

# Effect of Depth on the Growth and Carrageenan Content of Seaweed *Kappaphycus alvarezii* Cultivated Using Verticulture Method

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**Abstract.** Seaweed of *Kappaphycus alvarezii* is generally cultivated by the long line method through the water surface. The verticulture method might be an alternative method by using water column. This research aimed to evaluate the effects of depth on the growth and carrageenan content of *K. alvarezii* which was cultivated vertically. Cultivation was carried out in the floating raft (10 x 10 m)<sup>2</sup> contained of 36 vertical nets. The size of each net was 5 x 2 m<sup>2</sup> whereas the depth was 0.2, 1, 2, 3, 4, and 5 m. Seaweed seedlings of 50 g was each tied to the vertical nets; the distance between the knot was 20 cm. The results showed that the depth was significantly different to the daily growth rate of *K. alvarezii* ( $p < 0.05$ ) but did not affect carrageenan content ( $p > 0.05$ ). Therefore, this method might be applied as an alternative method to optimally increase seaweed production in narrow area without affecting seaweed quality.

## 1 Introduction

The type of red seaweed (*Rhodophyceae*) becomes the main commodity in fisheries and marine sector of Indonesia. One of the most important seaweed species is *Kappaphycus alvarezii* for its kappa-carrageenan content. Carrageenan is commonly used as stabilizer, gelling agent, emulsifier, and many more. Hence, these properties are utilized in many industries, such as food, pharmaceutical, cosmetic, textile, paint, toothpaste, and other industries [1,2]. Kappa-carrageenan in *K. alvarezii* seaweed produces stronger/thicker gelling agent, thus it costs higher and becomes the main commodity that generates foreign exchange for Indonesia [3].

Market demand for carrageenan continues to increase considering its important role in the industry [4,5]. To meet the market needs, the effort to trigger the productivity of *K.alvarezii* as carrageenan source in terms of quantity, quality, and sustainability is needed. Until today, seaweed is mostly cultivated using long-line method. However, applying this method means that seaweed farming is performed only on the water surface, while seaweed is ecologically able to grow in the water column on the condition that it still receives sunlight to perform photosynthesis. Moreover, conflict on water territory concerning seaweed cultivation occurs very often in the central area of seaweed farming development. For instance, if the long-line construction is too long then the shipping lane is hindered.

One of the solutions to overcome the problem is the development of verticulture method. The technique involves the use of rope as instrument to tie seaweed seeds vertically at certain

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depth in the water column that acts as the media until the water transparency limit [6]. The focus of this method is land optimization and water column use. Application of this method is expected to intensify land use and increase seaweed production.

Study concerning seaweed farming by using verticulture method continues to be developed, such as construction model of instrument in the form of vertical rope [7,8,9,10,11,12]. Furthermore, other studies that only focused on the aspect of increasing seaweed growth in weight was also reported [13].

It was found that *K. alvarezii* seaweed cultivated in the depth of 0.3-5 m using verticulture method in Barru Regency of South Sulawesi was only able to grow optimally at the depth of 1–4 m [7], while a study conducted by [12] showed that *K. alvarezii* seaweed farmed at the depth of 0.5-2 m in Pangkep Regency grew optimally at the depth of 1-1.5 m. Moreover, other study carried out by [10] indicated that seaweed cultivated at the depth of 0.3-1.5 m in Boalemo Regency of Gorontalo did not grow optimally, in fact it was vanished since the ocean current velocity was not strong enough to remove epiphytes and mud stuck to the thallus. In spite of the low current velocity in Boalemo Regency (0.1-0.2 m/s), it caused the vertical rope that tied the seaweed seeds got tangled and detached from the knot. It concluded that verticulture method is location-specific method and development of its technical application is needed to be adjusted to other areas with strong current.

The objective of this study is to investigate the effect of depth on the growth and carrageenan content of seaweed cultivated using verticulture method by using vertical net.

## 2 Methods

This study was conducted in March–June 2015 in coastal area of Doda Bahari Village, Sangia Wambulu Sub-district, Central Buton Regency, Province of Southeast Sulawesi. Analysis of water quality and carrageenan content was performed in the Laboratory of BPPBAP Maros, South Sulawesi.

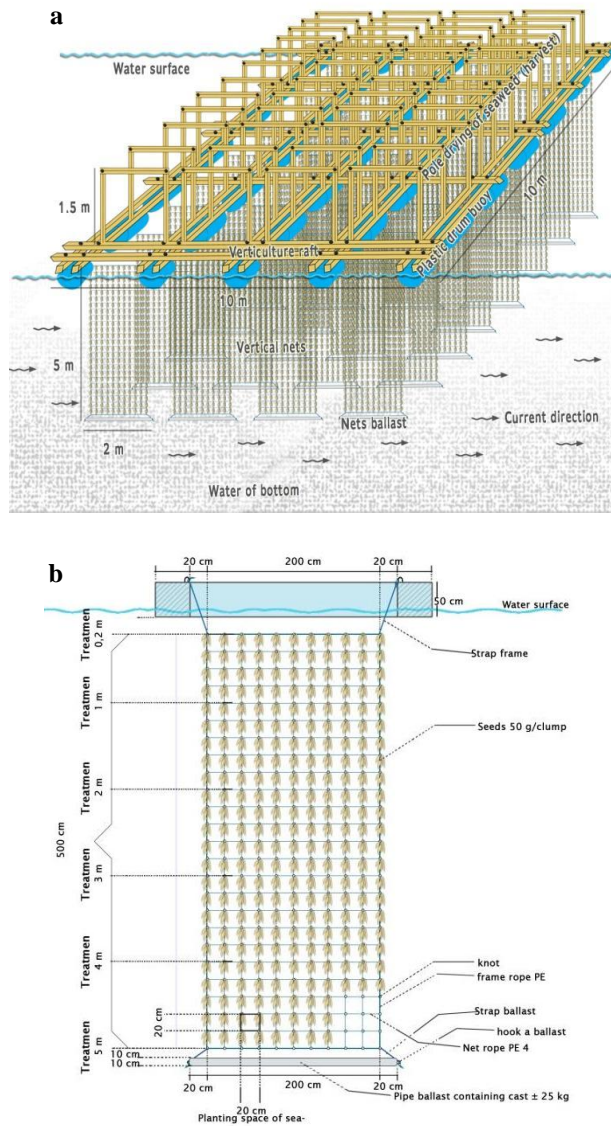
### 2.1 Preparation of seed

The seeds of *K. alvarezii* used in this study were obtained from local seaweed farmers. Seaweed seeds selected by the freshness and free from other seaweed species. The seeds were cleaned from dirt or biofouling organism, chopped and weighed for 50 g/clump.

### 2.2 Method of planting

Floating raft made from wooden beam of 10x10 m<sup>2</sup> used in this verticulture method. The construction was equipped with styrofoam floater and concrete anchor as barrier, thus it remained stable and would not be carried away by ocean currents.

Seaweed seeds were tied to polyethylene rope formed into net of 5x2 m<sup>2</sup>. Vertical net was equipped with ballast concrete-filled PVC pipe with distance between net strands of 1 m. The vertical net consisted of 11 vertical straps and 26 horizontal straps (Figure 1).



**Fig 1.** Construction of seaweed culture using verticulture method with vertical net application (a) full view and (b) side view.

At each point where vertical and horizontal ropes met, a knot was made so that rope ring to tie the seaweed seeds could be placed. The distance between knots was 20 cm, thus one vertical rope contained 26 points of seaweed clump and one horizontal rope consisted of 11 points of seaweed clump. Therefore, each net consisted of 286 points of seaweed clump.

One verticulture construction of 10x10 m<sup>2</sup> consisted of 36 nets, thus one construction contained 10,296 clumps. Among 36 verticulture nets, 18 nets were tagged and placed randomly for sampling purpose. Rearing period was performed for 3 cycles, each cycle was conducted for 30 days.

## 2.3 Observation

Observation of seaweed growth and carrageenan content was conducted at the beginning of stocking and 30<sup>th</sup> day after culture period. Moreover, water quality observation for parameter such as temperature, salinity, ocean current velocity, nitrate, phosphate, and total organic matter (TOM) measured every 15 days.

## 2.4 Method for carrageenan analysis

Harvested seaweed was sun-dried then weighed for 20 g each sample. The dry seaweed then washed to remove the salt and other pollutants. Thereafter, it was extracted on 90-95 °C using KOH for 30 minutes with comparison of solvent and raw material 40 mL: 1 g until pH of the solution reaches 8-9. The result of extraction then deposited in 100 mL of isopropyl alcohol and stirred then incubated for 15 minutes. The precipitate is dried in 50-60°C oven for 3 days. The result then milled and sifted with mesh filter (size 80). Carrageenan that has been obtained then calculated for the content. Carrageenan content is the extract result which is calculated based on the ratio between carrageenan content with the weights of dry sample used in each treatment [14].

$$\text{Rendemen (\%)} = \frac{\text{Carrageenan weight}}{\text{Seaweed weight}} \times 100 \quad (1)$$

## 2.5 Daily growth rate

Seaweed growth is determined by calculating the daily growth rate (DGR) using the following formula [15]:

$$DGR = \left\{ \frac{\ln W_t - \ln W_o}{t} \right\} \times 100\% \quad (2)$$

Description:

DGR = Daily growth rate (%/day)

$W_t$  = Final weight at time- $t$  (g)

$W_o$  = Initial weight (g)

$t$  = Culture period (day)

## 2.6 Data analysis

Effect of each treatment on observed variables was analyzed with Analysis of Variance (ANOVA) using SPSS (Ver. 16.0) program. If the result was significantly different, Tukey's test at a confidence level of 95% was further applied.

# 3 Result and discussion

## 3.1 Quality of water environment

The condition of water quality is an important factor that has to be considered in seaweed cultivation since water is the media that directly affects seaweed farming activity. Parameter of water quality that influences seaweed growth includes temperature, salinity, ocean current velocity, and nutrient [16]. Based on the measurement result of water quality parameter during *K. alvarezii* seaweed cultivation, the condition of water quality in the waters of Doda Bahari Village was in range for seaweed farming (Table 1).

**Table 1.** Parameter of water environmental quality

Depth (m)	Temperature (°C)	Salinity (ppm)	Ocean Current Velocity (m/s)	Nitrate (mg/L)	Phosphate (mg/L)	Total Organic Matter (mg/L)
0.2	29.8-30.3	34-35	0.4-1.0	0.03-0.26	0.07-0.09	29.40-42.54
1	29.2-30.0	34-35	0.4-1.0	0.02-0.46	0.05-0.11	38.16-58.18
2	29.0-29.8	34-35	0.4-1.0	0.03-0.25	0.04-0.07	40.04-64.44
3	29.1-29.6	34-35	0.4-0.9	0.15-0.24	0.04-0.06	36.91-63.81
4	28.9-29.5	34-35	0.3-0.9	0.17-0.19	0.07-0.08	40.66-63.19
5	28.6-29.2	34-35	0.3-0.6	0.22-0.24	0.06-0.08	40.66-52.55

The range of temperature was relatively different in each column, the deeper the water column, the lower the temperature (Table 1). This finding caused by the different sunlight exposure received in each column. The temperature during the study was in a range of 28.6-30.3 °C, while temperature tolerated by *K. alvarezii* seaweed is 26-30 °C [17].

Salinity measured in each water column during the study found to be similar in range of 34-35 ppt. According to [18], *K. alvarezii* is seaweed species that is not resistant to high salinity (stenohaline). Concentration of salinity suitable for seaweed growth ranges of 28-35 ppt.

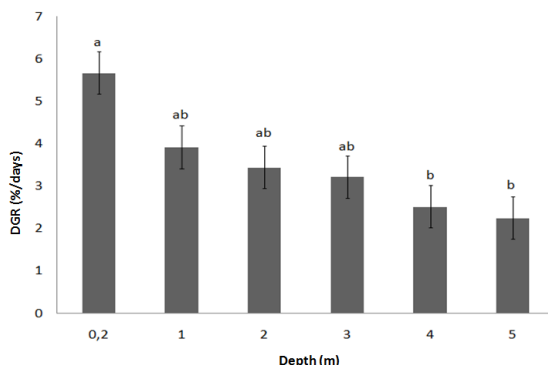
Location for seaweed farming should be protected from ocean currents (water movement) and strong wave because these two factors will destroy and sweep away seaweed. This condition will facilitate change and absorption of nutrient required by plants, but will not cause plant damage [6]. Measurement result for ocean current velocity during the study was 0.3–1 m/s.

Nitrate is the main form of nitrogen in natural waters. Nitrate is one essential nutrient compound in protein synthesis of animal and plant. High nitrate concentration in waters may stimulate the growth and development of aquatic organism if supported by nutrient availability. Result of nitrate measurement during the study ranged between 0.02–0.46 mg/L. Therefore, this nitrate concentration is considered suitable for the growth of *K. Alvarezii* seaweed. According to [19], the range of nitrate value for seaweed farming of 0.1-0.7 mg/L is characterized as excellent; 0.01-<0 mg/L is good, and <0.01 mg/L is considered poor. Phosphate concentration during the study ranged from 0.04-0.11 mg/L, which still in range the value tolerated by seaweed. Optimum range of phosphate for seaweed growth is 0.02-1.0 mg/L [20].

### 3.2 Seaweed Growth

*K. alvarezii* seaweed cultivated with verticulture method at a depth of 0.2 m obtained the highest DGR of 5.6%/day, while the lowest was found at 5 m depth of 2.2%/day (Figure 2). This result is caused by the different environmental conditions. Seaweed in each water column requires different sunlight intensity to be used as an energy source for photosynthesis

process. Different photosynthesis leads to different seaweed ability in each water column to obtain nutrient that will affect seaweed growth. Moreover, relatively different water movement or ocean current in each water column also affects nutrient absorption from seawater to seaweed thallus that correlates with seaweed growth



**Fig 2.** Effect of depth on daily growth rate of *Kappaphycus alvarezii* seaweed

Compared to 4-5 m depth, seaweed at a depth of 0.2 m was more exposed to sunlight as an energy source for photosynthesis process. This correlates with thermal stratification. At a depth of 0.2 m, temperature range of 29.8-30.3°C, while at 5 m depth, the range of temperature was about 28.6-29.2°C. Hence, deeper water means lower temperature.

Moreover, compared with ocean current at 4-5 m depth that ranged of 0.3-0.6 m/s, there was stronger water movement at 0.2 m depth of 0.4-1.0 m/s. Besides cleaning particles attached to seaweed thallus, ocean current also contains nutrients that will be absorbed by seaweed. Optimal photosynthesis process and ocean current facilitate seaweed to obtain nutrient for its growth, resulted in high DGR. Therefore, transparency and current velocity become the determining factor in seaweed farming with verticulture method. Similar result was reported by [21] that extremely low sunlight intensity will hinder seaweed growth since photosynthesis process will not be completely performed. Nutrient absorbed in seaweed cells and the release of metabolic residue will be hindered if seaweed is grown too deep or in area with less water movement.

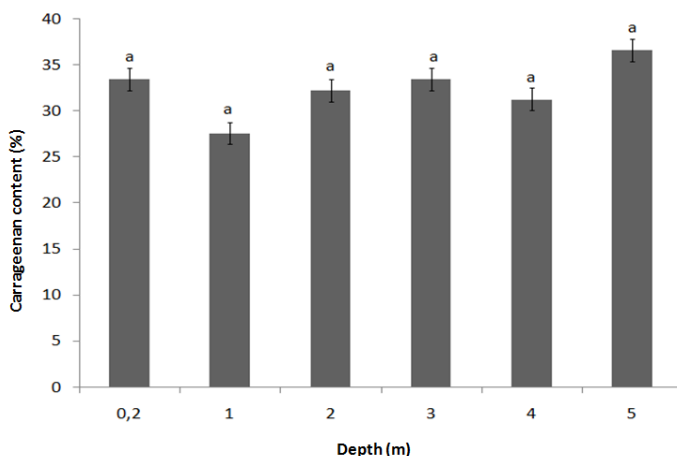
DGR of seaweed at 0.2-3 m depth in this study was categorized optimal since the growth value is higher than 3%/day [22]. This may be caused by the condition of waters that was quite fertile with concentration of nitrate of 0.15-0.46 mg/L, phosphate of 0.04-0.11 mg/L, and DOM of 29.40-64.44 mg/L (Table 1). According to [23], water that contains total organic matter above 26 mg/L is considered fertile.

Seaweed at 5 m depth was still able to grow well since water transparency in farming location was quite high, thus sunlight could penetrate into 14 m depth or around 50% of total depth of waters. Therefore, seaweed located close to the sea surface or up to 5 m depth were still reached by sunlight as energy source to perform photosynthesis. Water transparency influences sunlight penetration to the seawater. According to [24] and [25], transparency becomes an important factor that is related to the availability of sunlight for photosynthesis, thus seaweed farming should be performed in waters where sunlight is able to penetrate up to 5 m depth, at minimum. Turbid waters contain much fine particles that will attach to seaweed thallus and further inhibit food absorption and photosynthesis process.

Based on Figure 4, it is known that DGR of seaweed was not significantly different ( $p > 0.05$ ) at a depth of 0.2, 1, 2, and 3 m, but it was statistically different ( $p < 0.05$ ) at a depth of 4 and 5 m. It shows that seaweed cultivated using verticulture method in research location should be performed at 0.2-3 m depth to obtain optimal result.

### 3.3 Carrageenan Content

Seaweed cultivated through verticulture method contained quite fluctuated carrageenan content at each depth. *K<sub>2</sub> alvarezii* cultured at 5 m depth obtained the highest carrageenan content of 36.6%, while the lowest was obtained at 1 m depth of 27.6% (Figure 3). This finding is caused by environmental conditions such as salinity, phosphate, and DOM that obtained quite similar value in each water column (Table 1). Furthermore, it is also expected that seaweed at 5 m depth is protected from sunlight exposure that has a negative effect, i.e. UV radiation to carrageenan content of seaweed. According to [26], the quantity and quality of carrageenan produced from mariculture varied due to variety or species difference, age of planting, sunlight intensity, temperature, nutrients, and salinity.



**Fig 3.** Carrageenan content of *Kappaphycus alvarezii* seaweed

It is known that carrageenan content of seaweed was not significantly different ( $p > 0.05$ ) among the treatment of 0.2, 1, 2, 3, 4, and 5 m depth (Figure 3). It indicates that carrageenan content is not affected by depth, thus verticulture method can be applied as an alternative method to increase seaweed production in narrow area without affecting seaweed quality, carrageenan wise.

### 4 Conclusion

Depth affected the growth of *K. alvarezii* but it did not affect the carrageenan content of seaweed cultivated with verticulture method. This method can be applied as an alternative method to optimally increase seaweed production in narrow area without affecting seaweed quality.

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