Weight gain and carrageenan content of *Kappaphycus alvarezi* (Rhodophyta, *Solierisceae*) polycultured with *Sargassum polycystum* (Paeophyta, Sargassaceae)

R Syamsuddin\(^1\), Abustang\(^1\), Ruslaini\(^2\), A Tuwo\(^1\) and N R Aswar\(^1\)

\(^1\) Center of Excellence for Development and Utilization of Seaweed, Universitas Hasanuddin (PUI-P2R-UNHAS), Makassar, Indonesia

\(^2\) Faculty of Animal Husbandry and Fisheries, Universitas Tadulako, Palu, Indonesia

Email : rajuddin_syamsuddin@yahoo.com

**Abstract.** The objectives of this study were to analyse the weight gain and carrageenan content of the seaweed *Kappaphycus alvarezi* polycultured with *Sargassum polycystum* seed. The study was conducted in the coastal waters of Aeng Batu-Batu Village, Takalar Regency, South Sulawesi, Indonesia from July 13, 2016 to September 7, 2016. A Complete Randomized Design (CRD) was applied with 4 treatments and 3 replicates. The treatments were a control (*K. alvarezi* with no *S. polycystum*); the combination of 25 g *K. alvarezi* with 25 g *S. polycystum*; 25 g *K. alvarezi* with 30 g *S. polycystum*; and 25 g *K. alvarezi* with 35 g *S. polycystum*. High weight gain and carrageenan content of *K. alvarezi* in the presence of *S. polycystum* could be due to the shading effect of thallus morphology and the chemical content of *S. polycystum* that might protect the thallus of *K. alvarezi* from herbivorous animals, biofouling, and UV radiation. The antibiotic compounds, hormones, and phlorotannins content of *S. polycystum* may synergistically protect the red seaweed from ice-ice bacteria while promoting growth and carrageenan synthesis.

1. Introduction.

Seaweed culture in the intertidal zone has developed significantly in Indonesia. *Eucheuma cottonii* (*Kappaphycus alvarezi*) has been cultured in waters across the Indonesian Archipelago since it was first introduced [1]. Seaweed farming is a viable alternative source of income for small-scale fishermen and coastal communities. The red alga *K. alvarezi* is an important source of raw material for carrageenan (a colloidal substance used as gelling agent, stabilizer or emulsifier in food, cosmetics and other products). This species is generally cultured in shallow waters at a depth of about 10 meters, mostly using a longline technique [2].

Several environmental problems such as predation by herbivores, the disease *ice-ice*, and solar radiation can affect the growth, production, and carrageenan content of *K. alvarezi*. One environmentally friendly way to attempt to address these problems is to use a biological approach. The brown alga *Sargassum*, which is also widely distributed throughout the coastal waters of tropical and temperate countries, has a high commercial value. This alga could potentially be cultivated and processed for alginate production, providing a source income for coastal communities and for the country.

The brown alga *Sargassum* contains phlorotannins, a family of compounds which have secondary roles as chemical defences such as herbivore deterrents, antibacterial agents, and UV screens. This
algae could be polycultured with *K. alvarezii*. The objectives of the study were to analyse the influence of the presence of *S. polycystum* on the weight gain and carrageenan content of *K. alvarezii* when grown in a mixed culture (polyculture).

2. Materials and method

2.1. Study Site and Time.
The study was conducted in the waters of Jonggoa, Batu-batu Village, North Galesong District, Takalar Regency, 15 miles south of Makassar City in South Sulawesi Province, Indonesia. The study was conducted from July 13 to September 7, 2016. The seaweeds *K. alvarezii* and *Sargassum polycystum* were grown in polyculture with an intercropping pattern for 6 weeks. Carrageenan and alginate content of *K. alvarezii* and *Sargassum polycystum*, respectively were analysed at the Water Quality Laboratory of the Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar, Indonesia.

2.2. Culture Experiment.
*Kappaphycus alvarezii* and *Sargassum polycystum* (Figure 1) seeds were obtained locally in the study area. The long-line culture method was used. Depend on the treatments, 25 g *K. alvarezii* seeds were tied to the long line ropes, alternating with 25, 30, or 35 g *S. polycystum* seeds, or without *S. polycystum*. The long line ropes were tied to a bamboo raft (Figure 2).

2.3. Treatments and Experimental Design.
Randomized Complete Design (CRD) was applied with the following 4 (four) treatments, each with three replicates:

- 25 g *K. alvarezii* + 25 g *S. polycystum*
- 25 g *K. alvarezii* + 30 g *S. polycystum*
- 25 g *K. alvarezii* + 35 g *S. polycystum*

![Figure 1. Kappaphycus alvarezii (A) and Sargassum polycystum (B)](image)

![Figure 2. From frontage view of long line method applied](image)
25 g \( K. \text{alvarezii} + 0 \) g \( S. \text{polycystum} \) (control)

2.4. Biological Parameters.

Weight gain of \( K. \text{alvarezii} \) and \( S. \text{polycystum} \) was computed with the following formula:

\[
W = Wt - Wo
\]

\( W \) = weight gain (g)
\( Wt \) = final weight of seaweed (g)
\( Wo \) = initial weight of seaweed (g)

Carrageenan content of \( Kappaphycus \text{alvarezii} \) was computed based on the formula of [3,4,5] as follows:

\[
YC = WC.Wdw^{-1}.100;
\]

\( YC \) = carrageenan content (%)
\( WC \) = weight of carrageenan extract (g)
\( Wdw \) = dry weight of analysed thallus (g)

2.5. Analysis.

The weight gain data was statistically analysed using a Analysis of Variance (ANOVA) followed by a W-Tukey test when significant differences were found (at the 95% confidence level). Carrageenan content was analysed descriptively, supported by references.

3. Results.

Weight gain of \( K. \text{alvarezii} \) was significantly different with different initial weights of \( S. \text{polycystum} \) seeds. Growth of \( K. \text{alvarezii} \) was inversely proportional to the initial weight of \( S. \text{polycystum} \) seed (Figure 3). The highest weight gain of \( K. \text{alvarezii} \) (1387.33±48.09g) occurred without \( S. \text{polycystum} \) seeds (0 g), followed by with 25 g \( S. \text{polycystum} \) seeds.

3.3. Analysis

The weight gain ranges of \( K. \text{alvarezii} \) in this experiment were higher compared to the range of 186.23-340.80 g [6] and 114 – 192 g [7] reported from the monoculture of \( K. \text{alvarezii} \) at the same location.

The carrageenan content of \( K. \text{alvarezii} \) cultured singly (without \( S. \text{polycystum} \) seed) and polycultured with lowest initial size (25 g) of \( S. \text{polycystum} \) were relatively similar, at 33.70% and 34.27% respectively. These figures were lower compared to the values of 37.57% and 43.98% obtained when \( K. \text{alvarezii} \) was polycultured with 30 to 35 g \( S. \text{polycystum} \) seed (Figure 3).
Figure 4. Carrageenan content of *K. alvarezii* polycultured with *S. polycystum* at different initial *S. polycystum* seed weights

Recorded carrageenan content of *K. alvarezii* in this study when *K. alvarezii* seed with initial weight of 25 g was polycultured with 30 g of *S. polycystum* was 43.98%; this value meets the FAO standard (suitable) of 40% carrageenan content for export (Doty, 1985). Carrageenan content obtained from *K. alvarezii* mixed with *S. polycystum* seed of 25 -35 g initial weight (34.27 – 43.98%) was comparable to the 31-43% carrageenan content of *K. alvarezii* in [3] and relatively higher than other previous studies that obtained 40.7% from a green strain (Munoz et.al., 2004), 14.73 – 30.79%, for SRC (semi refined carrageenan) and 25 – 28% for RC (refined carrageenan) [2], all of which used monocultured *K. alvarezii*.

4. Discussion

The high weight gain of *K. alvarezii* when it was grown singly and when it was mix-cultured with smallest (25 g) *S. polycystum* seed could be related to better water motion between the plants, since water motion influences nutrient uptake kinetics and is an environmental factor regulating the growth of algae [8]. Water motion is reported as accounting for 81– 98% of the variation in weight gain of *K. alvarezii* [9], and compensating for a decline of nutrients in the growth media [10].

Higher growth rates of *K. alvarezii* in this polycultured system compared to the same species when previously cultured as a monoculture by several researchers could be due to the shading effect of the leafy morphology of *S. polycystum*. This morphology might provide some protection to the thallus of *K. alvarezii* from predation by herbivorous fishes and from biofouling (Figure 5).

In addition, the phlorotannins content of brown algae *S. polycystum* [11], which was exist in soluble form [12] and can form a complex with alginic acid present in the cell wall [13], could become diffused into the surrounding water by passing through the cell wall [14, 15, 16, 17, 18]. These compounds could rapidly react with both proteinaceous and carbohydrate substances of the seaweed thallus [19] and then be absorbed by the *K. alvarezii* thallus.
Figure 5. *S. polycystum* thallus covering *K. alvarezii* thallus

These compounds have secondary roles as chemical defences (herbivore deterents, antibacterial agents, and UV screens) [19, 11, 20, 21] which might drive the rabbit fish and other herbivores out from the lush canopy of *K. alvarezii*, protect the thallus from UV radiation [11, 13, 22], help the plant to avoid bacterial infection [21, 13]. In addition to the compounds may help protect the algae against predators and epiphytes [23], acting as antifouling substances [24], and even help protect the red algae from stress conditions [21]. All these functions could promote higher weight of the red seaweed species. Together with the antibiotics and hormones contained in the brown seaweed thallus, it is likely that these compounds synergistically protected the red seaweed from ice-ice bacteria and promoted growth.

Influence of the thallus morphology on carrageenan content might be related to effects on access to resources [25], such as nutrient uptake from the water column (“functional form”, surface/volume ratios) [26]. Higher carrageenan content of *K. alvarezii* mix-cultured with larger *S. polycystum* seed (30 – 35 g) compared to the lower (0 – 25 g) *S. polycystum* seed, as well as higher content compared to monocultured seaweeds of this species in several previous studies, could also be related to the shading effect of thallus morphology and the effects of the chemical content of *S. polycystum* on *K. alvarezii*. Carrageenan content is known to be influenced by temperature [27, 28] and light intensity [28]. When the thallus of the seaweed is directly exposed to UV, chlorophyll and other cell components can be damaged or destroyed. The leafy morphology of *S. polycystum* may shade the thallus of *K. alvarezii* from high (above optimum) temperatures and light intensity and from the negative effect of ultra violet (UV) radiation that falls on the water surface.

Acknowledgements
The authors gratefully thank the support from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through the World Class Professor program (no. 168.A10/D2/KP/2017).

References
[1] Adnan H and Porse H 1987 Culture of *Eucheuma cottonii* and *Eucheuma spinosum* in Indonesia *Hydrobiologia*. 151/152 355-358

[2] Wenno P A, Syamsuddin R, Zainuddin E N and Rappe R A 2015 Cultivation of red seaweed *Kappaphycus alvarezii* (Doty) at different depths in South Sulawesi, Indonesia *AACL Bioflux*
[3] Hayashi L, Paula E J D and Chow F 2007 Growth rate and carrageenan analyses in four strains of Kappaphycus alvarezii (Rhodophyta, Gigartinales) farmed in the subtropical water of Sao Paulo State, Brazil J. Appl. Phycol. 19 393-399

[4] Munoz J, Freile-Pelegrin Y and Robledo D 2004 Mariculture of K. alvarezii (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatan, Mexico Aquaculture 239 161-171

[5] Hung L D, Hori K, Nang H Q, Kha T and Hoa L T 2009 Seasonal changes in growth rate, carrageenan yield and lectin content in the red alga Kappaphycus alvarezii cultivated in Camranh Bay, Vietnam J. Appl. Phycol. 21 265-272

[6] Wenn P A 2014 Analysis of Growth and Carrageenan Quality of Red Alga Kappaphycus alvarezii (Doty) Cultivated in Deeper Seawater (Indonesia: Ph.D. Dissertation, Graduate Program, Hasanuddin University) 89p

[7] Alimuddin 2011 Growth and Carrageenan Content of Seaweed Kappaphycus alvarezii With Various Method on the Seagrass Ecosystem (Indonesia: TESIS Graduate Program, Faculty of Marine Science and Fisheries, Hasanuddin University) 54p

[8] Harrison P J and Hurd C L 2001 Nutrient physiology of seaweeds: Application of concepts to aquaculture Cah. Biol. Mar. 42 71-82

[9] Glenn E P and Doty M S 1992 Water motion affects the weight gains of Kappaphycus alvarezii and related seaweeds Aquaculture 108 233 – 246

[10] Kotiya A S, Gunalan B, Parmar H V, Jaikumar M, et al 2011 Growth comparison of the seaweed Kappaphycus alvarezii in nine different coastal areas of Gujarat coast, India Adv. Appl. Sci. Res. 2 99-106

[11] Hwang E K, Ay-Lin R L, Tsai C and Lee T 2004 Assessment of Temperature and Nutrient Limitation on Seasonal Dynamics Among Species of Sargassum From a Coral Reef in Southern Taiwan J. Phycol. 40 463–473

[12] Maina M H 2014 Structural Investigation of the Natural Products Composition of Selected South African Seaweeds (South Africa: Ph.D Dissertation, Department of Chemistry, Faculty of Natural Sciences University of the Western Cape) 282 p

[13] Singh J P and Sidana J 2014 5–Phlorotamins (India: National Institute of Pharmaceutical Education and Research (NIPER), India) (https://doi.org/10.1533/9780857098689.1.181)

[14] Carlson D J and Maye L M 1983 Relative influences of riverine and macroalgal phenolic materials on UV absorbance in temperate coastal waters Can. J. Fish. Aquat. Sci. 40 1258-1263

[15] Carlson D J and Carlson M L 1984 Reassessment of exudation by fucoid macroalgae Limnol. Oceanogr. 29 1077-1087

[16] Schoenwaelder M E A and Clayton M N 1998 Secretion of phenolic substances into the zygote wall and cell plate in embryos of Hormosira and Acrocarpia (Fucales, Phaeophyceae) J. Phycol. 34 969-980

[17] Arnold, T. M., and N. M. Targett. 2002. Marine tannins: the importance of a mechanistic framework for predicting ecological roles J. Chem. Ecol. 28 1919-1934

[18] Koivikko R, Loponen J, Honkanen T and Jormalainen V 2005 Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga Fucus vesiculosus, with implications on their ecological functions J. Chem. Ecol. 31 195-212

[19] Swanson A K and Drueh L D 2002 Induction, exudation and the UV protective role of kelp phlorotannins Aquat. Bot. 73 241-253

[20] Amsler C D and Fairhead V A 2006 Defensive and sensory chemical ecology of brown algae Adv. Bot. Res. 43 1-91

[21] Kim S K and Wijesekara I 2011 Anticoagulant Effect of Marine Algae Adv. Food Nut. Res. 64 235-244

[22] Kadam S U, Alvarez C, Tiwari B K and O’Donnel C P 2015 Extraction of Biomolecule From Seaweeds (Seaweed Sustainability Food and Non-Food Applications) eds K Brijesh, Tiwari
and J T Declan (United states: Academic Press) Chapter 9 pp 243-269
(http://doi.org/10.1016/B978-0-12-418697-2.00009-X)

[23] Ragan M A and Jensen A 1978 Quantitative studies on brown algal phenols II Seasonal variation in polyphenol content of Ascophyllum nodosum (L) and Fucus vesiculosus (L) J. Exp. Mar. Biol. Ecol. 34 245-258

[24] Steinberg C E W 2011 Stress Ecology-Environmental Stress as Ecological Driving Force and Key Player in Evolution (New york: Springer) 480 pp

[25] Edwards M S and Connell S D 2012 Competition, a Major Factor Structuring Seaweed Communities (Seaweed Biology, Ecological Studies) eds C Wiencke and K Bischof (New York: Springer-Verlag Berlin Heidelberg) Chapter 7 pp 135-156

[26] Hurd C L, Stevens C L, Laval B E, Lawrence G A and Harrison P J 1997 Seawater Flow Around Morphologically Distinct Forms of the Giant Kelp *Macrocystis integrifolia* from Wave -sheltered and exposed sites Limnol. Oceanogr. 42 156–163

[27] Mtolera M S P and Buriyo A S 2004 Studies on Tanzanian Hypneaceae: Seasonal Variation in Content and Quality of Kappa-Carrageenan from Hypnea musciformis (Gigartinales : Rhodophyta) Western Indian Ocean J. Mar. Sci. 3 43–49

[28] Mendoza W G, Ganzon-Fortes E T, Villanueva R D, Romero J B and Montano M N E 2006 Tissue Age as Factor Affecting Carrageenan Quantity in Farmed Kappaphycus striatum Bot. Mar. 49 57-64