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Seasonal changes in growth rate and carrageenan yield of *Kappaphycus alvarezii* and *Kappaphycus striatum* (Rhodophyta, Gigartinales) cultivated in Kolambugan, Lanao del Norte

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Abstract. The seasonal changes in growth and carrageenan yield of *Kappaphycus alvarezii* (var. wayway) and *Kappaphycus striatum* (var. sacol) cultivated in Barangay Manga, Kolambugan, Lanao del Norte was determined during the southwest and northeast monsoon. Some environmental parameters were also determined: water temperature, salinity, water flow and inorganic phosphate and nitrate. The growth rate and carrageenan content varied significantly with sampling periods; highest growth rate and carrageenan content occurred during the northeast monsoon from November to January while the lowest growth and carrageenan yield obtained during the southwest monsoon from June to August. *K. alvarezii* had higher growth rate and carrageenan yield compared to *K. striatum* in both seasons. A positive relationship between growth rate and carrageenan yield was detected in both species. Multiple stepwise regression analysis showed that water flow was the only environmental factor that affects the growth and carrageenan yield of *K. alvarezii* and *K. striatum*. The results show that the highest growth rate and carrageenan yield of *K. alvarezii* and *K. striatum* can be obtained during the northeast monsoon when water movement was strong.
Key Words: Alga, seaweed, K. *cottonii, Eucheuma cottonii*, red alga, southwest monsoon, northeast monsoon, hanging-monoline culture.

Introduction. *Kappaphycus,* commercially known as *"cottonii",* is composed of two major species, namely *Kappaphycus alvarezii* (Doty) Doty ex Silva and *Kappaphycus striatum* (Schmitz) Doty. They are the primary sources of the commercial hydrocolloid kappa-carrageenan in the world (Ask & Azanza 2002). Moreover, they have been successfully farmed in the Philippines since 1969 along with *Eucheuma* species (Lim & Porse 1981).

K. alvarezii and *K. striatum* varied consistently in terms of its morphology and physiology (Doty & Norris 1985). The most popular variety of *K. alvarezii* is "*tambalang*", which is characterized by its long cylindrical thallus and sparse branches with sharp pointed tips. "*Sacol*", the most common variety of *K. striatum* is characterized by stubby and thick cylindrical branches with blunt and forked tips hence resemble a "cauliflower" shape. The anastomosed and densely branched thallus of *K. striatum* is a limiting factor in mariculture because it is more susceptible to breakage and this creates difficulties during farm operation (Bulboa 2001). Conversely, it has been observed that *K. alvarezii* is more easily infected with "ice-ice" and epiphytes than *K. striatum*, especially during sudden changes in water condition such as increase in temperature (Hurtado et al 2008). Ice-ice disease in *Kappaphycus/Eucheuma* was thought of as mainly a non-infectious disease which could be triggered by unfavorable environmental condition such as extreme temperature, irradiance and salinity, and opportunistic bacterial pathogens, *Vibrio* sp. and *Cytophaga* sp. (Largo et al 1999). Moreover, Bulboa & de Paula (2005) observed physiological differences in terms of growth performance between *K. alvarezii* and *K. striatum* under a wide range of environmental conditions. In all tested conditions, *K. alvarezii* grew faster than *K. striatum*.

Since the cultivation of the commercial eucheumatoids began, seasonal patterns to growth have been noted (Doty & Alvarez 1975) and likewise with the carrageenan content (Trono & Lluisma 1992). Monsoons or seasonal events can exert environmental changes on a farming site resulting to changes in growth and carrageenan yield. Several authors have observed seasonality in growth and carrageenan yields of eucheumoid species in relation to changes in environmental factors (de Góes & Reis 2012; Hung et al 2009; Hayashi et al 2007; Wakibia et al 2006a,b; Bulboa & de Paula 2005; Muñoz et al 2004; Hurtado et al 2001; Ohno et al 1996; Ohno et al 1994; Prakash 1990). For example, in Fiji, high growth rates in *K. alvarezii* occur during the southeasterly trade winds in April to December (Prakash 1990). In Vietnam, *K. alvarezii* growth rates decreased 63 % during August, the warmest month (Ohno et al 1996). In the Philippines, maximum growth rates and carrageenan yield of *K. alvarezii* were registered during the northeast monsoon (September-February; Hurtado et al 2001). In Dizilam (Mexico), growth rates of *K. alvarezii* decreased by 34 – 47 % during warmer months (Muñoz et al 2004). In south-eastern Brazil, higher growth rates in *K. alvarezii* and *K. striatum* were registered during summer and autumn months (Bulboa & de Paula 2005). In southern Kenya, higher growth rate and carrageenan yield of *K. alvarezii* and *Eucheuma denticulatum* were obtained towards the end of southeast monsoon in September (Wakibia et al 2006a,b). In São Paulo, Brazil, higher growth rates in *K. alvarezii* were registered from February to May while higher carrageenan yield were obtained from August to December (Hayashi et al 2007). In Camranh Bay, higher growth rates of *K. alvarezii* were obtained during the northeast monsoon from September to February (Hung et al 2009). Moreover, in Sepetiba Bay, the growth rate of *K. alvarezii* tended to be higher in the end of summer to autumn and in spring while the carrageenan yield was higher in the middle of summer (de Góes & Reis 2012).

Study on seasonality in growth and carrageenan yield of *K. alvarezii* has already been done in the area (Orbita 2013) however no study has been done yet on *K. striatum.* On the other hand, the seaweed farmers in Kolambugan preferred to use *K. striatum* because of the observation that *K. alvarezii* is more easily infected with "ice-ice" disease, especially during sudden changes in water conditions such as increase in temperature. This preference requires scientific study as this selection is mostly based on anecdotal observations.

This study was conducted to compare the seasonality in growth and carrageenan yield of *K. alvarezii* and *K. striatum* in Barangay Manga, Kolambugan, Lanao del Norte. Specifically: (1) to determine some environmental parameters in the sampling area; (2) to determine the growth rate and carrageenan yield of *K. alvarezii* and *K. striatum* during the southwest and northeast monsoon; (3) to correlate the environmental factors with the growth rate and carrageenan yield of *K. alvarezii* and *K. striatum* and (4) to compare the growth rate and carrageenan yield of *K. alvarezii* and *K. striatum* during the southwest and northeast monsoon.

Material and Method. The study was conducted in Barangay Manga (08° 0.09' North Latitude and 123° 0.82' East Longitude), Kolambugan, Lanao del Norte (Figure 1). The area has been farmed with two varieties of *Kappaphycus*, *Kappaphycus alvarezii* (var. tambalang or wayway) and *Kappaphycus striatum* (var. sacol). *K. alvarezii* were grown at 2 – 3 m below the water surface (Ohno et al 1994) while *K. striatum* were grown at 50 - 100 cm below the water surface (Hurtado et al 2008; Gerung & Ohno 1997). The seaweed farmers used the hanging-monoline culture method and the area was composed of sandy-muddy substratum.

The measurement of Daily Growth Rate (DGR) in *K. alvarezii* and *K. striatum* was done during the southwest (habagat) and northeast (amihan) monsoon. The measurement of growth rate during the southwest monsoon or "habagat" was carried out once a month from June to August 2012. On the other hand, the growth rate measurement during the northeast monsoon was done from November 2012 to January 2013. Six bunches of fresh samples of *K. alvarezii* and *K. striatum* thalli were taken randomly from one hanging-monoline, placed in a labeled plastic bag, brought to the laboratory and weighed using a digital weighing balance.

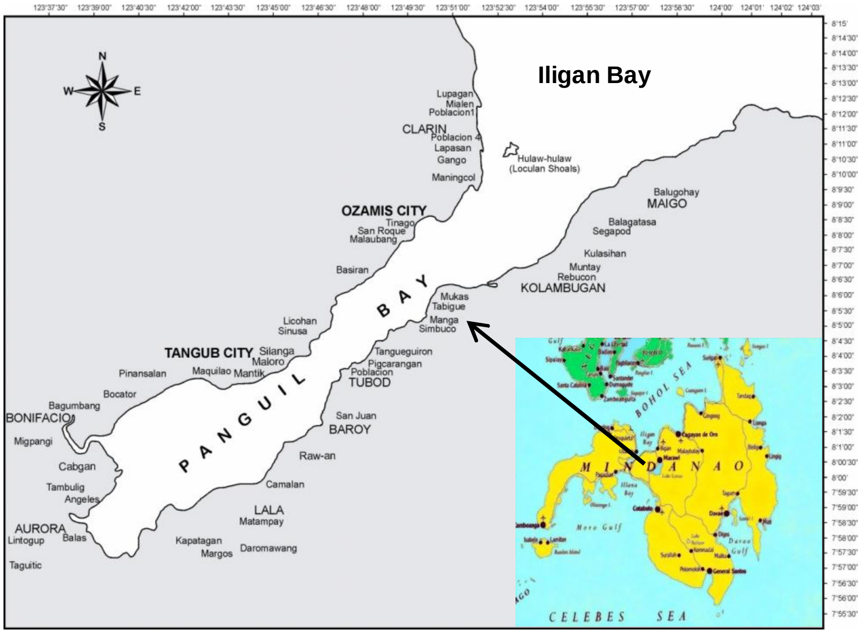

Figure 1. Map of Panguil Bay Northwestern Mindanao, showing the sampling area of Barangay Manga, Kolambugan, Lanao del Norte. Inset is the map of Mindanao with arrow pointing to the sampling area.

The increase in weight of plants was measured after 45 days of culture. The average daily growth rates (DGR = % day^{-1}) were measured and expressed as the percent increase in wet weight per day according to the formula (Hung et al 2009):

$$DGR = [(W_t / W_o)^{1-t} – 1] \times 100$$

Where: W_0 = initial wet weight, W_t = weight after *t* days, t = time intervals (days)

The samples of *K. alvarezii* and *K. striatum* that were collected for growth rate measurements were processed for the analysis of carrageenan content. The samples were cleaned with water and were air dried for 1 day or more. The samples were blended and were air dried again for 2 days. The dried samples were grounded into powder and were placed in a container prior to carrageenan extraction.

Carrageenan extraction was done following the method of Mtolera & Buriyo (2004) and Hayashi et al (2007). About 3 g of powdered seaweeds was hydrated in 105 mL distilled water for 12 h at room temperature under agitation (to remove internal salts). The hydrated material was boiled using 105 mL distilled water at 60 ^{0}C for 4 h with constant stirring (to dissolve algal particles and evaporate insoluble materials). The digested product was precipitated in three volumes of 95 % ethanol (210 mL). The precipitate was then filtered through nylon or cheese cloth, dried in an oven at 60 ^{0}C for 24 h and at 105 ^{0}C for 2 h to constant weight, and then weighed. The final carrageenan was grounded into powder for storage. The carrageenan yield (% dry weight) was determined according to the formula of Hung et al (2009):

$$Yield = (W_c / W_m) \times 100$$

Where: W_c = dry carrageenan weight, W_m= dry algal weight

Environmental parameters were measured randomly within the cultured area of *K. alvarezii* and *K. striatum*. Measurements of the environmental parameters were done once a month from June to August 2012 (southwest monsoon) and from November 2012 to January 2013 (northeast monsoon). Water temperature was measured *in situ* using an ordinary mercury thermometer while salinity was measured using a handheld refractometer. Water flow was estimated using clod cards (Doty 1971) and the calculation of water flow (cm^{-2}) was done based on the method of Anzai (2001). Nutrient analysis was carried out following the method of Grasshoff et al (1983). Data were determined by Analysis of Variance (One-Way ANOVA). Pearson's correlation analysis was used to determine the correlation coefficients between environmental factors with growth rates and carrageenan content. Stepwise multiple regression was used to determine the individual contribution of environmental factors (independent variables) to the total variance of growth rate and carrageenan content (dependent variables), considering $P<0.05$.

Results and Discussion. The average monthly values of the environmental parameters obtained in Barangay Manga varied significantly with sampling periods ($P<0.05$; Figure 2 and 3; Table 1). The highest water temperature occurred from June to August while the lowest temperature occurred in November to January. It ranges from 27.00 ^{0}C to 29.00 ^{0}C and were within the required levels for eucheumoid farming (Hurtado et al 2006). The pattern of salinity and nutrients seemed to follow that of temperature. Salinity values range from 27.40 ‰ to 32.20 ‰; phosphate ranged betwen 0.03 and 0.07 mg/L while nitrate ranges from 0.03 to 0.08 mg/L. The water flow was higher from November to January while it was lower in June to August. The highest water flow occurred during the northwest monsoon while the lowest water flow occurred during the southwest monsoon with values range from 7.28 cm s^{-2} to 11.36 cm s^{-2}.

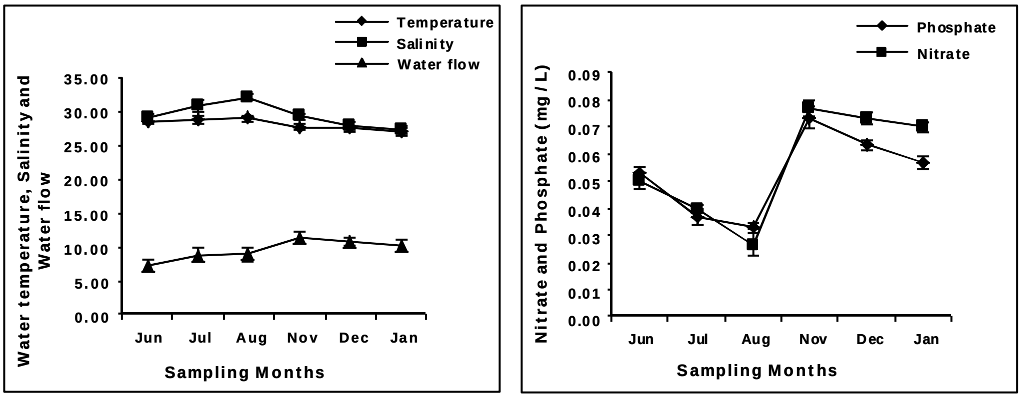

Figure 2. The average monthly values of the environmental parameters (temperature, salinity, water flow, phosphate and nitrate) measured in Barangay Manga, Kolambugan, Lanao del Norte (mean ± SD).

Table 1

Statistical analysis (One-Way ANOVA) of the environmental parameters with sampling periods

Parameters	*n*	*d.f.*	*F*	*p*	*Analysis*
Water temperature (^{0}C)	30	5	12.829	<0.05	Significant
Salinity (‰)	30	5	37.242	<0.05	Significant
Water flow (cm s^{-1})	30	5	15.318	<0.05	Significant
Phosphate (mg/L)	18	5	2.849	<0.05	Significant
Nitrate (mg/L)	18	5	5.005	<0.05	Significant

For the two studied species, the daily growth rates were higher in November and December while it was lower in June to August (Figure 3; Table 2). The high growth rate coincided with the northeast monsoon or *"amihan"* (November to February) which is characterized by high tides during the day and strong water motion. This result is similar to the study of Hurtado et al (2001) and Hung et al (2009) in which the growth rate of *K. alvarezii* was high during the northeast monsoon. On the other hand, previous study conducted from 2010 to 2011 (Orbita 2013) on *K. alvarezii* in the same cultivation area showed an opposite result, high growth rate occurred during the southwest monsoon or *"habagat".* This result shows that the highest growth rate of *K. alvarezii* cultivated in the coastal waters of Kolambugan, Lanao del Norte can be obtained during any season (both northeast and southwest monsoon). In the case of *K. striatum*, high growth rate can be obtained during the northeast monsoon.

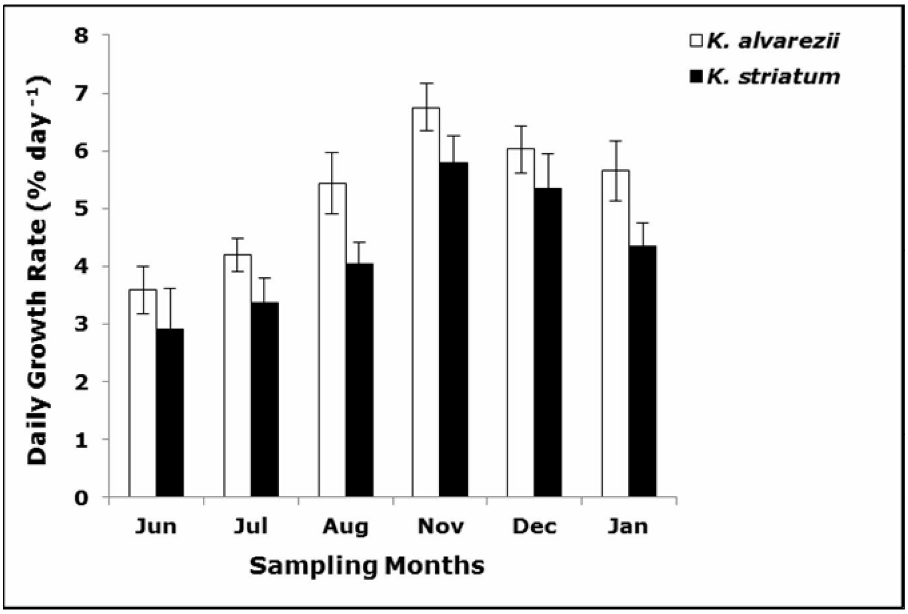

Figure 3. Monthly variation in daily growth rate of *Kappaphycus alvarezii* and *Kappaphycus striatum* in Barangay Manga, Kolambugan, Lanao del Norte (mean ± SD).

Table 2

Statistical analysis (One-Way ANOVA) of the daily growth rate of *Kappaphycus alvarezii* and *Kappaphycus striatum* with sampling periods

Source	*d.f.*	*F-statistics*	*p*	*Analysis*
K. alvarezii	5	36.398	<0.05	Significant
K. striatum	5	24.015	<0.05	Significant

Over a 6-month period, the average growth rate of *K. alvarezii* (3.59 - 6.75 % day^{-1}) was comparable to rates reported for *Eucheuma cottonii* (*K. alvarezii*) in different locations around the world: [2.5 - 3.5 % day $^{-1}$ (Adnan & Porse 1987)], 3.5 - 3.7 % day $^{-1}$ (Luxton et al 1987), 2.5 - 3.5 % day^{-1} (Mollion & Braud 1993), 0.1 - 8.4% day^{-1} (Dawes et al 1994), 0.13 - 8.12 % day^{-1} (Ohno et al 1994), 1.1 - 3.4 % day^{-1} (Hurtado-Ponce 1995), 4.0 - 11.0 % day^{-1} (Ohno et al 1996), 0.2 - 4.2 % day^{-1} (Hurtado et al 2001), 3.6 - 8.9 % day^{-1} (Paula & Pereira 2003), 2.0 - 7.1 % day^{-1} (Munoz et al 2004), 3.1 % day^{-1} (Hung et al 2009) and 1.1 - 5.3 % day^{-1} (de Góes & Reis 2012), indicating its commercial potential as reported by Doty (1987). On the other hand, the average growth rates of *K. striatum* (2.92 - 5.79 % day^{-1}) was comparable to rates (3.7 - 4.7 % day^{-1}) reported by Hurtado et al (2008) in Igang Nueva Valencia, Guimaras and in south-eastern Brazil [1.5 - 5.1 % day^{-1} (Bulboa & de Paula 2005)]. As reported by Ask & Azanza (2002), growth rates above 3.5 % day^{-1} are considered adequate for commercial eucheumatoid farming.

Similar to the daily growth rates, the carrageenan yield was higher (Figure 4; Table 3) during the northeast monsoon or *“amihan”* (November - December) and lower during the southwest monsoon or *“habagat”* (June - August). These seasonal growth patterns and carrageenan yield of *K. alvarezii* and *K. striatum* have been observed elsewhere and in Barangay Manga, Kolambugan, Lanao del Norte (Hung et al 2009; Bulboa & de Paula 2005; Muñoz et al 2004; Hurtado et al 2001; Ohno et al 1996; Prakash 1990). The average carrageenan yield (41.9 - 54.5 %) of *K. alvarezii* was within the range of the industrial requirements for this species [~38 % (Muñoz et al 2004)]. Also, the yield was within the values reported in Indonesia [45 % (Trono & Ohno 1989)], China [56.9 - 58.0 % (Li et al 1990)], Japan [27.6 - 43.5 % (Ohno et al 1994)], Mexico [30.3 - 40.7 % (Muñoz et al 2004)], Brazil [31 – 43 % (Hayashi et al 2007)] and Vietnam [40.9 - 46.8 % (Hung et al 2009)]. For *K. striatum*, the average carrageenan yield (39.7 - 50.3 %) was within the range (35.3 - 46.6 %) reported by Hurtado et al (2008).

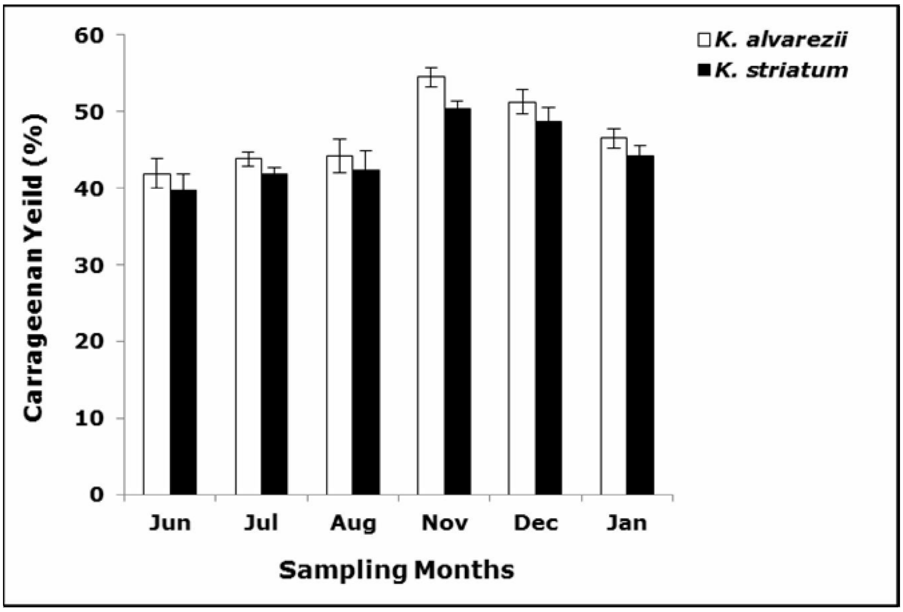

Figure 4. Monthly variation in carrageenan yield of *Kappaphycus alvarezii* and *Kappaphycus striatum* in Barangay Manga, Kolambugan, Lanao del Norte (mean ± SD).

Table 3

Statistical analysis (One-Way ANOVA) of the carrageenan yield of *Kappaphycus alvarezii* and *Kappaphycus striatum* with sampling periods

Source	*d.f.*	*F-statistics*	*p*	*Analysis*
K. alvarezii	5	47.773	<0.05	Significant
K. striatum	5	29.079	<0.05	Significant

Correlation coefficients among the environmental parameters analyzed for *K. alvarezii* and *K. striatum* are shown in table 4 and 5. The growth rate of *K. alvarezii* and *K. striatum* showed a significant positive correlation with the carrageenan yield indicating that the maximum carrageenan yield corresponds to the highest growth rate and vice versa. These results agree with those obtained by Hung et al (2009) and Naguit et al (2009), who found a similar trend in the growth and carrageenan yield of *K. alvarezii.* Also, the study of Wakibia et al (2006a) showed that the high carrageenan yield from the three eucheumoid species (brown *Eucheuma denticulatum*, green and brown *Kappaphycus alvarezii*) grown in southern Kenya corresponds to the period of high relative growth rate (Wakibia et al 2006b). Conversely, some authors have reported an inverse relationship between carrageenan content and growth rates in carrageenophytes

(Dawes et al 1994; Guist et al 1982; Hurtado et al 2008). It has been suggested that this negative correlation between growth and carrageenan yield was considered an advance especially for *K. alvarezii* culture since this species has the ability to produce high carrageenan content despite of its low growth rate (Ask & Azanza 2002). Furthermore, de Góes & Reis (2012) found no correlation between the carrageenan yield and growth rate of *K. alvarezii* cultured at Sepetiba Bay, southeastern Brazil.

Table 4

Correlation coefficients (R) between growth rate (% day^{-1}) and carrageenan yield (%) of *Kappaphycus alvarezii* with environmental parameters in Barangay Manga, Kolambugan, Lanao del Norte

Parameter	*DGR*	*CY*	*Temp*	*Sal*	*Water flow*	*Phosphate*	*Nitrate*
DGR	1						
CY	0.790**	1					
Temperature	-0.420	-0.416*	1				
Salinity	-0.214	-0.356	0.740**	1			
Water flow	0.754**	0.744**	-0.336	-0.336	1		
Phosphate	0.271	0.020	0.294	0.294	0.322	1	
Nitrate	0.421	0.186	0.503*	0.503*	0.269	0.436	1

DGR - Daily growth rate, CY - Carrageenan yield, Temp - Temperature, Sal – Salinity, * - represents significant differences at $p<0.05$, ** - represents significant differences at $p<0.01$.

Table 5

Correlation coefficients (R) between growth rate (% day^{-1}) and carrageenan yield (%) of *Kappaphycus striatum* with environmental parameters in Barangay Manga, Kolambugan, Lanao del Norte

Parameter	*DGR*	*CY*	*Temp*	*Sal*	*Water flow*	*Phosphate*	*Nitrate*
DGR	1						
CY	0.818**	1					
Temperature	-0.314	-0.401*	1				
Salinity	-0.322	-0.329	0.740**	1			
Water flow	0.792**	0.812**	-0.450*	-0.336	1		
Phosphate	0.371	0.047	0.017	0.294	0.322	1	
Nitrate	0.255	0.209	0.115	0.503*	0.269	0.436	1

DGR - Daily growth rate, CY - Carrageenan yield, Temp - Temperature, Sal – Salinity, * - represents significant differences at $p<0.05$, ** - represents significant differences at $p<0.01$.

Glenn & Doty (1992) demonstrated that water motion accounted for 81 – 98 % of the variation in growth rate of eucheumatoid species. In the present study, water flow was the main environmental factor affecting growth rates and carrageenan yield of *K. alvarezii* and *K. striatum.* Growth rates and carrageenan yield was positively correlated with water flow showing that the maximal growth and carrageenan yield corresponds to the highest water motion. Multiple stepwise regression analysis identified water flow as the only environmental factor that explained 62 % and 46 % of the observed variation (R^2 = 0.62 and 0.46) in *K. alvarezii* growth rate and carrageenan yield; 61 % and 72 % (R^2 = 0.61 and 0.72) variation in growth rate and carrageenan yield of *K. striatum.* Similar results have been found on a reef flat in Hawaii, where a positive correlation with water motion was observed with the growth of *K. alvarezii*, *K. striatum* and *E. denticulatum* (Glenn & Doty 1992). The high carrageenan yield of *K. alvarezii* and *K. striatum* during strong water motion could be an adaptive mechanism for the two eucheumoid species. *K. alvarezii* and *K. striatum* could have synthesized additional structural cell wall polysaccharides that caused an increase in its carrageenan content especially during the northeast monsoon when water motion was strong. Carrageenan as one type of phycocolloids provides flexible structural support in response to water currents and wave action in seaweeds (Kloareg & Quatrano 1988). Moreover, the strong water motion in the sampling area could also have provided sufficient nutrients (inorganic phosphate and nitrate) to support high growth rates of *K. alvarezii* and *K. striatum.*

Table 6 shows the difference in the daily growth rate and carrageenan yield of *K. alvarezii* and *K. striatum* obtained during the northeast and southwest monsoon. The average growth rate and carrageenan yield differed significantly between the two species ($P<0.05$). *K. alvarezii* had higher growth and carrageenan yield compared to *K. striatum* in both seasons.

Table 6

Statistical analysis (One-Way ANOVA) of the daily growth rate and carrageenan yield between *Kappaphycus alvarezii* and *Kappaphycus striatum* obtained during the southwest and northeast monsoon in Barangay Manga, Kolambugan, Lanao del Norte

Source of variation	*Mean*	*F-statistics*	*p*	*Analysis*
		DGR (SWM)		
K. alvarezii	4.41±0.89	11.02	<0.05	Significant
K. striatum	3.45±0.67			
		DGR (NEM)		
K. alvarezii	6.14±0.63	14.64	<0.05	Significant
K. striatum	5.16±0.77			
		CY (SW)		
K. alvarezii	43.33±1.92	7.08	<0.05	Significant
K. striatum	41.36±2.13			
		CY (NEM)		
K. alvarezii	50.75±3.63	6.15	<0.05	Significant
K. striatum	47.73±2.99			

DGR - daily growth rate; CY - carrageenan yield; SWM - southwest monsoon; NEM - northeast monsoon.

A similar result was reported for *K. alvarezii* and *K. striatum* grown under laboratory and field condition in south-eastern Brazil (Bulboa & de Paula 2005). The differences in growth rate and carrageenan content between these two species could be attributed to their physiological and morphological differences. According to Doty & Norris (1985), *K. alvarezii* and *K. striatum* were found to be consistently different in their physiology and morphology. For example, Bulboa & de Paula (2005) found that the highest growth rate of *K. alvarezii* (5.7 % day^{-1}) was observed at 30 ^{0}C and 100 µmol photons m^{-2}s^{-1}, whereas for *K. striatum* the highest growth rate (4.8 % day^{-1}) occurred at 30 ^{0}C and 150 µmol photons m^{-2}s^{-1} when grown under laboratory conditions. In addition, the anastomosed and densely branched thallus of *K. striatum* could be another reason for its low growth rate and carrageenan content because it is more susceptible to breakage especially during increased water movement (Bulboa 2001). The morphology of *K. striatum* was considered as a limiting factor in mariculture and this creates difficulties during farm operation (Bulboa 2001). In fact, this is one reason why farmers prefer to cultivate the seaweed for shorter periods of time (Hurtado et al 2008). Furthermore, differences in growth rate and carrageenan yield could also be due to individual conditions at locations and the performance of each species (Hurtado et al 2008). *K. striatum* preferred to grow in shallower area at a depth of 1 m below the water surface (Gerung & Ohno 1997) while *K. alvarezii* preferred to grow at 3 m below the water surface (Ohno et al 1994).

Conclusions. The present study reveals the strong variations found in cultured *K. alvarezii* and *K. striatum*, as demonstrated by the values obtained for growth rate and carrageenan content. The monsoons or seasonal events can exert major environmental changes in the farm area and consequently affecting the growth and carrageenan yield. The results suggest that the highest growth rate and carrageenan yield of *K. alvarezii* and

K. striatum can be obtained during the northeast monsoon. It is suggested that the seaweed farmers should plant more during this season in order to increase their harvest hence their income. The seaweed farmers should be encouraged to plant more of *K. alvarezii* over that of *K. striatum* as this species yielded higher growth rate and carrageenan yield.

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