Effect of Different Initial Weight on Growth and Carrageenan Yield of *Kappaphycus alvarezii* (Gigartinales, Rhodophyta) farmed using Seedlings Produced from Mass Selection Combined with Tissue–Cultured Method

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**Abstract.** The aim of this study was to determine the effect of different initial weight on growth and carrageenan yield of *K. alvarezii*. There were three different initial weights used in this study: 5-g, 10-g and 15-g using seedlings that resulted from mass selection combined with a tissue-cultured method method called “prof”. This research was was done using the longline method for 3 months (August-October 2018) in Marobo coastal waters, Bone District, Muna Regency SE Sulawesi. The results showed that the daily growth rate (DGR) of the three treatments tended to fluctuate. However, seedlings seedlings at 5-g initial weight showed highest DGR (5.72 ± 0.71%day⁻¹) followed by 10-g (4.84 ± 1.22%day⁻¹) and 15-g (4.25 ± 0.93 %day⁻¹). Ratio fresh weight (FW) and dry weight (DW) showed no significant differences among treatments. FW: DW ratio showed the 5 g had the highest ratio (9.85:1) followed by 10 g (8.49:1) and 15 g (7.88:1). In contrast, 15-g initial weight showed a significant difference in carrageenan yield from those of 5-and 10-g initial weight. 15-g initial weight showed the highest carrageenan yield (37.04 ± 1.03%) followed by 10 g (34.80 ± 1.45%) and 5 g (33.11 ± 2.64%). Epiphyte (*Sargassum polychyrum*) and ice-ice disease were found during this study. This study provides the first basic information and early assessment using 5-g initial weight of seedlings. Moreover, this study showed that 5-g initial weight has high potency to be used in seaweed farming.

1. **Introduction**

The red seaweed, *Kappaphycus alvarezii* locally known as "kottonii", has become a significant income-generating activity in Indonesia. This seaweed requires low investment, simple technology and is a potential source of income and employment in coastal areas [1-4]. This seaweed farming has high production because it is fast growing (within 45 days) and does not need greatly skilled cultivators. Thus, *Kappaphycus* seaweed has a clear advantage for the farmers [5].
In the last 3 years, total production of seaweed in Indonesia has decreased. Production figures 2015 to 2017 were; 11.27 million tons, 11.05 million tons and 10.46 million tons respectively [4]. This decrease is owing to the impoverishment of natural seaweed seedlings populations. Additionally, continuous vegetative propagation caused deteriorating quality in Kappaphycus seedlings; which lowered growth rates and increased the occurrence of ice–ice disease. To improve the productivity, a better quality of Kappaphycus seedlings is certainly needed.

One alternative method to improve the productivity is the tissue-culture method of seedlings. In recent years, this method has already been used in many countries, resulting in higher production that that using vegetatively-produced seedlings [6,7]. Furthermore, since 2013 [8], a new method which combines mass selection and tissue-cultured seedlings has been developed and this method produces better results than those from tissue-cultured seedlings.

Seedlings produced from the combination of these two methods are locally known as “prof”. They show different morphologies from tissue-cultured seedlings. Tissue-cultured seedlings have longer thalli than “prof” seedlings. Moreover, “prof” seedlings have more dense and irregular branches at primary and secondary branches (Figure 1).

![Figure 1. Morphological differences of seaweed seedlings. A, Tissue-cultured seedling; B, “prof” Seedling](image)

The “prof” seedlings are expected to produce higher growth than tissue-culture seedlings. Unfortunately, until now, no research was done to examine the growth using different initial weight of the seedlings. Initial weight is one parameter of seaweed culture used for gauging the high biomass production and efficiency of seedlings use. In India, 150 g seed material planted initially grows up to 500 to 1000 g (3.5 to 6.5 times) in 45 days [9], but when this seaweed was planted using 100-g initial weight, a 10-fold increase in weight was obtained [10]. It seems initial weight influences the production and efficiency of seaweed farming.

Many studies have already been done using different initial weights of vegetatively-produced seedlings. They used lighter sizes (around 50 g or less) [11, 12], or heavier sizes (around 100 g or more [9,13-16]. In contrast, seaweed farmers in Southeast (SE) Sulawesi always use lighter seedlings, around 10 g in weight [3,17].
There is no any study has been done for initial size of “prof” seedlings of less than 10 g. Therefore, we conducted a study, using “prof” seedlings, of how different initial weights affected the growth and carrageenan yield of *Kappaphycus alvarezii*.

2. Method

This study was done in Marobo coastal waters, Marobo District, Muna Regency, Southeast (SE) Sulawesi (50°8’9.06” S/122018’48.49” E). Analysis of carrageenan yield was conducted in the Analysis Laboratory of the Faculty of Fisheries and Marine Sciences (FFMS), Halu Oleo University, Kendari, Indonesia.

2.1 Seedling preparation

The healthier, younger, more densely branched “prof” thalli with no signs of ice-ice diseases were selected as seedlings for planting purposes. Three treatments of different initial weights of “prof” seedling were tested: (a) 5-, (b) 10-, and (c) 15-g. For each treatment, five replicates were made. Plants were cultured for 45 days. The “prof” seedlings of *K. alvarezii* used in this study were derived from previous study [8]. Before being used, all seedlings were cleaned from deposited silt, sediments or attached organisms.

2.2 Planting of seaweed seedlings

The prepared seedlings were then tied on the prepared rope, with a 10-cm distance between seedlings, using a hanging long-line method. Before planting, all the tied seedlings were soaked to prevent dehydration. In this study, all seedlings were cultivated. Before planting, all the tied seedlings should be immersed in the seawater to prevent desiccation.

2.3 Seaweed cultivation and maintenance

During the 45 days cultivation period, periodical weeding and maintenance were done at least twice a week in situ to ensure continuing optimum growth by checking for any damage such as rope deterioration, and cleaning the dirt, epiphyte or other organisms from the ropes and seaweed thalli.

2.4 Parameters observed

The parameters observed during the study were:

1. The daily growth rate (DGR) for all the treatments at 9-day intervals using the formula: 
   \[
   \text{DGR} = \frac{Wf/W0}{t}/(t-1) \times 100\%\ , \text{ where } W0 \text{ is the initial weight, } Wf \text{ is the final weight (g) after } t \text{ days} \left[18\right]. \text{ Data were expressed as mean } \pm \text{ SD for all the harvested treatments of the respective growth periods} \left[19\right].
   \]

2. Ratio of fresh weight to dry weight (FW: DW). The ratio was obtained after cleaning the harvested seaweed to remove sand and other attached organisms from each treatment, both in the field and in the laboratory. All freshly harvested seaweed were weighed (g). After drying for 2-3 days in open sunlight using a hanging method, the final dry weight (g) was measured. Ratio of fresh weight to dry weight was then calculated. The resulting data was expressed as mean ± SD for all treatments [20].

3. Analysis of carrageenan yield. In the laboratory, 5 g of dry seaweed from each treatment used as a sample was weighed and the samples washed in freshwater. Using aquadest, the samples were soaked for 12 hours, then sterilised in an autoclave for 30 minutes at 121°C. The samples were then smoothed in a blender and filtered with filter. After that, the samples were precipitated with 100 ml Iso-propanol. Finally, the samples were analyzed by drying in an oven for 24 hours to obtain carrageenan yield (%). The carrageenan yield (%) was determined according to the formula: Yield (%)= \( \frac{Wc}{Wm} \times 100\% \) where \( Wc \) is weight of carrageenan extract (g) and \( Wm \) is the dry seaweed weight (g) used for extraction [20]. The data were presented as mean± SD obtained from the three treatments.

4. Epiphytes and ice-ice disease found during the culture period were also recorded.
2.5 Data analysis
Statistical analyses were determined using ANOVA and the means were compared by a Tukey post hoc test. Subsequent analysis with Tukey's HSD test was computed when there were significant differences among treatment with level of significance $p<0.05$.

3. Results and Discussion

3.1 Daily Growth Rate (DGR)
The DGRs of “prof” seedlings using 5-g initial weight tended to be highest and were significantly different from those of the other two treatments (10-, and 15-g initial weight) (Figure 2, Table 1). The DGRs of “prof” using 5-g initial weight were $5.72 \pm 0.71\% \text{day}^{-1}$ followed by 10-g ($4.84 \pm 1.22\% \text{day}^{-1}$) and 15-g initial weight ($4.25 \pm 0.93\% \text{day}^{-1}$). In addition, the means final weight after 45 days in cultivation of 5-g initial weight were $68.92 \pm 7.37 \text{g}$, followed by 10-g ($107.02 \pm 12.92 \text{g}$) and 15-g initial weight ($140.30 \pm 8.64 \text{g}$). So the 5-g initial weight seedlings had a 13.78-fold increase in weight; which was greater than than 10-g initial weight seedlings (10.72-fold increase) and 15-g initial seedlings (9.35-fold increase). These findings of this study are higher than those observed in the Mexican study done by [21], which recorded a 10-fold increase.

These DGRs were comparatively higher than the other DGRs recorded elsewhere for similar Kappaphycus species: In Brazil, the DGRs were 4.07 and 5.12\%\text{day}^{-1} [22], in India 3.76 $\pm$ 0.07\%\text{day}^{-1} [23], in Madagascar 5.46 $\pm$ 0.09\%/day [24] and in Gorontalo, Indonesia 2.22-5.21\%/day [12]. In contrast, compared to tissue-cultured in this study were nearly comparable to those found by studies in Malaysia (6.3 $\pm$ 0.1%/day) [25] and in Philippines (5.8-7.2%/day) [26].

![Figure 2](image-url) Daily growth rates (DGRs) in of seaweed (K. alvarezii) of “prof” seedlings using different initial weight (5-, 10-, and 15-g)

| Days | Initial Weight (g) | DGR (%.day$^{-1}$) | Tukey test | Significance ($p<0.05$) |
|------|--------------------|--------------------|------------|------------------------|
| 9    | 5                  | 6.12 $\pm$ 1.43    | 6.12$^a$   | 0.003                  |
|      | 10                 | 4.31 $\pm$ 0.60    | 4.31$^a$   | 0.029                  |
|      | 15                 | 3.58 $\pm$ 0.62    | 3.59$^a$   | 0.468                  |
| 18   | 5                  | 6.48 $\pm$ 0.40    | 6.48$^b$   | 0.002                  |
|      | 10                 | 6.44 $\pm$ 0.41    | 6.43$^b$   | 0.003                  |
|      | 15                 | 5.34 $\pm$ 0.39    | 5.34$^a$   | 0.985                  |
Furthermore the DGRs found from this study were lower than those from a previous study done by [27] which recorded a DGR 6.27± 0.31%/day\(^1\). The research period (August-October) for the lower DGRs in the present study coincided with those observed at Okha, Gujarat [28]. The lower DGRs were mainly caused by biomass loss of seedlings due to the breakage of thalli. The breakages could be attributed to strong water motion, ice-ice disease and increased temperature and salinity. A high temperature (31.0°C) and salinity (31.0 ppt) seem to inhibit the growth of seedlings and to limit the chance for seedling survival. In addition, based on farmers’ experiences and personal observation in Marobo coastal waters, the productive season for seaweed \textit{K. alvarezii} was December-April whereas May-November is less productive. This is nearly similar to the productive season in Gorontalo, Indonesia, which occurs in November-April, while the non-productive season occurs May-October [12, 29]. The results indicated that the growth rates were mainly owing to the farming locations and seasonally prevailing environmental parameters at the site. Therefore, further need to be done in May to December to obtain more comprehensive data about DGRs.

### 3.2 Ratio of wet weight and dry weight

The ratio of fresh weight and dry weight (FW: DW) showed significant difference among treatments (Table 2). 5-g initial weight had the highest ratio of FW: DW. The ratio of 5- and 10-g initial weight were significantly different from 15-g initial weight. The ratios of FW: DW of 5-, 10-, and 15-g of initial weight were 9.85:1, 8.49:1 and 7.88:1, respectively. The ratio FW: DW of 5-g initial weight obtained during this study were comparatively very similar to a previous study done in Indian waters [19], in which they found (9.89 ± 0.13). Therefore, 5-g initial weight of seedlings shows very promising prospects and feasibility as a new initial seedling weight for commercial cultivation.

### Table 2. Tukey test of ratio of wet weight and dry weight of “prof” seedlings of \textit{K. alvarezii} using different initial weight (g).

| Initial weight (g) | W0 (g) | Fresh weight/FW (g) | Dry weight/DW (g) | FW:DW | Tukey Test | p Value |
|--------------------|--------|---------------------|-------------------|-------|------------|---------|
|                    | 1      | 2                   | 3                 | 4     | 5          | 6       | 7       |
| 5                  | 5      | 68.92 ± 8.24        | 7.00              | 9.85 : 1 | 10.44 \(^a\) | 0.040  |
| 10                 | 10     | 107.02 ± 14.44      | 12.60             | 8.49 : 1 | 8.48 \(^a\)  | 0.122  |
| 15                 | 15     | 140.30 ± 9.66       | 17.80             | 7.88 : 1 | 7.89 \(^a\)  | 0.798  |
| Mean               |        | 105.41 ± 10.78      | 12.47             | 1 : 8.74 |            |         |

*values followed by different letters are significantly different at \( p < 0.05 \)

### 3.3 Carrageenan yield
The highest carrageenan yield was obtained for the 15-g initial weight (40.7%), whereas the mean carrageenan yields for the 5-g initial weight were 33.11% and 34.81% for the 10-g initial weight. There were no significant differences found between the three treatments (Table 3).

Table 3. Carrageenan Yield of Seaweed (*K. alvarezii*) of “prof” seedlings using different initial weight (g).

| Initial Weight (g) | Tukey Test       | Significant (p<0.05) |
|--------------------|------------------|----------------------|
| 5                  | 33.11± 2.64      | 0.064                |
| 10                 | 34.81± 1.45      | 0.268                |
| 15                 | 37.04± 1.03      | 0.532                |

*values followed by different letters are significantly different at p<0.05

The carrageenan yields obtained for this seaweed are higher than those reported from previous studies done in India (24.52-31.10 %) [23] and 33.30-38.50 % [30] and these yields are comparable with those reported from other countries. The yields in Brazil were 31-43% [31] and 35.3-46.1 % [32], while in Mexico they were 30.3-40.7% [10]. Differences in carrageenan yield from different countries appear to be dependent on environmental parameters such as culture site, season of harvest time. Therefore, to obtain more detailed information regarding the carrageenan yield using different initial weight of seedlings, further studies are really needed.

3.4 Epiphyte and Ice-ice Disease

Epiphyte and disease were found during this study (Figure 3). Biofouling by epiphyte on seaweed thalli was *Sargassum polychystum* while the ice-ice disease was attached to the tip of the seaweed thalli. *Ice-ice* was mostly found on the 18th and the 36th days of the culture period. These outbreaks of epiphyte and ice-ice occurred in August-October and they appeared to correspond with increasing seawater temperature [24]. However, the reasons for this apparent correlation need to be clarified. The epiphyte was more common and simultaneously occurred with Ice-ice disease.

![Figure 3. Epiphyte and Ice-ice diseases found during the study. A) The epiphyte, *S. polychystum*; B) Ice-ice disease on the seaweed thallus](image)

4. Conclusion

This study provides the first basic information and early assessment of using 5-g initial weight of seedlings. The growth and carrageenan yield using 5-g initial weight were within the commercial requirement desirable for commercial cultivation. Therefore, planting of 5-g initial
weight could optimize seaweed production and could develop sustainable seaweed culture not only in Marobo coastal waters but also in Indonesia and in other countries.

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