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## Research Article New Methods on Cultivation of *Eucheuma denticulatum* and *Kappahycus alvarezii* in Indonesia

<sup>1</sup>Ma'ruf Kasim, <sup>1</sup>Ahmad Mustafa, <sup>1</sup>Idul Male, <sup>2</sup>Muzuni and <sup>3</sup>Wardha Jalil

## **Abstract**

**Background and Objective:** Eucheumatoid cultivation is increasing and a variety of methods exist that can increase production. However, new cultivation approaches are rarely reported. The objective of study was to examine a new method of seaweed cultivation: The floating cage. **Materials and Methods:** The growth rate of *Eucheuma denticulatum* and *Kappahycus alvarezii* was assessed in floating cages from March-November, 2015 and compared with that in the traditional longline approach. Propagule wet weight was measured daily to document growth rates. Correlation coefficients between growth rate and environmental factors were calculated using simple linear models (Pearson's) and statistically analyzed by SPSS Version 24. **Results:** Growth rates of both species in floating cages were faster than on longline and thallus morphology was better. For *E. denticulatum*, the daily specific growth rate (SGR) in floating cages varied from a low of 2.68% to a high of 3.32%, but the respective rates on longline were 1.67 and 2.91%. For *K. alvarezii* cultivated in floating cages, the highest and lowest rates were 3.1 and 2.1% but, when cultivated on longline, the respective rates were 2.9 and 1.71%. **Conclusion:** Therefore, it was concluded that cultivation of *E. denticulatum* and *K. alvarezii* using floating cages resulted in higher growth rates compared to cultivation on longline. The difference in results comes from reduced herbivore attack when the plants are in the protective cages.

Key words: Floating cage, seaweeds, herbivorous attack, growth rate, production, cultivation, methods

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Corresponding Author: Ma'ruf Kasim, Faculty of Fishery and Marine Sciences, Halu Oleo University, Andounohu Kendari City, Southeast Sulawesi, Indonesia Tel: +624013193620

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

<sup>&</sup>lt;sup>1</sup>Faculty of Fishery and Marine Sciences, Halu Oleo University, Andounohu Kendari City, Southeast Sulawesi, Indonesia

<sup>&</sup>lt;sup>2</sup>Faculty of Mathematic and Natural Science, Halu Oleo University, Andounohu Kendari City, Southeast Sulawesi, Indonesia

<sup>&</sup>lt;sup>3</sup>Faculty of Fisheries, Dayanulksanuddin University, Baubau City, Southeast Sulawesi, Indonesia

### **INTRODUCTION**

Eucheuma cultivation continues to expand, with the most commonly cultivated types being Kappahycus alvarezii, Eucheuma striatum and Eucheuma denticulatum. Commercial cultivation of K. alvarezii began in the Philippines around 1960<sup>1-3</sup>. Eucheuma cultivation continues to increase in scale and several countries such as India, Japan, Indonesia, Tanzania, Fiji, Hawaii and South Africa are high producers4. The methods developed in these different countries vary depending on the community's ability to use specialized cultivation equipment. Fiji developed a raft method for K. alvarezii cultivation in 1970<sup>5,6</sup>, while Tuvalu<sup>7</sup> and Malaysia<sup>2</sup> developed raft approaches between 1977 and 1978. Indonesia began to develop raft methods in 1985, but switched to longline (tie-tie)8-11. Maldives, India and Tanzania began to develop cultivation in the 1980s<sup>12-15</sup> and some countries such as Vietnam, Brazil and Venezuela began to develop various methods as late as 1990<sup>16-18</sup>. Longline, known as tie-tie in the Philippines, is the most common method used to cultivate K. alvarezii and Eucheuma sp. 19,20. Longline binds the seaweed together to improve growth. This method is also applied in Indonesia, India, Malaysia, Tanzania, Vietnam, Brazil, Kenya and Madagascar<sup>6,11,17,21-25</sup>. Some countries also developed a similar method, in which the bound seaweeds grow on the surface of the sea. A disadvantage of longline cultivation is herbivorous grazing on the seaweed<sup>12</sup> and Eucheumatoid seaweeds are highly preferred by herbivores.

Grazing by herbivores, together with problems of pests and ice-ice disease, can reduce the production of *K. alvarezii* by 60%<sup>11,24,25,26</sup>. Such crop losses prompted several countries to begin developing methods that protect seaweed from herbivorous fish. However, protective culture approaches are still rarely used by farmers. Philippines began using cages made from net and bamboo<sup>27</sup> but only for small-scale production. In Brazil, farmers are developing a floating raft system with a protective tubular net that showed an improvement over the tie technique during a study across one cultivation cycle<sup>4</sup>. In Indonesia, seaweed cultivation methods have not been altered. The present study reports the daily growth rates of *K. alvarezii* and *E. denticulatum* cultivated using a new method, the floating cage.

### **MATERIALS AND METHODS**

This study was conducted in the coastal water cultivation area Lakeba, Bau-Bau, Southeast Sulawesi Province, Indonesia from March-November, 2015. The floating cage is a new method for cultivation of *Eucheuma* in Indonesia. The cages are boxes (100×200×60 cm) constructed using PVC pipe. The boxes were wrapped with multifilament nets with a mesh size of 1 cm, but the top of the cage was unwrapped to allow access to the plants (Fig. 1). Thus, the cages were designed to be submerged to a depth of 50 cm<sup>11,26</sup>, but with a small region exposed at the water's surface. Propagules of *K. alvarezii* and *E. denticulatum* were scattered in the cage.

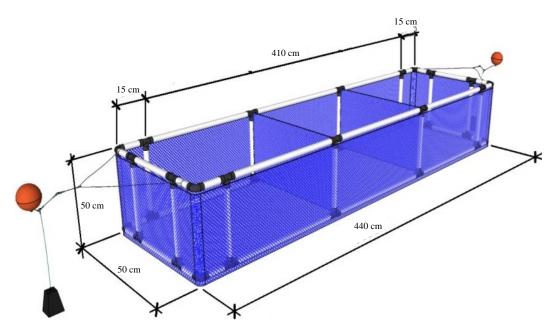


Fig. 1: Floating cage method used during the study

The specific growth rate (SGR) was calculated by daily measurement of propagule wet weight in each cage over a period of 90 days. The formula of Luhan and Sollesta<sup>28</sup> was used:

$$SGR (\%) = \frac{\ln Wt/\ln Wo}{t} \times 100$$

where, SGR is the specific growth rate (% in wet weight perday), Wt is the weight after t days, Wo is the initial weight and t is the time in days.

The wet weight of *K. alvarezii* and *E. denticulatum* was entered as Mean±Standard Deviation (SD). Total nitrogen and phosphorus were determined using the spectrophotometric methods described by Strickland and Parson<sup>29</sup>.

**Statistical analysis:** Statistical analyses were performed using the SPSS package 16. Fresh weight of *K. alvarezii* was entered as Mean±Standard Deviation. Correlation coefficients between growth rate and environmental factors were calculated using simple linear models (Pearson's analysis)<sup>30</sup>. Statistical analyses were performed using the SPSS Package 24 with probability level (p>0.05).

### **RESULTS**

**Growth rate comparison:** Growth of *E. denticulatum* and of *K. alvarezii* in floating cages and on longline varied from May-November. Propagule morphology of *E. denticulatum* and *K. alvarezii* in floating cages was better than when cultivated on longline (Fig. 2).

Fresh weights of *E. denticulatum* cultivated in floating cages for 40 and 50 days were 74 and 78 kg, respectively, but on a longline, the fresh weights were 63 and 70 kg, respectively (Fig. 3). Likewise, the daily growth rates of *K. alvarezii* in floating cage were different from those on longline. The fresh weight of *K. alvarezii* in the floating cages after 50 and 60 days were 75.0 and 73.3 kg, respectively (Fig. 4). Taken together, the results showed that the floating cage method yields a higher wet weight than does the longline approach.

The growth rate of both seaweeds in floating cages and on longline differed. For floating cages, the average growth of *E. denticulatum* propagules after 45 days in August, September and October was 97, 73.3 and 47.5 g, respectively (Fig. 5). The average growth of *K. alvarezii* during those same months was, respectively, 61.8, 45.5 and 40 kg (Fig. 6).



Fig. 2: Propagules appearance of K. alvarezii in (a) Floating cage and (b) longline

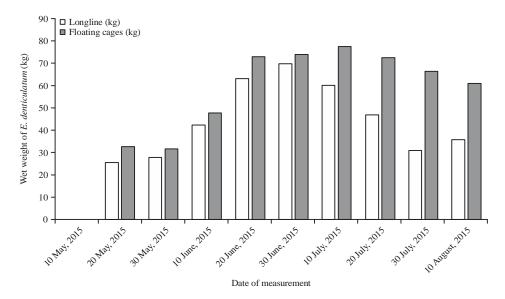


Fig. 3: Different production of *E. denticulatum* cultivated in floating cage and longline during 90 days

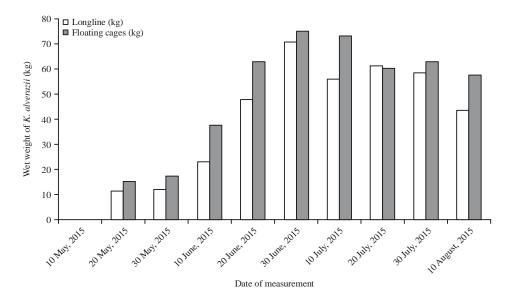


Fig. 4: Different production of K. alvarezii cultivated in floating cage and longline during 90 days

The SGR of *E. denticulatum* cultivated on longline for 45 days decreased in July (1.67%/day) but increased in August (2.91%/day) (Fig. 7). However, a difference was noted when using a floating cage. The SGRs were the lowest in April (2.68%/day) and the highest in June (3.32%/day) (Fig. 8).

The SGR for *K. alvarezii* cultivated on longline was the highest in July (2.9%/day) and the lowest in April (1.71%/day) over the cultivation period of 45 days (Fig. 9), but the rates when cultivated in floating cages were the highest in July (3.1%/day) and the lowest in April (2.1%/day) (Fig. 10). A positive correlation was found

between seaweed growth and the level of nitrate and phosphate, particularly between June and September (Fig. 11, 12).

**Environmental parameters:** During the study, the temperature in the morning was 25-27°C and in the afternoon, the temperature ranged 27-32°C. The current speed typically ranged from 0.01-0.167 m sec<sup>-1</sup> and the highest current velocity was seen in July and August (0.163 and 0.167 m sec<sup>-1</sup>, respectively). Salinity ranged from 30-32%.

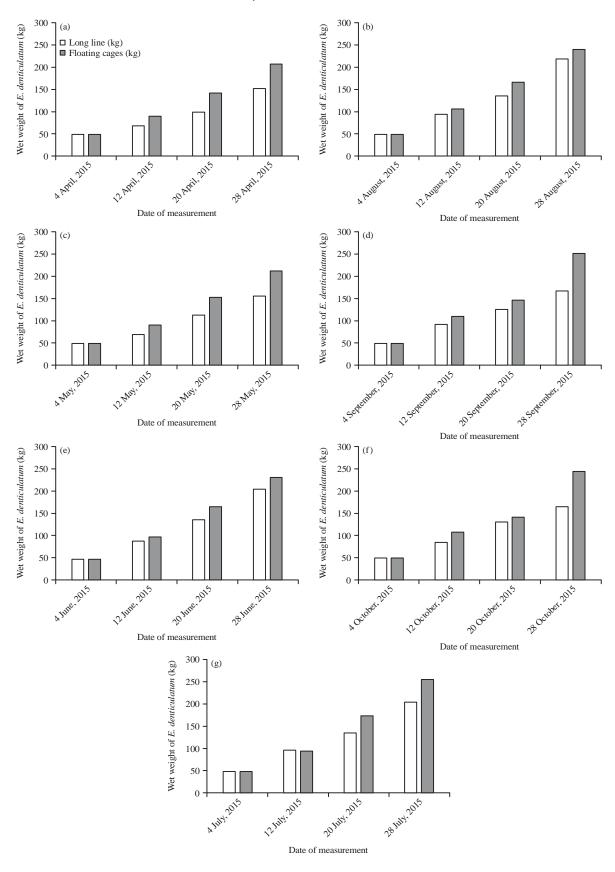


Fig. 5(a-g): Monthly growth rates of E. denticulatum during 35 days cultivation periods in Floating cage and longline

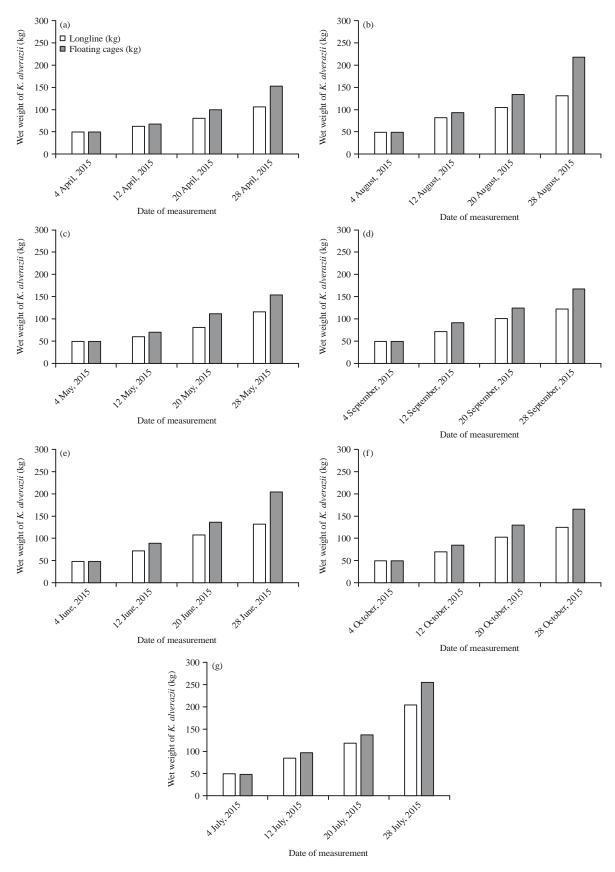


Fig. 6(a-g): Monthly growth rates of K. alvarezii during 35 days cultivation periods in floating cage and longline

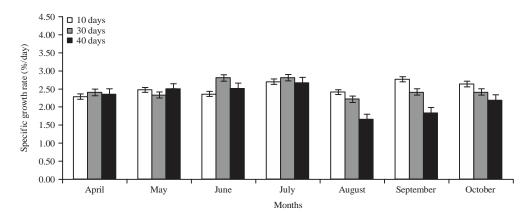


Fig. 7: Specific growth rate (%/day) of *E. denticulatum* cultured using longline (error bars as standard deviation)

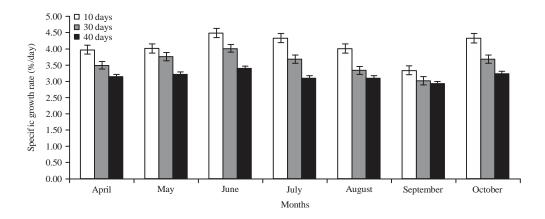


Fig. 8: Specific growth rate (%/day) of E. denticulatum cultured using floating cage (error bars as standard deviation)

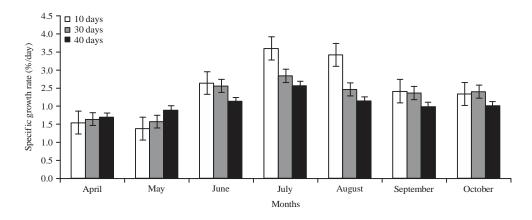


Fig. 9: Specific growth rate (%/day) of K. alvarezii cultured using Longline (error bars as standard deviation)

Nitrate concentration reached a high of 0.838 mg  $L^{-1}$  in June, followed by a continuous decrease to 0.0136 mg  $L^{-1}$  in October. Phosphate concentration in May, July and August was 0.0099, 0.0086 and 0.0085 mg  $L^{-1}$ , respectively.

A negative correlation was found between growth rate of *K. alvarezii* and temperature, even though positively correlated with salinity, nitrate and phosphate. Nevertheless, a positive correlation existed between SGR and environmental factors, particularly current velocity (p>0.05) (Table 1).

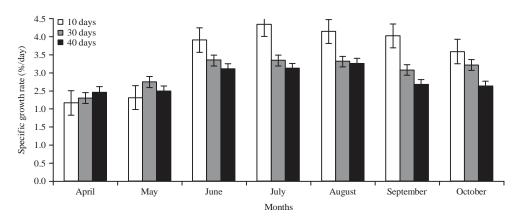


Fig. 10: Specific growth rate (%/day) of *K. alvarezii* cultured using floating cage (error bars as standard deviation)

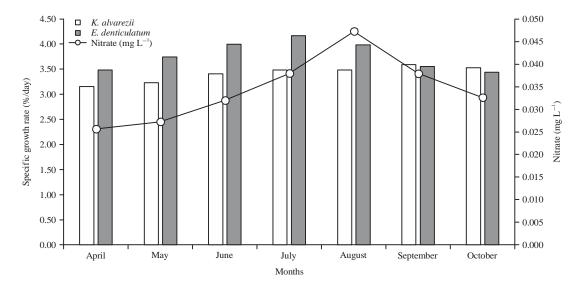


Fig. 11: Correlation between SGR of K. alvarezii and E. denticulatum with Nitrate

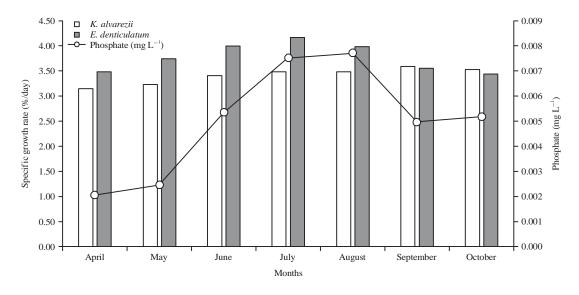


Fig. 12: Correlation between SGR of K. alvarezii and E. denticulatum with Phosphate

Table 1: Correlation coefficient between SGR of K. alvarezii and environmental factors

	Growth rate at	Temperature	Current	Salinity	Nitrate	Phosphate
Growth rate at Longline (%/day)	floating cage (%/day)	(°C)	velocity (m sec <sup>-1</sup> )	(%)	$(mg L^{-1})$	$(mg L^{-1})$
Growth rate at Longline (%/day)	0.779*	-0.021	0.503	0.327	0.317	0.307
Growth rate at Floating cage (%/day)		-0.152	0.807*	0.326	0.692	0.404
Temperature (°C)			0.031	-0.785*	-0.107	0.445
Current velocity (m sec $^{-1}$ )				0.192	0.421	0.410
Salinity (%)					0.226	0.017
Nitrate (mg $L^{-1}$ )						0.673
Phosphate (mg L <sup>-1</sup> )						

<sup>\*</sup>Correlation is significant at the 0.05 level

### **DISCUSSION**

The difference in growth of Eucheuma between floating cages and longline was less prominent over 40-50 days in April-July when herbivorous fish were not present. However, a substantial decrease in growth was seen in E. denticulatum on days 50-70 on longline. On days 50 and 70, wet weights of E. denticulatum reached 76 and 36 kg, respectively. These results differed from those for the floating cage, in which the weights were 74 and 61 kg, respectively. Growth of both E. denticulatum and K. alvarezii decreased after 50 days. The main reason for loss in wet weight on longline was that propagules were damaged and carried away by the current and some were grazed by herbivorous fish. In contrast, cultivation in floating cages can protect seaweeds from pests and strong current. Broken propagules will remain in the net. However, after 50 days of growth, Eucheuma biomass became very dense and covered almost the entire cage. Such conditions resulted in poor growth and led to a decrease in weight. This suggests that the cultivation period of Eucheuma in most sites in the world should not exceed 50 days. Optimum production occurs between 40 and 50 days. The average growth rate of K. alvarezii and E. denticulatum in August decreased drastically when the number of herbivores was high around the cultivation area. Production of *K. alvarezii* in Igang, Guimaras, Philippines, during February and March was 862 and 575 g m<sup>-1</sup> per line. The growth rate of *K. alvarezii* increased during April and May to reach 1877 and 2237 g m<sup>-1</sup> per line, an effect attributable to the protection from herbivorous fishes provided by the cages. Furthermore, April and May are good growing periods for K. alvarezii<sup>27</sup>. In Bongao, Southern Philippines, K. alvarezii growth rate can reach up to 300% of the biomass after cultivation for 4-7 weeks<sup>31</sup>. In Vietnam, K. alvarezii cultivated by longline method at a depth of 0.5-1 m during January-August showed a daily average growth from 6.14-6.26%, respectively and the growth rate increased from May-June in the range<sup>32</sup> of 9.14-10.8%/day. In Ubatuba Bay, Sao Paulo, Brazil, K. alvarezii cultured with the monoline method grew well on the water surface and at a depth of 0.5 m. The average growth rate ranged from 5.2-7.2%/day for a cultivation period of 28 days.

However, the growth was noted to decline when the cultivation was continued for 59 days<sup>22</sup>. In Madagascar, the highest growth rate of *E. denticulatum* was recorded in April and February, while in March, the growth rate decreased due to herbivore grazing and ice-ice disease. An SGR of 2.2%/day was recorded at each planting season<sup>21</sup>. In Yucatan State, Mexico, the growth rate of Eucheuma isiforme increased over 25 days to 2.21%/day. Herbivorous pests and ice-ice disease greatly affect Eucheuma production<sup>33</sup>. At Vizhinjam village, Kerala, India, the growth and production of K. alvarezii in shallow waters during March and May were high during 45-60 days of cultivation at 24 and 36 kg, respectively<sup>23</sup>. In the same study in 2014, it was found that growth increased using the longline method when no herbivorous fish are present in the cultivation area. The use of floating cages in the present study protected K. alvarezii and E. denticulatum from herbivore attacks and growth was good at all times, even though many herbivorous fish was present in the experimental sites. Increasing growth throughout the year is an indication of the absence of pest attacks on propagules of K. alvarezii and E. denticulatum<sup>11,26</sup>.

In the present study, environmental factors also affected the growth of *E. denticulatum* and *K. alvarezii*. Growth was good during June and July, when the temperature ranged from 26-29°C, salinity ranged from 30-31% and current velocity was 0.163-0.177 m sec<sup>-1</sup> compared with the other months. During June-July, the nitrate concentration ranged from 0.564-0.0838 mg L<sup>-1</sup> and phosphate ranged from 0.0084-0.0086 mg L<sup>-1</sup>. Good *K. alvarezii* growth in Vietnam correlated with the following period and environmental conditions: May, with a temperature range of 27.2-30°C and salinity range of 31.4-34%<sup>16</sup>. In the Philippines, a good temperature for the growth of *K. alvarezii* is 28-30°C and salinity of 33-35%<sup>34</sup>.

The presence of many herbivores at cultivation areas of *K. alvarezii* and *E. denticulatum* can have a severe negative impact. The best way to overcome this is by using a method that can protect both *K. alvarezii* and *E. denticulatum* against the existing herbivorous fish population. The floating cage method is currently the best solution to avoid grazing and thereby improve production.

### **CONCLUSION**

Herbivorous fishes were able to graze on *K. alvarezii* and *E. denticulatum* when these seaweeds were cultivated by longline methods. The floating cage method protected the seaweeds from the various herbivores. In the present study, the production and growth rate of *K. alvarezii* and *E. denticulatum* cultivated in floating cages were much higher than that on longline, especially in August when many herbivorous fishes are present in the cultivation area.

### **SIGNIFICANCE STATEMENTS**

This study clarifies the utilization of new methods in seaweed cultivation. Floating cage is a new method that successfully avoids grazing fish and herbivorous animals against cultivated seaweed. The use of floating cage will be possible to increase the production of seaweed, especially *Kappaphycus alvarezii* and *Eucheuma denticulatum*.

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