Open access • Journal Article • DOI:10.1007/S10811-016-0792-9

Growth and biochemical composition of Kappaphycus (Rhodophyta) in customized tank culture system — Source link

Wahidatul Husna Zuldin, Suhaimi Yassir, Rossita Shapawi

Institutions: Universiti Malaysia Sabah

Published on: 28 Jan 2016 - Journal of Applied Phycology (Springer Netherlands)

Topics: Kappaphycus alvarezii and Kappaphycus

Growth and biochemical composition of Kappaphycus (Rhodophyta) in customized tank culture system

Article *in* Journal of Applied Phycology · January 2016

DOI: 10.1007/s10811-016-0792-9

CITATIONS
8

READS
535

3 authors, including:

Wahidatul Husna Zuldin
Universiti Malaysia Sabah (UMS)
5 PUBLICATIONS **13** CITATIONS
SEE PROFILE

Rossita Shapawi
Universiti Malaysia Sabah (UMS)
73 PUBLICATIONS **539** CITATIONS
SEE PROFILE

J Appl Phycol
DOI 10.1007/s10811-016-0792-9

Growth and biochemical composition of *Kappaphycus* (Rhodophyta) in customized tank culture system

Wahidatul Husna Zuldin[1] · **Suhaimi Yassir**[1] · **Rossita Shapawi**[1]

Received: 6 August 2015 / Revised and accepted: 10 January 2016

Abstract The study was conducted to determine the growth and biochemical composition of *Kappaphycus* cultivated in a customized tank culture system. Two red seaweed species (*Kappaphycus alvarezii* and *Kappaphycus striatum*) were selected and cultivated using suspension culture method in the tank. Three cycles of 40-day culture trials were performed during September to December 2014, and both *K. alvarezii* and *K. striatum* were successfully grown in the tank. This is the first report on the success of seaweed culture in Malaysia involving land-based facility. Interestingly, *K. striatum* was found to grow better than *K. alvarezii* in the tank. The daily growth rate (DGR) and daily weight productivity (DWP) of *K. alvarezii* ranged from 1.96 ± 0.08 to 2.29 ± 0.11 % day^{-1} and 3.70 ± 0.20 to 4.55 ± 0.34 g DW m^{-2} day^{-1}, and those of *K. striatum* ranged from 2.25 ± 0.06 to 2.96 ± 0.02 % day^{-1} and 4.48 ± 0.19 to 6.17 ± 0.18 g DW m^{-2} day^{-1}, respectively. These values were influenced by the changes in the water quality variables during the culture period. On the other hand, the biochemical composition of *K. alvarezii* and *K. striatum* was not significantly different ($p > 0.05$) from each other. Both growth and biochemical composition of *K. alvarezii* and *K. striatum* in the present study were comparable with those cultured in the open sea. In conclusion, the findings indicate the ability of *Kappaphycus* to grow well in land-based cultivation system which can be further explored to support the development of local seaweed farming industry especially for the high-quality seed production.

✉ Rossita Shapawi
rossita@ums.edu.my

[1] Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

Keywords *Kappaphycus* · Seaweed · Growth · Productivity · Customized tank culture · Biochemical composition

Introduction

The increase in world production of farmed seaweed more than doubled from 2000 to 2012 especially for *Kappaphycus* in Southeast Asia such as in Indonesia, China, and the Philippines (FAO 2014). In fact, seaweed farming is one of the top priorities set for development in Malaysia due to the increasing world demand for raw (biomass) and processed seaweed, especially the commercial red seaweed that is used as the main source of carrageenan (Bindu and Levine 2011). The seaweed farming industry was introduced to Malaysia in 1978, and Sabah is the only state producing seaweed commercially (Sade et al. 1987). Recently, seaweed farming activities have become an economically important industry for Malaysia.

Most of the seaweed farming in the South East Asian countries involves open sea cultivation using the fixed line culture method (Azanza-Corrales et al. 1996; Hurtado et al. 2014; 2015). This method is also the most common technique used for seaweed cultivation in Sabah, Malaysia. The open sea cultivation of *Kappaphycus* occurs in the Semporna waters of Sabah, especially for *Kappaphycus alvarezii* and *Kappaphycus striatum*. The cultivation method practiced by local seaweed farmers in Semporna involves the repetitive vegetative propagation method using fixed long-line "tie-tie" culture system (Ali et al. 2014). Vegetative propagation of *Kappaphycus* is easily done by breaking off and planting individual large pieces of the thalli offshore which can be harvested after 30 to 60 days (Doty 1985; Neish 2003).

Considering the high potential of seaweed aquaculture industry in the tropical region, a sound research program

Published online: 28 January 2016

especially on high-quality seed production should be considered which normally involves a land-based cultivation facility. For example, high-quality seaweed seedling in nursery through selective breeding, hybridization and grafting must be explored. Research on the in vitro micropropagation of *Eucheuma* seaweeds in Universiti Malaysia Sabah has been initiated, and the result showed that the *Eucheuma* species had higher growth rate with the optimum condition including continuous aeration, suitable culture medium, temperature of 25 to 30 °C, and salinity of 32 ppt (Yong et al. 2011). Nevertheless, problems were encountered during the field trial of the micropropagated *K. alvarezii* even though the seaweeds had gone through a successful acclimatization process in an outdoor nursery system (Yoong et al. 2013a, b). Therefore, a suitable tank design must be invented to solve this problem. Currently, little is known about land-based seaweed cultivation using tank culture method, especially in Malaysia. Common practice is to culture seaweed with other aquatic animals such as fish and mussels in round or rectangular tanks placed indoors or outdoors. However, a customized tank design can be easily manufactured to optimize the water condition and quality in the tank culture system.

In the present study, *Kappaphycus* were selected as the target species based on commercial value as a carrageenophyte. In terms of reproduction, *Kappaphycus* has the ability to reproduce both asexually and sexually (Bulboa et al. 2008) (Ask and Azanza 2002). In fact, vegetative propagation is highly applicable for seaweed cultivation in tank culture (Halling et al. 2005). Therefore, *Kappaphycus* might be able to survive and grow in tank culture system through vegetative reproduction. The present study aimed to determine the growth performance and biochemical composition of *Kappaphycus* cultivated in the customized tank culture system.

Materials and methods

Seedling collection and translocation

Kappaphycus alvarezii and *Kappaphycus striatum* were collected from a local seaweed farm located at Selakan Island, Semporna, Sabah (N 04° 34.066′, E 118° 40.673′). The seaweeds were translocated from the farm to Universiti Malaysia Sabah (UMS) Shrimp Hatchery. Young and healthy seaweeds with good quality and high number of potential growing thalli were selected and weighed about 100 to 150 g as initial seedlings. The seedlings were washed thoroughly in fresh sea water before being transferred to the customized tank for visible epiphytes removal.

Customized tank culture system

Three 40-day trials of *Kappaphycus* cultivation were performed from September to December 2014 using the customized tank culture system. The system consisted of a 750-L filter tank and a 4480-L flow-through tank with partition screens (1120 L reservoir and six equal-sized 560 L partitions) as shown in Fig. 1. The filter tank consisted of polyvinyl chloride (PVC) tank filled with different sizes of corals together with a back-wash system for daily cleaning purposes. The tank was equipped with continuous water flow with inlet and outlet flow-rates of 35 and 33 L s^{-1}, respectively. Each partition was labeled as A1, A2, A3, B1, B2, and B3. *Kappaphycus alvarezii* was placed in A1, A2, and A3; *K. striatum* was placed in B1, B2, and B3. Approximately, 1000 g (nine to ten 100–150 g seaweed thalli) of *Kappaphycus* was loaded in each partition as initial seedlings. There was no nutrient enrichment or fertilizers added to the tank. Aeration was provided using super bubble-disc air stone, a 4-in flat round air disc.

Water quality analysis

The physical water quality variables in each culture tank were monitored "in situ" (dissolved oxygen level (DO), pH, temperature, salinity, and light intensity) twice daily (8 a.m. in the morning and 3 p.m. in the afternoon) using HANNA Multiparameter Equipment and Lux meter. The water concentration of nitrite, nitrate, and ammonia was measured using methods described by Hansen and Koroleff (1999) once in a week using colorimetric analysis.

Fig. 1 The customized tank used to culture the seaweed

Daily growth rate and dry weight productivity analysis

Wet weight of each plant was recorded every 10 days. Growth was measured by calculating the daily growth rate (DGR, % day^{-1}) for each tank according to the formula (Yoong et al. 2013a, b):

$$\mathrm{DGR}\left(\%\ \mathrm{day}^{-1}\right) = \left[\left(\frac{W_t}{W_0}\right)^{\frac{1}{t}} - 1\right] \times 100\%$$

where W_t = final fresh weight at day t (g), W_o = initial fresh weight (g), and t = number of culture days.

The daily dry weight productivity was also measured in each of the partitions in the tank using the formula:

$$\text{Dry weight productivity}\left(\mathrm{g\ DW\ m^{-2}\ day^{-1}}\right) = \frac{0.20 \times [\mathrm{FW}-\mathrm{IW}]}{a}/t$$

where 0.20 = the estimated percentage of the seaweed dry weight, t = no. of culture days, FW = final fresh weight, IW = initial fresh weight, and a = area of the partition.

Sample preparation

The seaweeds were harvested at day 40 and washed thoroughly using tap water to remove visible epiphytes and unwanted particles. The cleaned samples were oven dried at 60 °C until constant weight. The dried sample was ground into a fine powder using a FOSS Tecator Cyclotec Sample Mill for further biochemical composition analysis.

Proximate analysis

The proximate analysis was done using AOAC (2000) Standard Method for estimation of moisture, ash, crude fiber, and crude protein. The moisture content of seaweed sample was determined using the oven method at 105 °C until constant weight was obtained. Ash content was determined gravimetrically after heating the sample in a muffle furnace at 550 °C for 5 h. The crude fiber content was analyzed using subsequent steps of chemical treatments using a Fibertec machine. Crude protein content was determined using the Kjedahl method with a conversion factor of 6.25 to convert total nitrogen to protein.

Semi-refined carrageenan extraction

The semi-refined carrageenan extraction method was performed as described by Istinii et al. (1994). Fifty gram of dried sample of *K. alvarezii* and *K. striatum* were weighed (*Ws*) and washed with tap water to remove excess sand and salt. The samples were then cooked in 6 % potassium hydroxide, KOH, solution pre-heated at 80 °C for 30 min. The cooked seaweed was cooled and washed with distilled water several times. The semi-refined carrageenan was extracted by filtration using a pressure pump. Precipitated semi-refined carrageenan was then dried at 50 °C for 24 h in a drying oven. The dried semi-refined carrageenan was weighed at the end (*Wc*) and the carrageenan yield was expressed as percentage according to the formula (de Goes and Reis 2011):

$$\text{Carrageenan yield}\ (\%) = \frac{Wc}{Ws}\ \mathrm{x}\ 100$$

Statistical analysis

The data were subjected to further analysis of significant differences on the growth rate, dry weight productivity, water quality variables, and biochemical composition of the tank-cultured *Kappaphycus* by two-way ANOVA and post hoc test using SPSS software version 21 (SPSS Inc.). *T* test was used to analyze any significant difference on the daily growth rate between the species.

Results

Both *K. alvarezii* and *K. striatum* grew successfully in the customized tank. *Kappaphycus striatum* was found to grow

Fig. 2 **a** The *K. alvarezii* at day 5, **b** the harvested *K. alvarezii* at day 40, **c** the *K. striatum* cultured at day 5, and **d** the harvested *K. striatum* at day 40

Table 1 The average daily growth rate and dry weight productivity of *Kappaphycus* in tank for cycles 1, 2, and 3

Cycle #	*Kappaphycus alvarezii*		*Kappaphycus striatum*	
	Daily growth rate/DGR (% day^{-1})	Dry weight productivity (g DW m^{-2} day^{-1})	Daily growth rate/DGR (% $day^{-1)}$	Dry weight productivity (g DW m^{-2} day^{-1})
1	2.29 ± 0.11^{a}	4.55 ± 0.34^{a}	2.81 ± 0.06^{d}	6.17 ± 0.18^{d}
2	2.13 ± 0.03^{b}	3.93 ± 0.06^{b}	2.96 ± 0.02^{e}	6.59 ± 0.07^{e}
3	1.96 ± 0.08^{c}	3.70 ± 0.20^{c}	2.25 ± 0.06^{f}	4.48 ± 0.19^{f}

Different letters indicated significant differences. The ± values refer to standard errors, $n = 30$

better than *K. alvarezii* in the tank (Fig. 2). The average daily growth rate and dry weight productivity of *K. alvarezii* were found to be higher during cycles 1 and 2 (September 2014 to November 2014) than during cycle 3 (Table 1). There were significant differences ($p < 0.05$) on the growth rate between the two different *Kappaphycus* species within every cycle. The decreasing trend of the DGR and daily weight productivity (DWP) was related to the changes in the water quality variables during the cycle.

The measured water quality variables during cycle 1 (September 2014 to October 2014) were salinity (30.54 to 33.59 ppt), temperature (29.67 to 32.18 °C), dissolved oxygen (DO) level (5.25 to 6.51 mg L^{-1}), pH (7.20 to 8.34), and light intensity (102.33 to 146.15 μmol photons m^{-2} s^{-1}) as shown in Table 2. During cycle 2 (October 2014 to November 2014), the readings were salinity (30.57 to 34.13 ppt), temperature (29.89 to 32.27 °C), dissolved oxygen (DO) level (5.29 to 6.47 mg L^{-1}), pH (7.17 to 8.32), and light intensity (118.38 to 153.30 μmol photons m^{-2} s^{-1}); and during cycle 3 (November 2014 to December 2014), the readings were salinity (29.68 to 33.66 ppt), temperature (28.21 to 31.67 °C), dissolved oxygen (DO) level (5.35 to 6.52 mg L^{-1}), pH (7.15 to 8.42), and light intensity (92.54 to 132.96 μmol photons m^{-2} s^{-1}). The average water nitrogen contents were NO_3-N (0.141 ± 0.02 mg L^{-1}), NO_2-N (0.042 ± 0.01 mg L^{-1}), and NH_3-N (0.048 ± 0.01 mg L^{-1}) (Table 3).

The average content of moisture, crude ash, crude protein, crude fiber, and carrageenan in tank-cultured *K. alvarezii* and *K. striatum* during the three cycles is shown in Table 4.

Discussion

Our findings indicate that both *Kappaphycus* species were able to grow in the tank culture system with good daily growth rates, and dry weight productivity. Specifically, *K. striatum* showed a better growth rate than the *K. alvarezii* in the culture tank. This differs from Naguit et al. (2009) who reported that the brown variety of *K. alvarezii* showed a higher growth rate compared to *K. striatum* when cultured in the open sea open sea (Naguit et al. 2009). Orbita and Arnaiz (2004) also reported a higher growth rate of *K. alvarezii* compared to *K. striatum* when cultured during the Northeast monsoon season in Philippines. The higher DGR and DWP of *K. alvarezii* and *K. striatum* were found to be during cycles 1 and 2 in September to November 2014. The growth performance decreased during cycle 3 (November to December 2014) which might be related to changes in the water quality variables. To our knowledge, this is the first report on the performance of *K. striatum* in a tank culture system. The good growth of *K. alvarezii* and *K. striatum* in the tank indicated that sufficient nutrients were available from the supplied seawater.

The propagation of *Kappaphycus* in tank culture is the possibility of seaweeds being attacked by predators such as herbivorous fishes and turtles. According to Korzen et al. (2011), grazing by herbivorous fishes and invertebrates strongly affects algal cover and biomass. In fact, the grazing of seaweed tissues by herbivores can result in inconsistent crop yields making commercial seaweed farming a less economically viable venture (Ganesan et al. 2006; Ateweberhan

Table 2 pH, dissolved oxygen (DO), temperature, salinity, and light intensity in the culture tank during cycle 1 (September to October 2014), cycle 2 (October to November 2014), and cycle 3 (November to December 2014)

Water quality variables Culture cycle	pH	DO level (mg L^{-1})	Temperature (°C)	Salinity (ppt)	Light intensity (μmol photons m^{-2} s^{-1})
1	7.20–8.34	5.25–6.51	29.67–32.18	30.54–33.59	102.33–146.15
2	7.17–8.32	5.29–6.47	29.89–32.27	30.57–34.13	118.38–153.30
3	7.15–8.42	5.35–6.52	28.21–31.67	29.68–33.66	92.54–132.96

The ± values refer to standard errors, $n = 30$

Table 3 Dissolved inorganic concentration (nitrate, nitrite, and ammonia) recorded in cycles 1, 2, and 3

Cycle	NO_3^-–N (nitrate) mg L^{-1}	NO_2^-–N (nitrite) mg L^{-1}	NH_3-N (ammonia) mg L^{-1}
1	0.141 ± 0.14	0.039 ± 0.03	0.051 ± 0.08
2	0.137 ± 0.08	0.043 ± 0.04	0.041 ± 0.03
3	0.146 ± 0.15	0.044 ± 0.04	0.053 ± 0.05

The ± values refer to standard errors, $n = 12$

et al. 2015). Thus, the use of land-based culture facilities for cultivating seaweeds might reduce the risks of predation to maximize growth performance and production. Land-based seaweed cultivation is beneficial for maintaining seaweed seedling in good condition avoiding inbreeding phenomenon that might lower the quality especially on the genetic traits and also might deviate the seed over time if they were continually re-seeded from the farm for years.

Throughout the trials, the recorded water quality variables (salinity, temperature, pH, dissolved oxygen (DO) level, and light intensity) in the culture tank fall within the range of optimum requirements for field-cultured *Kappaphycus* species except for the light intensity sourced from direct sunlight to the tank during cycle 3 (November to December 2014). According to Sahoo and Yarish (2005), the optimum mariculture condition of *Kappaphycus* in the open sea are a water temperature of about 27 to 30 °C, a salinity of 30 to 33 ppt, DO levels of 5 to 6 mg L^{-1}, pH from 7 to 9, light intensity of 112 to 149 μmol photons m^{-2} s^{-1}, and a water level of 0.5 to 1.0 m during low tide and 2.0 to 3.0 m during high tide. The depth of the tank used in the present study was 0.8 m which is still favorable for the mariculture of *Kappaphycus* species. Light intensity was one of the important key factors for seaweed growth. During cycle 3, the available sunlight in the tank ranged from 92.54 to 132.96 μmol photons m^{-2} s^{-1} which was lower than in cycles 1 and 2, and this could be the reason for the decreasing growth rate of *Kappaphycus* in the tank during that culture period. The weather during November to December 2014 was unpredictable, and there was heavy rainfall during cycle 3 for more than 10 days. The changes in salinity during cycle 3 might also have caused stress to the cultured seaweed in the tank.

In terms of the seawater nutrient content in the tank, the average concentrations of nitrite, nitrate, and ammonia in all treatment tanks were below 0.1 mg L^{-1}, indicating good water quality (Luhan et al. 2015). Nitrate is the principal form of fixed dissolved inorganic nitrogen assimilated by algae (Najafpour 2012). The nutrients available in the tank appeared to be sufficient in supporting good growth of *Kappaphycus* without fertilizer supplementation.

The biochemical composition (moisture, ash, crude protein, and crude fiber content) of tank-cultured *K. alvarezii* and *K. striatum* were in the range reported by Ahmad et al. (2012). The percentage of ash content is in a high proportion for both *K. alvarezii* and *K. striatum*. According to Pena-Rodriguez et al. (2011), the high ash content in marine algae is related to their ability to absorb minerals and trace elements from the surrounding environment. The moisture and crude fiber content for the tank-cultured *K. alvarezii* and *K. striatum* were also in the range similar to the study of Ahmad et al. (2012). There were no significant differences between the moisture, ash, crude fiber, and protein of the tank and field-cultured *Kappaphycus* species. The carrageenan content of both *Kappaphycus* species in the present study was within the values reported by Yong et al. (2014) and Hurtado et al. (2008) and was comparable to *Kappaphycus* cultured in the open sea. These results suggest that land-based seaweed cultivation does not have any negative effects on the biochemical composition of the seaweed. Therefore, the current customized tank design is proven useful, not only for the high-quality seed production research but also on the manipulation of carrageenan content.

In summary, both *Kappaphycus* species (*K. striatum* and *K. alvarezii*) can be successfully grown and propagated in a tank culture system with good growth performance and survival. The findings from this study are significant and provide fundamental data for the purpose of expanding research on land-

Table 4 The biochemical composition of *K. alvarezii* and *K. striatum* cultivated in the customized tank

Cycle #	1		2		3	
Species	*K. alvarezii*	*K. striatum*	*K. alvarezii*	*K. striatum*	*K. alvarezii*	*K. striatum*
Biochemical composition (dry matter basis)						
Moisture content (%)	78.27 ± 0.31	77.25 ± 0.13	79.51 ± 0.98	79.97 ± 0.79	78.39 ± 0.63	78.12 ± 0.23
Ash content (%)	22.04 ± 0.19	22.98 ± 0.83	21.48 ± 0.62	20.45 ± 1.01	22.15 ± 0.42	22.36 ± 0.93
Crude fiber content (%)	5.12 ± 0.21	5.32 ± 0.17	5.27 ± 0.14	5.41 ± 0.05	5.01 ± 0.27	5.29 ± 0.17
Crude protein content (%)	5.43 ± 0.27	5.48 ± 0.34	5.90 ± 0.46	5.62 ± 0.11	5.38 ± 0.19	5.40 ± 0.26
Carrageenan content (%)	52.91 ± 0.42	41.17 ± 0.28	54.25 ± 1.11	43.68 ± 1.97	51.48 ± 0.38	40.75 ± 0.29

The ± values refer to standard errors, $n = 12$

based seaweed cultivation. Indeed, it is important to facilitate seaweed research in producing high-quality seedlings for the benefit of the industry. Therefore, more research on the land-based seaweed cultivation systems should be considered to explore the full potential of land-based seaweed farming.

Acknowledgments The authors would like to thank the Ministry of Education Malaysia for funding the research under the potential HiCoE program (project COE0005). We would also like to express our gratitude for BMRI (UMS) and Pulau Selakan Seaweed Farm Management Staff.

References

Ahmad F, Sulaiman MR, Saimon W, Chye FY, Matanjun P (2012) Proximate compositions and total phenolic contents of selected edible seaweed from Semporna, Sabah, Malaysia. Borneo Sci 31(1): 85–96

Ali MKM, Wong JVH, Sulaiman J, Juli JL, Yasir SM (2014). Improvement of growth and mass of *Kappaphycus striatum* var. sacol by using plant density study at Selakan Island, Semporna Malaysia. International Conferences on Biological, Chemical and Environmental Science Proceedings pp 58–63.

AOAC (2000) Official methods of analysis of AOAC international, 17th edn. AOAC International, Md., USA

Ateweberhan M, Rougier A, Rakotomahazo C (2015) Influence of environmental factors and farming technique on growth and health of farmed *Kappaphycus alvarezii* (cottonii) in south-west Madagascar. J Appl Phycol 27:923–934

Ask EI, Azanza RV (2002) Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research. Aquaculture 206:257–277

Azanza-Corrales R, Aliaza TT, Montano NE (1996) Recruitment of *Eucheuma* and *Kappaphycus* on a farm in Tawi-tawi, Philippines. Hydrobiologia 116:235–244

Bindu MS, Levine IA (2011) The commercial red seaweed *Kappaphycus alvarezii*—an overview on farming and environment. J Appl Phycol 23:789–796

Bulboa C, de Paula EJ, Chow F (2008) Germination and survival of tetraspores of *Kappaphycus alvarezii* var. *alvarezii* (Solieriaceae, Rhodophyta) introduced in subtropical waters of Brazil. J Phycol Res 56:39–45

Doty MS (1985) *Eucheuma alvarezii* sp. nov. (Gigartinales, Rhodophyta) from Malaysia. In: Abbot IA, Norris JN (eds) Taxonomy of economic seaweeds with reference to some Pacific and Carribean species. California Sea Grant College Program, University of California, La Jolla, California, pp 37–45

FAO (2014) The State of World Fisheries and Aquaculture: Opportunities and Challenges. Food and Agriculture Organization of the United Nations, Rome, pp 18–64

Ganesan M, Thiruppathi S, Sahu N, Rengarajan N, Veeragurunathan V, Bhavanath J (2006) *In situ* observations on preferential grazing of seaweeds by some herbivores. J Curr Sci 91:1256–1260

Góes HG, Reis RP (2011) An initial comparison of tubular netting versus tie-tie methods of cultivation for *Kappaphycus alvarezii* (Rhodophyta, Solericiaceae) on the South Coast of Rio de Janeiro State, Brazil. J Appl Phycol 23:607–613

Halling C, Aroca G, Cifuentes M, Buschmann AH, Troell M (2005) Comparison of spore inoculated and vegetative propagated cultivation methods of *Gracilaria chilensis* in an integrated seaweed and fish cage culture. Aquacult Internat 13:409–422

Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrahardt M (eds) Methods of Seawater Analysis, 3rd edn. Wiley-VCH, Weinheim, Germany, pp 159–228

Hurtado AQ, Critchley AT, Trespoey A, Bleicher-Lhonneur G (2008) Growth and carrageenan quality of *Kappaphycus striatum* var. *sacol* grown at different stocking densities, duration of culture and depth. J Appl Phycol 20:551–555

Hurtado AQ, Gerung GS, Yasir S, Critchley AT (2014) Cultivation of tropical red seaweeds in the BIMP-EAGA region. J Appl Phycol 26: 707–718

Hurtado AQ, Neish IC, Critchley AT (2015) Developments in production technology of *Kappaphycus* in the Philippines: more than four decades of farming. J Appl Phycol 27:1945–1961

Istinii S, Ohno M, Kusunose H (1994) Methods of analysis for agar, carrageenan and alginate in seaweed. Bulletin of Marine Science and Fisheries 14:49–55

Korzen L, Israel A, Abelson A (2011) Grazing effects of fish versus sea urchins on turf algae and coral recruits: possible implications for coral reef resilience and restoration. J Mar Biol 2011:1–8

Luhan MRJ, Avancena SS, Mateo JP (2015) Effect of short-term immersion of *Kappaphycus alvarezii* (Doty) Doty in high nitrogen on the growth, nitrogen assimilation, carrageenan quality, and occurrence of "ice-ice" disease. J Appl Phycol 27:917–922

Naguit MRA, Tisera WI, Lanioso A (2009) Growth performance and carrageenan yield of *Kappaphycus alvarezii* (Doty) and *Eucheuma denticulatum* (Burman) Collins et Harvey, Farmed in Bais Bay, Negros Oriental and Olingan, Dipolog City. The Threshold 4:38–51

Najafpour M (2012) Applied photosynthesis: nitrate assimilation. In: Chow F (ed) The Role of In Vitro Nitrate Reductase Assay as Nutritional Predictor. InTech Publication, Europe, pp 105–118

Neish CI (2003) The ABC of *Eucheuma* Seaplant Production. Agronomy, biology and crop handling of *Betaphycus*, *Eucheuma* and *Kappaphycus* the gelatinae, spinosum and cottonii of commerce SuriaLink. pp.1–82.

Orbita MLS, Arnaiz JA (2004) Seasonal changes in growth rate and carrageenan yield of *Kappaphycus alvarezii* and *Kappaphycus striatum* (Rhodophyta, Gigartinales) cultivated in Kolambugan, Lanao del Norte. AAB Bioflux 6(2):134–144

Pena-Rodriguez A, Mawhinney TP, Ricque-Marie D, Cruz-Suarez LE (2011) Chemical composition of cultivated seaweed *Ulva clathrata* (Roth) C. Agardh. Food Chem 129:491–498

Sade A, Ali I, Mohd Ariff MR (1987) The seaweed industry in Sabah, East Malaysia. J South East Asia Stud 11:97–107

Sahoo D, Yarish C (2005) Mariculture of seaweeds. In: Preisig R (ed) Phycological Methods: Historical Review of Algal Culturing Techniques. Academic, Zurich, pp 1–12

Yong WTL, Ting SH, Chin LW, Rodrigues KF, Anton A (2011) In vitro micropropagation of *Eucheuma* seaweeds. Proceedings. 2nd International Conference on Biotechnology and Food Science. IACSIT Press, Singapore. 7:58–60

Yoong SY, Thau WLY, Anton A (2013a) Analysis of formulae for determination of seaweed growth rate. J Appl Phycol 25: 1831–1834

Yoong SY, Thau WLY, Vun YT, Su EN, Anton A (2013b) Acclimatization of micropropagated *Kappaphycus alvarezii* (Doty) Doty ex Silva (Rhodophyta, Solieriaceae) in outdoor nursery system. J Appl Phycol 27:413–419

Yong SY, Thau WLY, Vun YT, Su EN, Anton A (2014) Acclimatization of micropropagated *Kappaphycus alvarezii* (Doty) Doty ex Silva (Rhodophyta, Solieriaceae) in outdoor nursery system. J Appl Phycol 25:1831–1834