronutrients needed include nitrogen, phosphorus and potassium. Nitrogen can be function as a plant growth regulator. The growth regulator contained in liquid organic fertilizer had less effect than hydroponic nutrient fertilization (inorganic) to increase growth rate, in contrast, Atonic growth regulator has an impact to increase in vitro growth of *Eucheuma cottonii* [20]; [21]. The availability of nutrients is very influential on the growth of seaweed [5]. As a comparison, *C. lentillifera* which is maintained in land ponds using the primary spread method grows at 5.2% per day [22].

The content in hydroponic nutrition includes macro elements and micro elements. The macro elements are nutrients needed in large quantities such as N, P, K, S, Ca and Mg. In addition, microelements are nutrients needed in small amounts, including Mn, Cu, Zn, Cl, Na, and Fe. The results of this research show that *Caulerpa racemosa* seaweed treated with hydroponic nutrient fertilizer at a dose of 15ml/L produces the highest growth, this is because the nutrients provided can increase plant growth if the dose is appropriate to the plant's needs [10]. Chlorophyll-a is a green leaf substance found in all algae, and is essential in the reaction center [23]. Chlorophyll-a has many benefits, including helping absorb nutrients and maintaining body balance. Chlorophyll is also needed to improve the immune system [24]. Based on the results of this study, it can be seen that the chlorophyll-a content at the beginning and the end of the rearing showed different results. At the beginning of culturing,

the chlorophyll-a content was 136.9±2.5 µg per gww (mean±SD), after adding hydroponic nutrient fertilizer and liquid organic fertilizer, the chlorophyll content was changed. In the control (without fertilizer) the chlorophyll-a content further decreased to 121.2±10.2 µg/gww, while in HN treatment the chlorophyll-a content increased to 222.0±4.5 µg/gwwand LO treatment the chlorophyll-a content increased to 208.6±6.6 µg/gww. The highest chlorophyll-a content in HN treatment showed a highest growth rate of *C. racemosa*. These chlorophyll-a contents of *C. racemosa* were higher than the chlorophyll-a content of *Kappaphycus alvarezii* (85.23-120.06 µg/gww) cultured under controlled light and temperature condition [25]. Increasing cholorphyll-a can increase photosynthetic rate to provide energy for biochemical activities.

C. racemosa is a type of green algae. Based on research, *C. racemosa* can produce secondary metabolites that function as antioxidants. Apart from that, Caulerpa contains caulerpenin, which has anticancer, antitumor, and antiproliferation properties that show human bioactivity. The vitamin content in Caulerpa is quite high, such as vitamin A, vitamin C, and iron [2].

As with aquaculture commodities in general, water quality parameters can affect growth. Temperature, salinity, pH, light intensity and nutrients affect the growth of seaweed [8]. Nitrogen is one of the important element constituents of chlorophyll, which is needed in photosynthesis. The results of temperature, salinity, and pH measurements are still within the optimal range. The temperature dynamics experienced increases and decreases between 23.39-30.36 °C, where this temperature range is outside of the optimal value for sea grape cultivation [26], but the lowest temperature (23.39 °C) occurred one time at last fourth week (Figure 4.), so, the lowest temperature was not affected survival rate.

5 Conclusions

Results of this study provided important information for the land-based culturing of *C. racemosa*. The nutrient level of the culture media significantly affects the growth rate of *C. racemosa*. The highest specific growth rate of *C. racemosa* occurred in media given hydroponic nutrient fertilizer at 2.70±0.02% per day, followed by *C. racemosa*, which was given liquid organic fertilizer (2.07±0.03% per day). In contrast, the lowest growth rate occurred in the control (without fertilizer) treatment, 1.65±0.01% daily. These three media culture treatments gave a response that did not affect the survival of *C. racemosa*. At the same time, the chlorophyll-a content showed a parallel effect to the growth rate. Where *C. racemosa* had the highest growth rate, its water was found to have the highest chlorophyll-a content too.

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References

1. A. Rahmawati, D. Surilayani, *Pengelolaan pesisir desa lontar untuk kegiatan budidaya rumput laut*, in Prosiding Seminar Nasional Perikanan dan Kelautan, Bandung, Indonesia (2016) (in Indonesian)
2. S. Ridhowati, Asnani, Jurnal Oceana **61**, 4 (2016)
3. F. Ferdouse, L. Holdt, S. Smith, R. Murúa, P. L. Yang, *The Global Status of Seaweed Production, Trade and Utilization*, in FAO Globefish Research Program **124** (2018)
4. H. Long, X. Gu, Z. Zhu, C. Wang, X. Xia, N. Zhou, X. Liu, M. Zhao, J. Appl. Phycol **12** (2019)
5. E. R. Sitorus, G. W. Santosa, R. Pramesti, Jurnal of Marine Research **9**, 1 (2020)

6. K. Setiaji, G. W. Santosa, Sunaryo, Jurnal of Marine Research **1**, 2 (2012)
7. A. Mary, V. Mary, A. Lorella, J. R. Matias, Current Science **97**, 10 (2009)
8. F. B. Budiyani, K. Suwartimah, Sunaryo, Jurnal of Marine Research **1**, 1 (2012)
9. S. Sentana, *Pupuk Organik, Peluang dan Kendalanya*, in Prosiding Seminar Nasional Teknik Kimia "Kejuangan", Yogyakarta, Indonesia (2010) (in Indonesian)
10. L. Hidayanti, T. Kartika, Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam **16**, 9 (2019)
11. E. S. Ginting, S. Rejeki, T. Susilowati, Journal of Aquaculture Management and Technology **4**, 5 (2015)
12. N. Zonneveld, E. A. Huisman, J. H. Boon, Prinsip-prinsip budidaya ikan (PT. Main Library Gramedia, Jakarta, 1991) (in Indonesian)
13. Lideman, G. N. Nishihara, T. Noro, R. Terada, Aquaculture Sci **59**, 8 (2011)
14. R. J. Porra, W. A. Thompson, P. E. Kriedemann, Biochimica et Biophysica Acta **975** (1989)
15. N. W. Suniti, I. K. Suada, Agrotrop **2**, 4 (2012)
16. H. Guo, G. Yao, Z. Sun, D. Duan, J. Appl. Phycol **27** (2014)
17. S. N. Iskandar, S. Rejeki, T. Susilowati, Journal of Aquaculture Management and Technology **4**, 6 (2015)
18. W. Safia, Budiyanti, Musrif, JPHPI **23**, 10 (2020)
19. Burhanuddin. J. Balik Diwa **5**, 5 (2014)
20. M. Kasnir, Harlina, Lideman, M. I. Wamnebo, A. Hamdillah, AACL Bioflux **14**, 10 (2021)
21. E. Supriyono, Lideman, Y. P. Hastuti, A. R. Arifka, *Combination effect of atonic growth regulator with PES (Provasoli Enrich Seawater) on Seaweed (Eucheuma Cottonnii) growth*, in Proceedings of the 5th EMBRIO International Symposium: Sustainable Development of Fisheries and Marine Resource Amidst Covid-19 Era And Beyond, 6 September 2021, Bogor, Indonesia (2022)
22. Lideman, D. Hasbullah, J. T. Anggadiredja, Prosedur operasional produksi bibit Gracilaria melalui pengembangan spora (STP Press, Jakarta, 2023) (in Indonesian)
23. C. S. Lobban, P. J. Harrison, Seaweed Ecology and Physiology (Cambridge University Press, Cambridge, 2000)
24. W. Merdekawati, A. B. Susanto, Squalen. **4**, 7 (2009)
25. Syahrul, E. Supriyono, K. Nirmala, Lideman, *Growth and quality performances of seaweed (Kappaphycus alvarezii) with different combinations of temperature and light parameters*, in Proceeding of The 4th International Conference on Fisheries, Aquatic and Environmental Science, The 4th ICFAES 2022, 25 October 2023, Banda Aceh, Indonesia (2023)
26. A. B. Cahyanurani, R. M. R. Ummah, J. Ilmu Perikanan. **11**, 7 (2020)