Comparative study on the growth, carotenoid, fibre and mineral content of the seaweed *Caulerpa lentillifera* cultivated indoors and in the sea

R Syamsuddin 1,2, H Y Azis 1,2, Badraeni 1,2, Rustam 1,2

1Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar
2Center of Excellence for Development and Utilization of Seaweed (PUI-P2RL), Hasanuddin University, Makassar, Indonesia

Email: rajuddin_syamsuddin@yahoo.com

**Abstract.** This research series consisted of 4 (four) research activities, two implemented indoors at the Center for Brackishwater Aquaculture (BPBAP) Takalar in July - August 2017, and two carried out in the coastal waters of Aeng Batu - Batu Village, Galesong District, Takalar Regency, South Sulawesi, Indonesia in May - July 2018. The objectives of the studies were to analyse growth and content of specific nutritional components (carotenoids, fibre, and minerals) of the seaweed *Caulerpa lentillifera*. The *C. lentillifera* cultivated indoors and in coastal waters had the same cultivation period and initial seedling weight. There were differences in *C. lentillifera* growth and nutritional content between the two treatments. Growth and mineral content were higher indoors; likely due to the presence of readily absorbed minerals in the substrate provided (a mixture of sand and coral fragments). Higher carotenoid and fibre content in *C. lentillifera* cultivated in coastal waters was likely due to carotenoid synthesis by the seaweed to protect chlorophyll from damage, and increased photosynthesis producing more complex carbohydrates (fibre). Although lower than in some previous studies, the mineral and fibre content of *C. lentillifera* in both treatments was higher than the levels found in most land plants seaweeds.

1. **Introduction**

Indonesia is an archipelagic country with a coastline of approximately 81,000 km and high potential for seaweed production. Several species of seaweed (macroalgae) have become export commodities, earning foreign exchange source for the country and income for coastal communities. *Caulerpa lentillifera* is one of the seaweed species with an economic value and potential for aquaculture development in Indonesia. This species is widespread in tropical and subtropical waters and can survive at relatively low water temperatures.

Because the shape of the thallus somewhat resembles bunches of grapes, some people call *Caulerpa lentillifera* the "sea grape"; the shape and taste are also considered by some to resemble caviar, earning *C. lentillifera* the name of "green caviar". Also known as *latoh* (Javanese), *bulung boni* (Bali), *lawi-lawi* (Makassar), and *umi budo* (Japan), this seaweed has long been consumed as a source of fresh food (vegetables) throughout its distribution, including Japan, the Philippines, and Indonesia, in particular in South Sulawesi. Due to demand from the local market, this seaweed species has begun to be cultivated in South Sulawesi, providing benefits for coastal communities and opening opportunities for becoming an Indonesian fishery product of the future.
Aside from being a source of functional food components beneficial to human health with a high nutritional content (protein, carbohydrates, minerals and vitamins) [1], *C. Lentillifera* could be used for medical purposes. *C. Lentillifera* contains antioxidants, and is reported as being beneficial for several health problems including diabetes, heart disease, high blood pressure, worm infestation, and fungal infections. This seaweed is also believed to be effective in increasing appetite, mitigating the effect of cancer drugs, promoting wound healing, and increasing immune system function. The antioxidants could help reduce the formation of free radicals, and reduce blood cholesterol levels, as well as facilitating good digestion [2]. The carotenoids and fibre contained in *C. lentillifera* are organic compounds necessary for human health. Therefore, cultivation of *C. lentillifera* offers good economic prospects, for both domestic and export markets. Until very recently, the *Caulerpa* traded and consumed by Indonesian people has been extracted from the wild, and supply has been dependent on the seasonal abundance of this species.

The coastal waters around coral reefs, especially the inter-tidal zone, as the natural *Caulerpa* habitat, are a potential environment for *Caulerpa* cultivation. However, *C. lentillifera* is currently only cultivated in brackish water ponds by coastal communities. One reason *C. lentillifera* has not been cultivated in the sea is the consideration that this type of seaweed is easily damaged and washed away by currents and waves and is also readily grazed by herbivorous fauna. Indoor cultivation, using seawater and substrate as a medium for growing *C. Lentillifera*, has not been reported. This study comprised a series of research activities with the aim of analysing and comparing the growth as well as the carotenoid, fibre and mineral content of *C. lentillifera* cultivated in two different environments, indoors and in the sea.

2. Materials and Methods

2.1. Study Site and Time

The four experiments in this study were carried out in the same area of the Makassar Strait, in Takalar Regency, about 10 km south of Makassar City, South Sulawesi Province, Indonesia. Two indoor studies (in a greenhouse) were implemented from July 1, 2017 to August 5, 2017 at the Brackish Water Aquaculture Center (BPBAP) Takalar, Mappakalompo Village, South Galesong District, Takalar District. The other two studies were conducted in the coastal waters of Aeng Batu-batu Village, Galesong District, also in Takalar Regency, from May 2018 to July 2018, with a preparation period and 30-40 days of cultivation.

2.2. *Caulerpa lentillifera* Seed

*Caulerpa lentillifera* seeds (Figure 1) were sourced from aquaculture ponds in Laikkang Village, Mangarabombang District, Takalar Regency, South Sulawesi, Indonesia.

![Figure 1. Seaweed *Caulerpa lentillifera* Seeds.](image-url)
2.3. **Indoor Cultivation**

Two factors were addressed using an indoor cultivation system. The first was the effect of 3 (three) substrate composition treatments, each with 4 replications. These were (based on dry weight): 75% sand + 25% coral fragments; 50% sand + 50% coral fragments; 25% sand + 75% coral fragments. The other factor was seed spacing distance, with four treatments: 5 cm, 10 cm, 15 cm, and 20 cm, on the mixed substrate of 50% sand and 50% coral fragments.

The same containers were used for all treatments. These were styrofoam boxes measuring 38 cm (length) x 25 cm (width) x 30 cm (height). The bottom surface of the container was covered to a depth of 5 cm with substrate, in which the seaweed holdfast was embedded (Figure 2).

![Indoor cultivation in polystyrene boxes. Above: substrate treatments. Below: seaweed treatments.](image)

Before being used as substrate, the sand and coral fragments were washed in sea water and then dried in direct sunlight to remove any toxic compounds and living organisms that might be present in the sand particles and coral fragments. The depth of water in each container was maintained at 20 cm. The water was pumped directly from the sea and stored in a reservoir, passing through a filter system before use. Salinity during the experiments ranged from 30-35 ppt.

Replacement of the seawater in the containers (80% every two days) was carried out carefully using an aeration hose in order to avoid turbidity and movement of the seaweed thalli. To maintain seed condition and avoid overheating, the *Caulerpa lentillifera* seeds were planted in the morning.

2.4. **Cultivation in the Sea**

Cultivation trials of *Caulerpa lentillifera* in the sea sued two methods: a surface method, with the containers (trays) containing seaweed seed positioned 20 cm below the sea surface, and an off bottom method with the trays positioned 20 cm above the sea floor. To evaluate the effect of seed weight there were three treatments with 50g, 100g and 150g seeds/tray, each with 3 replicates. The plastic trays
were of uniform dimensions and colours, measuring 49 cm in length, 35 cm in width, and 17 cm in height (Figure 3).

Figure 3. Plastic trays used containers for *C. lentillifera* cultivation in the sea.

The cultivation containers were tied to nylon ropes equipped with floats. In order to remain hung in the same position in the water column and avoid any shift from the cultivation location, the trays were tied to anchors placed on the sea floor (Figure 4). The distance between the containers was 30 cm.

Figure 4. Construction of *C. lentillifera* cultivation facilities in the sea

2.5. **Measurement of seaweed growth, carotenoid, fibre and mineral content**

Growth (net increase in weight, in g) of *C. Lentillifera* was measured at the end of each cultivation treatment. Each thallus was removed from the container, cleaned using seawater, and then weighed using electric scales. The absolute growth was calculated using the following equation:

- \[ W = W_t - W_o; \]
  
  Where:
  
  \[ W = \text{net growth (weight gain) (g)} \]
  
  \[ W_t = \text{final weight (g)} \]
  
  \[ W_o = \text{initial weight (g)} \]

Carotenoid, fibre, and mineral content were analysed at the Water Quality Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University at the end of the study. The carotenoid content was analysed with the method proposed by [3], and was calculated using the following formula:

- \[ C = (V)(E_{1%}B)^4 \]
where:

\[ C = \text{carotenoid content (ppm)} \]
\[ V = \text{Volume of extract (mL)} \]
\[ E = \text{Extension coefficient (absorbency) at spectrophotometer settings 1\% and A460} \]
\[ B = \text{sample extracted weight (g fresh weight)} \]

The fibre content was analysed with the method proposed by [4], and calculated using the formula:

- Crude fibre content (\%) = \((a-b)\cdot a^{-1}\cdot 100\%\)
  
    where:
    
    \[ a = \text{sample weight (g) before drying in the oven} \]
    \[ b = \text{sample weight (g) after drying in the oven} \]

The mineral content (ash) was determined with the method proposed by [4], calculated using the formula:

- % ash = \((W_1-W_2)\cdot W^{-1}\cdot 100\%\)

  Where;
  
  \[ W_1 = \text{weight of cup + weight of ignited sample (g)} \]
  \[ W_2 = \text{weight of cup (g)} \]
  \[ W = \text{sample weight before ignited (g)} \]

2.6. Experimental Design and Data Analysis

The experimental design applied was a Completely Randomized Design (CRD). Growth data were compared between treatments using Analysis of Variance (ANOVA) followed by W-Tukey's post-hoc test. All statistical analyses were implemented in SPSS. The carotenoid and mineral content were analysed descriptively.

3. Results and Discussion

The net growth of \textit{C. lentillifera} was highest (48.52 - 80.12 g) with indoor cultivation, seed weight 80 g/tray and substrate composed of a mixture of sand and coral fragments. Net weight gain of seeds cultivated in the sea did not exceed 25.15 g (Table 1).

\textbf{Table 1.} Comparison on the Growth, Carotenoid, Fibre, and Mineral Content of \textit{C. lentillifera} Cultivated Indoor System versus Cultivated in the Sea.

| Cultivation Method                      | Net Growth (g) | Carotenoids (ppm) | Fibre (%) | Minerals (%) |
|----------------------------------------|----------------|-------------------|-----------|-------------|
| Indoor with substrate (3 types)        | 48.52 - 80.12  | 1.49 - 1.55       | 5.03 - 5.48 | 51.03 - 52.79 |
| Indoor with substrate (3 types)        | 1.17 - 3.82    | 1.31 - 2.11       | 5.05 - 5.56 | 49.92 - 50.28 |
| High Density (5 cm spacing)            | 1.66 - 17.20   | 1.88 - 2.21       | 7.64 - 8.65 | 33.97 - 36.60 |
| In the sea using trays, Floating Method 1| 14.20 - 25.15  | 1.71 - 2.29       | 7.73 - 8.19 | 32.04 - 36.25 |
| In the sea using trays, Off Bottom Method 2 | 14.20 - 25.15 | 1.71 - 2.29 | 7.73 - 8.19 | 32.04 - 36.25 |

Note: bold type indicates the highest value for each parameter.

Seaweeds of the genus \textit{Caulerpa} live and grow on coral reef beaches [5]. The seaweed is generally firmly embedded in the substrate and the holdfast, which resembles the roots of higher plants, is not easily detached from its anchorage. The holdfast of \textit{C. lentillifera} readily adheres to substrate with indents or pore-shaped holes, such as fragments of coral, and then develops into a new thallus [6].
There are many nutrient compounds in coral fragments which come from coral reef ecosystems that store many nutrients [7]. Of the 74 types of minerals contained in powdered coral, some are macro elements (needed in large quantities) present in high concentrations, including elements which are essential for seaweed growth. These include carbon (126,000 ppm), calcium (348,000 ppm), magnesium (25,600 ppm), phosphorus (46.9 ppm), potassium (121 ppm), silica (870 ppm), sodium (650.3 ppm), and sulphur (569 ppm) [8]. The nutrients are derived from the metabolism of coral animals and their symbiotic zooxanthellae, the excreta produced by fish in the coral ecosystem, as well as from plants (phytoplankton), and the decomposition of benthic algae [7]. With cell walls consisting of polysaccharides and proteins composed of carboxyl anions, phosphate, and sulphate groups, the seaweed C. lentillifera can absorb these nutrients [9], which could explain the high growth in net weight of C. lentillifera in indoor cultivation on a substrate of sand mixed with coral fragments.

The carbonate contained in coral fragments in the substrate could have been a source of the carbon dioxide (CO₂) which is essential for the process of photosynthesis. Calcium, magnesium, carbon, and phosphorus content in the substrate could provide essential nutrients for the formation of cell walls, synthesis of chlorophyll, forming organic compounds, and energy-forming elements in C. lentillifera. Another function of high Ca and Mg content is that these minerals can diffuse into water and agglomerate (flocculate) clay particles suspended in the water [10]. The clumps of clay particles then settle down to the bottom, preventing clay particles from becoming attached to the thallus of seaweed, a process which can reduce sunlight penetration and reduce the diffusion and absorption of nutrients by the seaweed. Therefore cultivation with an indoor system using suitable substrate can spur photosynthetic activity and growth of C. lentillifera cells. The amount and quality of light is very influential in the process of photosynthesis as well as cell division and growth [11].

Lower growth with cultivation in the sea may have been caused by limited concentrations of nutrients that can be absorbed by seaweed and by sunlight intensity. In the floating method, the light intensity reaching the seaweed thallus may have exceeded the optimal intensity for this species, in particular the exposure to ultraviolet light in the upper layer of sea water. In the off-bottom method, the seaweed was very close to the nutrient source (seabed). However light intensity was low, while clay and sand particles attached to the thallus which may have reduced nutrient diffusion and absorption.

The very low growth in one indoor system treatment (1.17 - 3.82 g) was caused by the number of seedlings grown in one tray exceeding 80 g. The growth of seaweed was strongly influenced by the spacing of seeds grown. So, differences in C. lentillifera growth can be caused by differences in the number (or size) of initial seedlings used in a given space [12].

Mineral (ash) content is the inorganic substances left over from burning seaweed, and includes metal salts [13]. High mineral content of C. lentillifera was also obtained by indoor cultivation using trays, namely 51.03 - 52.79% with 80 g/tray and 49.92 - 50.28% with 80 g at a planting distance of 5 cm - 20 cm/tray. The difference in mineral content was most likely partly due to differences in the rate of photosynthesis [14] and environmental factors [15,16].

In the indoor systems, the intensity of light received by seaweed could be easily controlled so that it was optimal for photosynthesis and active absorption of nutrients. The high concentration of various nutrients in the substrate used for indoor cultivation was another very important factor for high mineral content of this seaweed. Nutrients can be directly absorbed by the seaweed thallus which is directly attached to the substrate. In addition, polysaccharides and proteins with carboxyl anion, phosphate and sulphate groups from the cell wall can bind and store nutrients in the form of metals [9]. Conversely, the low mineral content of C. lentillifera cultivated in the sea was most likely mainly due to light intensity above the optimal level, and the lower availability of nutrients in the water column. Even though one treatment was an off-bottom method, the seaweed could not directly touch the substrate accumulating nutrients in the sea.

The mineral content of C. lentillifera in this study was high compared to the ranges recorded by several previous researchers for this species and other macroalgae, e.g. 14.10 ± 0.76% [17] in Sabah Malaysia, 10.93 - 35, 94% [12], 10.64 ± 0.40% in C. racemosa [17], 35.08 ± 1.89% in the seaweed
Phylum chlorophyta (a green seaweed) [18] and 7-38% [19] for seaweeds in general. Caulerpa is generally rich in minerals needed for human health, including iodine (480,665 µg in 100 g of wet weight according to [20]), silica, arsenic, copper, cobalt, cadmium, molybdenum, lead, and chrome [21], iron, zinc, magnesium, calcium, potassium, and sodium [14]. Therefore Caulerpa is used as an ingredient in anti-fungal drugs. With a strong ability to bind and store metals [9], the mineral content of C. lentillifera was much higher than that of most land plants [13] and higher than that reported for algae such as Gracilaria gigas, G. verrucosa, Sargassum sp. and Eucheuma cottoni.

Caulerpa contains carotenoids (including β carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin, and neoxanthin [22] and fibre which is a type of carbohydrate, and is the main product of photosynthesis. The fibre content in Caulerpa consists mainly of polysaccharides.

Green seaweeds (including C. lentillifera) generally contain carotenoids. Carotenoids function as antioxidants that are beneficial to human health and can help reduce the formation of free radicals which affect cell growth. While the growth rate and mineral content were higher in indoor cultivation with a substrate, the carotenoid and fibre content were higher in C. lentillifera cultivated in the sea, with both floating and off-bottom methods. The high carotenoid content in C. lentillifera cultivated in the sea is likely a result of exposure to excessive light intensity, in particular ultraviolet light that can damage chlorophyll (due to photo-inhibition, photo-oxidation, photodamage) [23]. Under such conditions, carotenoids tend to be synthesized to protect the chlorophyll from damage [24]. The low carotenoid pigment content of C. lentillifera grown indoors was probably due to the optimal light intensity reaching the thallus [25].

The fibre content of algae consists of cellulose, lignin and various types of carbohydrates [26]. The highest fibre content (7.64 - 8.65%) of C. lentillifera cultivated in the sea was higher compared to the content recorded by [12] (2.15 - 8.19%). These values are low compared to the finding of [27] that seaweed generally contains 9.62% of fibre, and the fibre content reported in C. racemosa from Sabah, Malaysia (11.29 ± 0.47%) [17], and the Kenya Coast, Africa (12.38 ± 0.10%) [18]. Variation in fibre content can be caused by environmental conditions [28], including season, temperature, salinity, water transparency and nutrient concentrations. Another influential factor is the ability of algae to absorb nutrients, related to the growth phase, photosynthetic activity, and differences in seaweed species [29].

The fibre content in Caulerpa should be beneficial for human health and facilitate digestion [2]. Soluble fibre can also reduce blood cholesterol levels and help reduce the risk of coronary heart disease due to the ability of fibre to ensnare fat in the intestine thus preventing absorption of fat in the body. Soluble fibre binds to bile acids (cholesterol end products) in the digestive tract which are then released with faeces, so that the higher consumption of soluble fibre, the more bile acid and fat are released by the body [30].

4. Conclusion
After 30 days of cultivation, the growth rate and mineral content were higher in C. lentillifera cultivated under indoor conditions compared to maricultured C. Lentillifera. However, when cultivated in the sea, the high carotenoid and fibre content of C. lentillifera were higher.

Acknowledgements
The authors wish to thank the Dean of the Faculty of Marine Sciences and Fisheries, Hasanuddin University, and the Director of the Center of Excellence for Development and Utilization of Seaweed (PUI-P2RL), Hasanuddin University for moral and material support enabling the implementation of this research and participation in this symposium. Thanks are also due to Ms Fitri and the technicians of the Water Quality Laboratory, UNHAS FIKP for assistance with the chemical analysis and to Dg. Sau (seaweed cultivator) for his assistance, in the field.

References
[1] Turagan F A C 2001 Pertumbuhan, Variasi Intraspesisif, Biomassa Total dan Kandungan Nutrisi Alga Hijau Caulerpa racemosa (Forskal) J. Agardhdi Perairan Tongkaine, Kota
Manado Sulawesi Utara J. Perikanan–UNSRAT

[2] Winarno F G 1996 Teknologi Pengolahan Rumput Laut (Jakarta: Pustaka Sinar Harapan)
[3] Shahidi F, Metusalach and Brown J A 1997 Carotenoid pigments in seafoods and aquaculture Crit Rev Food Sci Nutr. 38 1–67
[4] Sudarmadji S, Haryono B and Suhardi 1997 Prosedur Analisa Untuk Bahan Makanan dan Pertanian (Yogyakarta: Liberty)
[5] Dawson E Y 2004 How to Know The Sweed. (Dubuque, Lowa: Wm C.Brown)
[6] Teaching Science as Inquiry (TSI) 2018 Aquatic Plants and Algae; Structure and Function. Exploring our Fluid Earth
[7] McCormick M I, Barry R P and Allan B J M 2017 Algae associated with coral degradation affects risk assessment in coral reef fishes Sci. Rep. 7
[8] Schick K 2018 Coral Calcium Powder Chemical Analysis of 74 Trace Minerals Mar. Bio
[9] Davis T A, Volesky B and Mucci A 2003 A review of the biochemistry of heavy Metal biosorption by brown algae Water Res. 37 4311–30
[10] Donskova K and Norton L. 1990 Effect of Changeable Ca : Mg Ratio on Soil Clay Flocculation, Infiltration and Erosion
[11] Geider W 2006 Fotosintesis Pada Alga dan Bakteri
[12] Syamsuddin R, Rustam N A, Abustang N A and Fitra I 2019 Carotenoid Content and Weight Gain of Caulerpa racemosa (Chlorophyta, Caulerpaceae) at Several Light Intensities JISRAT 6 211–9
[13] Mayer A M S, Rodriguez A D, Berlinck R G and Fusetani N 2011 Marine pharmacology in 2007-8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; Affecting the immune and nervous system, and other miscellaneous mec Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 153 191–222
[14] Krishnaiah D R, Sarbatly D M R, Prasad and Bono A 2008 Mineral Content of Some Seaweeds from Sabah’s South China Sea sian J. Sci. Res. 1 166–70
[15] Rupérez P 2002 Mineral content of edible marine seaweeds FoodChemistry 79 23–26
[16] Mendis E and Kim S J 2011 Present and Future Prospects of Seaweeds in Developing Functional Foods Adv. Food Nutr. Res. 64 1–15
[17] Ahmad A, Sulaiman M R, Saimon W, Chye F Y and Matanjun P 2012 Proximate Composition and Total Phenolic Contents of Selected Edible Seaweed from Semporna, Sabah, Malaysia Borneo Sci. 31 85–95
[18] Mwalugha H, Wakibia J, Kenji J and Mwasaru W 2015 Chemical Composition of Common Seaweeds from the Kenya Coast J. Food Res. 4 28–38
[19] Zailanie K and Kortikaningsi H 2016 Dietary fibre and fatty acids in the Thallus of brown alga (Sargassum duplicatum J.G. Agardh) Int. Food Res. J. 23 1584–9
[20] Maslukah L, Rudiana E and Pringgenies D 2004 Kajian tentang kandungan iodium pada ekstrak beberapa jenis rumput laut di perairan Jepara dan sekitarnya (Semarang: Universitas Diponegoro)
[21] Hampel K 2013 The Characterization of Algae Grown on Nutrient Removal Systems and Evaluation of Potential Uses for the Resulting Biomass (Western Michigan University)
[22] Burtin P 2003 Nutritional value of seaweed J. Agric. Food Chem. 2 1–6
[23] Glenn E P and Doty M S 1981 Photosynthesis and Respiration of the Tropical Red Seaweed Eucheuma striatum (Tambalang and Elkhorn Varieties) and E. denticulatum in Hawaii Aquat. Bot. 10 353–64
[24] Kabinawa I N K 2006 Spirulina Ganggang Penggempur Aneka Penyakit (Jakarta: Agromedia Pustaka)
[25] Olaizolal M and O D E 1990 Effects of light intensity and quality on the growth rate and photosynthetic pigment content of Spirulina platensis J. Appl. Phycol. 2 97–104
[26] Seniczak A, Ligocka A, Seniczak S and Paluszak Z 2016 Effects of green algae and napa
cabbage on life-history parameters and gut microflora of *Archegozetes longisetosus* (*Acari: Oribatida*) under laboratory conditions *Biol. Lett.* **53** 67–78

[27] Chaidir A 2007 *Kajian rumput laut sebagai sumber serat alternatif untuk minuman berserat* (Institut pertanian Bogor)

[28] McDermid K, Stuercke B and Haleakala O J 2005 Total dietary fibre content in Hawaiian marine algae *Bot. Mar.* **48**

[29] Siddique M A M, Aktar M and Khatib M A M 2013 Proximate Chemical Composition and Amino Acid Profile of Two Red Seaweeds (*Hypnea pannosa* and *Hypnea musciformis*) Collected From St. Martins Island, Bangladesh *J. Fish. Sci.* **7**

[30] Winarti S 2010 *Makanan Fungsional* (Yogyakarta: Graha Ilmu)