



# Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, México

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## Abstract

Three color strains of the  $\kappa$ -carrageenan producing red alga *Kappaphycus alvarezii* were cultured in Dzilam, Yucatán, over a 6-month period using the fixed off bottom monoline culture method to determine the technical viability of producing commercially this seaweed in the tropical waters of the Yucatán peninsula. Seawater temperature ranged from 28 to 31 °C, with a daily fluctuation range between 25.9 and 31.9 °C. Irradiance showed a strong fluctuation along the study period with maximal recorded irradiance during April (881.8  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ). Ammonia accounted for 55.1–89.8% of total DIN depending on the season, and salinity did not change significantly. Temperature was the only environmental factor that explained 96% of the observed variation in *K. alvarezii* growth rate ( $R^2=0.96$ ). The highest average growth rates obtained were  $6.5 \pm 1\% \text{ day}^{-1}$  for the red strain,  $7.1 \pm 1.8\% \text{ day}^{-1}$  for the brown strain, and  $8.1 \pm 1.6\% \text{ day}^{-1}$  for green strain during June. Lowest growth rates were obtained during August and September for the green strain ( $2.0 \pm 0.6\% \text{ day}^{-1}$ ). Despite the significant differences in growth between months no significant differences among color strains were found ( $P>0.05$ ). The growth rates of *K. alvarezii* were exponential during April to June. A 10-fold increase in weight was obtained after 30 days in cultivation for all the colored strains. As propagule weight increased the growth rate was reduced. Further, biomass yield (% weight gained) fluctuated as propagule weight increased and averaged  $33.2 \pm 8.4\%$  for the green,  $35.4 \pm 6.0\%$  for the red and  $36.3 \pm 10.8\%$  for the brown strain after 15 days. The highest carrageenan yield was obtained for the green strain (40.7%), whereas average carrageenan yields for the red strain were  $32.7 \pm 3.9\%$  and  $37.5 \pm 1.1\%$  for the brown. This study shows that *K. alvarezii* can be grown in the tropical waters of the

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Yucatán peninsula during the dry and part of the wet season. This period coincided with the closed season of the main fisheries. Thus, seaweed farming could be proposed as an alternative activity in the area.

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## 1. Introduction

*Kappaphycus alvarezii* (Doty) Doty ex Silva is an economically important red tropical seaweed highly demanded for its cell wall polysaccharide, being the most important source of  $\kappa$ -carrageenan in the world (Bixler, 1996). The market of carrageenan continues to grow and current sources of cultivated eucheumatoids seem incapable of meeting demand, at least in quality, price and volume for the requirements of the processing industry (Ask et al., 2003). Commercial cultivation of *K. alvarezii* was developed in the Philippines during the latter half of the 1960s using local varieties selected from the wild (Parker, 1974). The existence of color strains of *K. alvarezii* with varied pigmentation (green, red and brown) has been reported in the literature for decades. Differences in their physiological characteristics including growth performance and photosynthesis have been reported from laboratory studies (Dawes et al., 1994; Aguirre von Wobeser et al., 2001); however, in field studies or cultivation trials only one or two color strains (preferentially brown and green) have been studied so far (Ohno et al., 1994; Hurtado et al., 2001).

The selected and cultivated varieties of *K. alvarezii* have been introduced to numerous parts of the world for the purpose of research or the development of a commercial cultivation industry; though, only the Philippines, Indonesia, Malaysia (Sabah), Fiji and Tanzania are commercial producers today, selling quantities of 1000 Mt or more on a substantial continuous basis. In these countries seaweed farming has become a livelihood in coastal fishing communities, generating a cash income for more than 1500 fishing families in Indonesia, 80,000 in the Philippines and 400 in the Republic of Kiribati (Hurtado et al., 2001). In the last 30 years, commercial production of eucheumatoid species has increased from less than 1000 dry weight Mt to over 100,000 Mt that are produced annually by about 40,000–50,000 families worldwide (Ask and Azanza, 2002). Therefore, numerous tropical countries with coastlines are searching for seaweed cultivation as a sustainable alternative livelihood for coastal villagers, particularly as part of coastal management programs. Thus, countries including Cuba (Areces, 1995), Venezuela (Rincones and Rubio, 1999) and Brazil (Paula and Pereira, 2003) have introduced *K. alvarezii* in the warm waters of the Caribbean and the Western Atlantic in order to evaluate the feasibility of producing biomass for the carrageenan industry.

It is generally accepted that *Kappaphycus* requires warm sea water, high light levels, nutrient-enriched water and a high degree of water motion for successful cultivation (Glenn and Doty, 1990). The tropical coastline of the Yucatán peninsula, inside the Gulf of

Mexico, has the necessary environmental and oceanographic conditions for seaweed farming. Among its most notable morphological and oceanographic characteristics are the width of the continental shelf, known as Campeche Bank; an upwelling on the Yucatán Shelf that has been described as one of the most important upwelling regions on the western oceanic margin (Merino, 1997); currents associated with tides ( $1\text{--}4\text{ cm s}^{-1}$ ) and wind surface current circulation towards the west with velocities of  $5\text{--}18\text{ cm s}^{-1}$  (Martínez-López and Parés-Sierra, 1998). Moreover, from the economic point of view the demand for carrageenan in Mexico during 1990 exceeded 1000 Mt, and in 1994 the carrageenan imports surpassed \$14 million in value (Zertuche, 1996) increasing significantly in the last 10 years. The environmental and oceanographic conditions, together with the necessity of alternative livelihood for fishermen, and the internal and external demands for carrageenan, determine the potential for seaweed cultivation in the area. However, the development of a commercial size farm requires cultivation trials and environmental monitoring, considering the possibility of problems associated with seasonality in the growth of target seaweed.

This study reports on the seasonality of *K. alvarezii* using three colored strains and culture days over a 6-month period as factors in assessing growth rate and feasibility of producing this seaweed in tropical waters of the Yucatán peninsula. Carrageenan content was also evaluated as a measure of quality of the cultivated material.

## 2. Material and methods

### 2.1. Environmental factors

The Yucatán coast is a limestone platform characterized by marked seasonal differences in environmental conditions that affect physiological performance and biochemical constituents of seaweed species as reported by Orduña et al. (2002). In the cultivation area, the substrate consists of coarse sand and pebbles with small limestone outcrops where macroalgae such as *Gracilaria cornea* J. Agardh and *Euclima isiforme* (C. Agardh) J. Agardh are attached. The seagrasses *Thalassia testudinum* Kőning and *Syringodium filiforme* Kützing together with six species of *Caulerpa* occur on the surrounding sandy areas. Irradiance and seawater temperature were registered over the study period at regular intervals (2 and 1 h, respectively) using submersible data loggers (HOBO®, Onset Computer Pocasset, MA, USA) tied to the monolines in culture. Mean monthly temperature ( $\pm$ S.D.) was obtained by averaging all the values obtained for a single month. Light readings during the sunlight period of the day (13 h) were converted from Lux to photon flux density (photosynthetic active radiation, PAR, 400–700 nm, in  $\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ ) using an equation derived from linear regression obtained through the use of a Li-Cor 4 $\pi$  quantum sensor (Li-Cor, Lincoln, NE, USA). Two separate seawater samples were collected in the cultivation area each month for nutrient analysis. Dissolved inorganic nitrogen (DIN=sum of  $\text{N-NO}_3+\text{N-NO}_2+\text{N-NH}_4$ ) and dissolved reactive phosphorus (DRP= $\text{P-PO}_4$ ) were determined according to the method described by Strickland and Parsons (1972).

## 2.2. Cultivation of seaweed

The color strains of *Kappaphycus alvarezii* (red, green and brown) used in this study were kindly provided by Edison José de Paula (University of São Paulo, Brazil) in December 1999. These strains have been in cultivation at an experimental farm in Ubatuba, Brazil, since 1995 (Paula et al., 2002; Paula and Pereira, 2003). The plants were originally introduced from Northern Bohol, Philippines, and selected from vigorous and sterile plants propagated in Uranochi Inlet in Tosa Bay, Japan (Ohno et al., 1994). In Mexico, plants were kept for 3 years in quarantine at our research laboratory in CINVESTAV-Unidad Mérida, 30 km from the coast. Quarantine protocol considering the Mexican Official Code NOM-011-PESC-1993 was used as proposed for the introduction of commercial eucheumatoids by Ask et al. (2003).

Plants were out planted to an experimental seaweed farm located at Dzilam ( $21^{\circ} 10' \text{ N}$ – $89^{\circ} 45' \text{ W}$ ), Yucatán (Fig. 1). Seedlings of the selected varieties were transported to the farm site into Styrofoam boxes. *K. alvarezii* cultivation experiments were performed during 6 months from April to September of 2002, coinciding with the dry and wet season. These months present the best environmental conditions that might be favorable for *K. alvarezii* farming at this site. The dry season occurs between March and June, and is characterized by

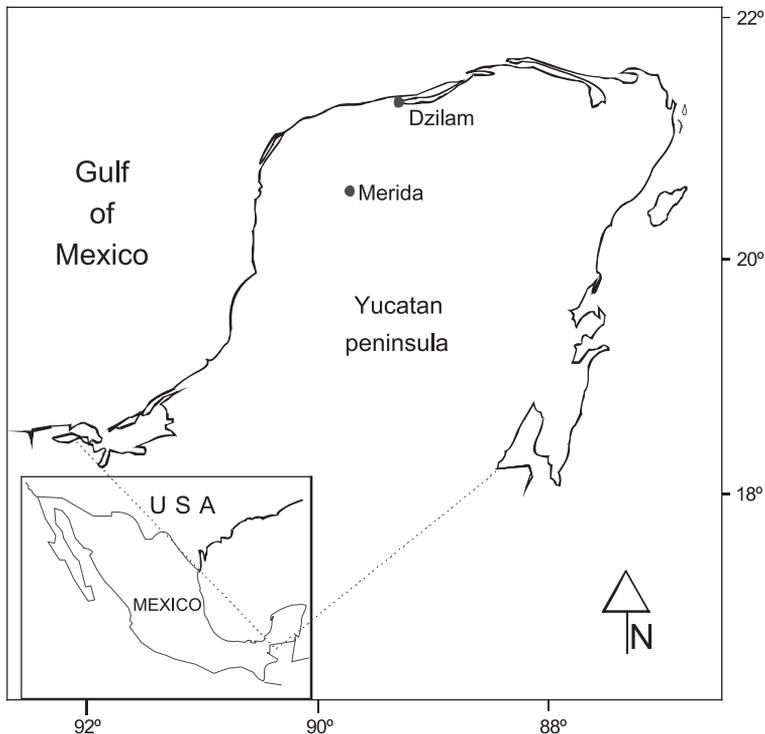


Fig. 1. Map showing location of experimental farm site at Dzilam, Yucatán peninsula, México.

sunny days and calm water, while the wet season occurs between July and October, and is characterized with tropical rainfall and high temperatures. Cold season that occurs from November to February was not considered in this study due to stormy weather, prevailing northern trade winds, high water turbidity ( $>160 \text{ mg L}^{-1}$  SPM) and low temperature.

The standard off bottom monoline farm was employed (Trono, 1992) using twenty-seven 10-m nylon monofilament lines (200 lb) spaced 0.7 m apart and deployed in an area of  $200 \text{ m}^2$ . The cultivation area was confined with fishing net (1 in. mesh size) to avoid fragment dispersion. In the fixed off bottom monoline culture method wooden stakes ('Tabché', local hard wood *Conocarpus erectus* Linnaeus) were arranged in rows. The monolines were positioned parallel to the direction of the prevailing current (east to west). The initial weight of inoculum was  $\sim 100 \text{ g}$ , tied 25 cm of each other with 3-mm polypropylene line allowing a density of 40 fragments per line. Each plant was tagged to follow individual weight increase. Each line supported  $\sim 4\text{-kg}$  cuttings.

The seaweeds were harvested at 15-day intervals in order to determine the daily growth rate ( $\text{DGR} = \% \text{ day}^{-1}$ ). Daily growth rate was calculated using a formula that takes into account exponential growth as shown for other field studies (Dawes et al., 1994).

$$\text{DGR} = \ln(W_t/W_0)/t \cdot 100 \quad (1)$$

where  $W_0$  is the initial weight (g),  $W_t$  is the final weight (g) after the number of culture days ( $t$ ). The data are presented as mean daily growth rates per 15 days.

Each month plants reached approximately 1 kg, then thalli were fragmented into new 100-g fragments. The biomass gained during this study was used as new seeding material in a subsequent experiment to evaluate the effect of initial propagule weight (from 100 to 2000 g) on daily growth rate and biomass increase or net yield (% weight gained) after 15 days in cultivation. The above experiment followed the same cultivation method and growth rate determination previously described.

### 2.3. Carrageenan extraction and viscosity determination

At the end of the cultivation period 1 kg fresh weight of each strain was harvested for carrageenan extraction. Seaweed was washed with tap water to eliminate sand, debris and epiphytes, sun dried (with 35% humidity content), milled and stored until carrageenan extraction. Native carrageenan extraction was performed in triplicate according to Dawes et al. (1977) with the following modifications: each extraction used 5 g dry weight of *K. alvarezii* that were soaked in 500-ml distilled water for 12 h and heated at  $85 \text{ }^\circ\text{C}$  for 3 h. The mixture was ground with a commercial blender, mixed with Celite and finally hot pressure filtered. The filtrate was maintained at  $66 \pm 1 \text{ }^\circ\text{C}$  and adjusted to 8.5–8.6 pH adding NaOH 0.3 N. Filtrates were precipitated with 250 ml of 2% hexadecyl–trimethyl–ammonium bromide (CTAB) in 9:1 distilled water/acetone. Carrageenan was recovered with paper filter over a funnel. The pellet was washed three times with 63 ml ethanol 95% nearly saturated with sodium acetate to remove CTAB residues, and three additional times with 95% ethanol to remove sodium acetate residues. The carrageenan was recovered into

glass Petri dishes, oven dried at 60 °C for 24 h, weighed, and the carrageenan content or yield (%) determined according to the formula:

$$\text{Yield} = (W_c / W_{ds}) \cdot 100 \quad (2)$$

where  $W_c$  is the extracted carrageenan weight (g) and  $W_{ds}$  is the dry seaweed weight (g) used for extraction. The data are presented as mean yield obtained from three replicates.

Triplicate samples of the above-extracted carrageenan (0.3 g) were dissolved in 20-ml hot distilled water (1.5% solution) for 20 min. Samples were homogenized and allowed to stabilize in a recirculating water bath at 75 °C. Viscosity was determined with a Cole-Parmer® viscometer on samples run at 20 rpm for 30 s using the spindle No. 8. These values are expressed as centipoises (cP).

#### 2.4. Statistical analysis

Data were tested for normality and homogeneity of variance using Stat-graphics Plus V. 3.0. Pearson's correlation analysis was used to determine the correlation coefficient between environmental factors and daily growth rates. Stepwise multiple regression was used to determine the individual contribution of the environmental factors (independent variables) to the total variance of growth rates (dependent variable). Analysis of variance (ANOVA) was also conducted to determine the differences in growth rate among color strains (red, green, brown), among seasons (dry, wet), and also for carrageenan yield and viscosity ( $P=0.05$ ).

### 3. Results

#### 3.1. Environmental factors

Monthly changes in environmental factors at the experimental farm are shown in Fig. 2. Seawater temperature around Dzilam ranged from  $28 \pm 0.7$  °C in April to  $30.9 \pm 0.8$  °C in September with a daily fluctuation range between 25.9 and 31.9 °C. Salinity did not change significantly, with the lowest value (37) during April and the highest value (38) recorded in September. Irradiance showed a strong fluctuation along the study period. The maximal recorded irradiance was  $881.8 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  during April with a monthly average of  $76 \pm 2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ . During late June the lowest irradiance was recorded with a monthly average of  $5.5 \pm 4.6 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  which corresponds to the start of the wet season, reflecting the conditions of cloudy days and increasing seawater turbidity.

Nutrients ranged from 5.9 to  $10.45 \mu\text{M L}^{-1}$  for dissolved inorganic nitrogen (DIN) and 0.8 to  $1.1 \mu\text{M L}^{-1}$  for dissolved reactive phosphorus (DRP). Ammonia accounted for 55.1–89.8% of total DIN depending on the season. The highest DIN values were found in May and July, whereas the lowest was obtained in September. In contrast, the lowest DRP was found in May and increased during the wet season months.

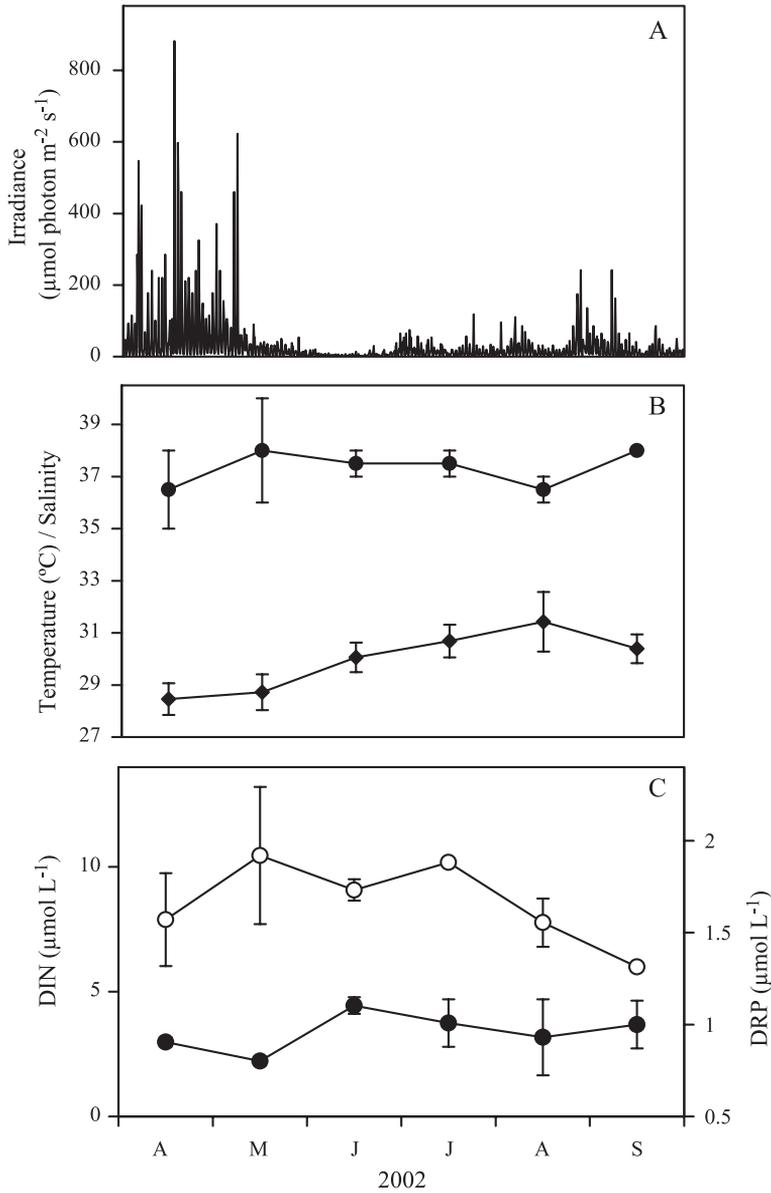


Fig. 2. Monthly variation of environmental factors over the cultivation period in Dzilam. (A) Irradiance, (B) temperature (◆) and salinity (●), and (C) nutrients: DIN (○) and DRP (●). Standard deviation indicated ( $\pm$ S.D.).

### 3.2. Growth rate

A clear seasonal variation in *K. alvarezii* growth rates was observed (Fig. 3). The highest average growth rates obtained were  $6.5 \pm 1\% \text{ day}^{-1}$  for the red strain (Fig. 3C),

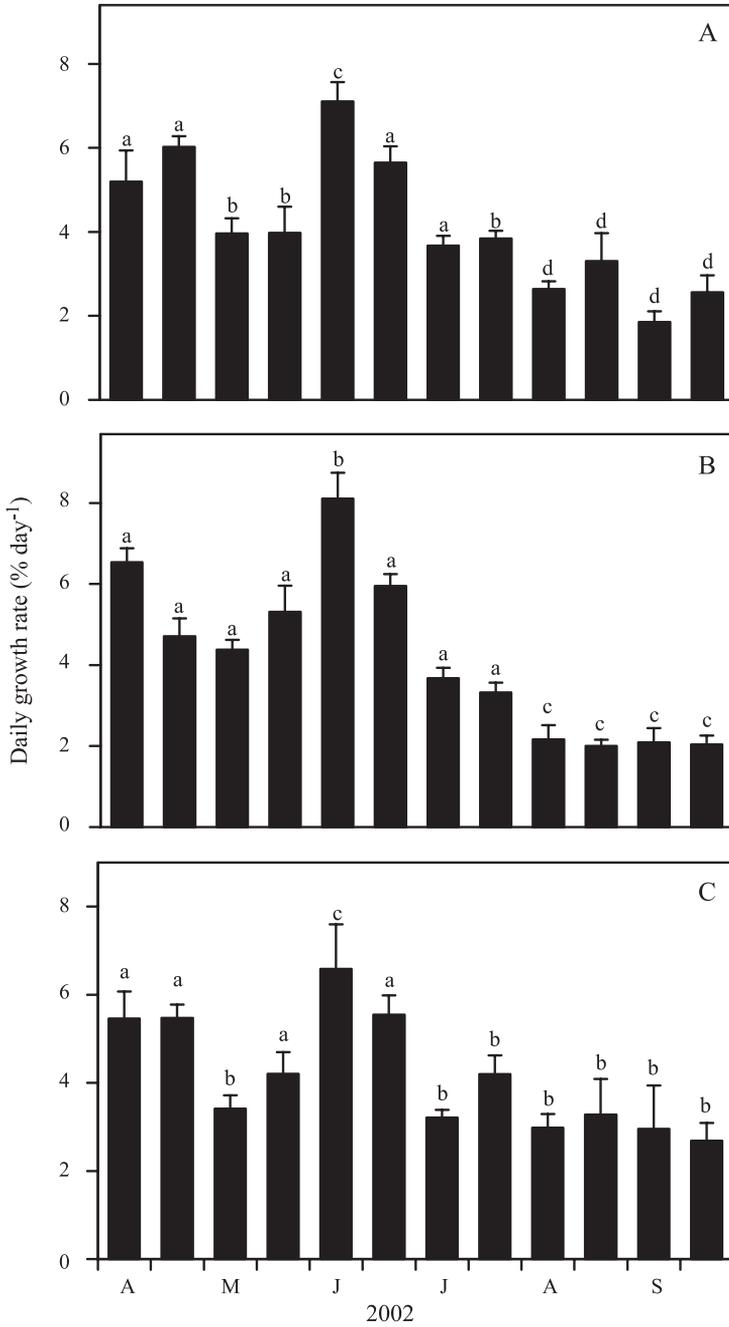


Fig. 3. Daily growth rate of *K. alvarezii* over the cultivation period in Dzilam. (A) Brown, (B) green, and (C) red color strain. Bars with the same letters are not significantly different ( $P < 0.05$ ). Standard error indicated ( $\pm$ S.E.).

$7.1 \pm 1.8\% \text{ day}^{-1}$  for the brown strain (Fig. 3B) and  $8.1 \pm 1.6\% \text{ day}^{-1}$  for the green strain (Fig. 3B) during June, and the lowest were obtained during August and September for the green strain ( $2.0 \pm 0.6\% \text{ day}^{-1}$ ). Reduction in growth rate was evident during August–September for the brown and green strains when compared to other months. No significant

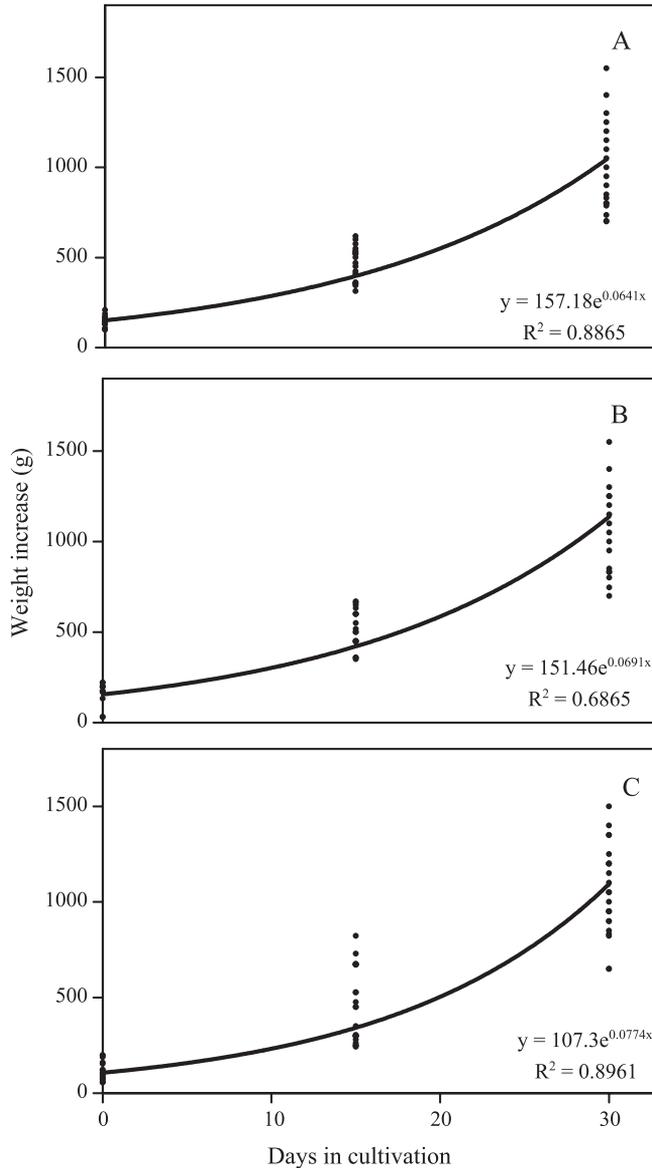


Fig. 4. Exponential growth curves of *K. alvarezii* after 30 days in cultivation at Dzilam. (A) Brown, (B) green, and (C) red color strain. The lines represent the best fit using at least 15 replicates.

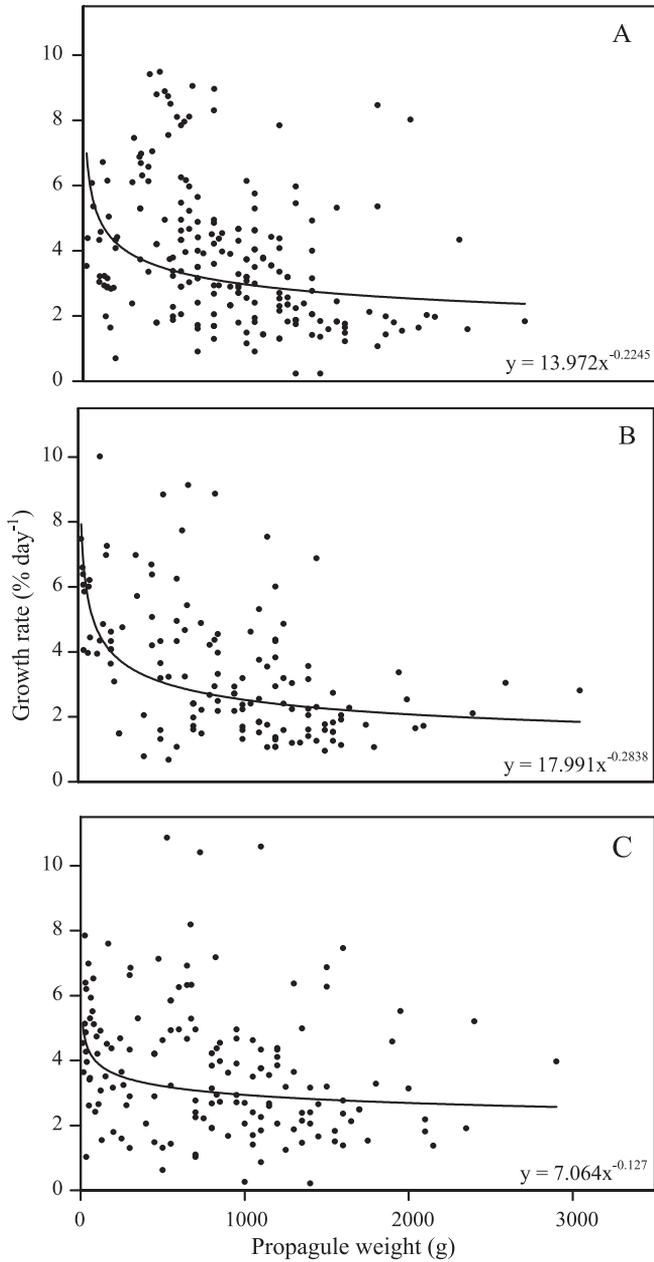


Fig. 5. *K. alvarezii* growth rate as a function of propagule weight. (A) Brown, (B) green, and (C) red color strain. The lines represent the best fit using growth rate data for brown ( $n=210$ ), green ( $n=139$ ), and red color strain ( $n=155$ ).

differences in growth rate for the green strain were found between April, May and July, whereas for the red strain no significant differences were found between the first part of May and June throughout September. Despite the significant differences in growth between months the ANOVA showed no significant differences among color strains ( $P>0.05$ ).

The growth rates of the three color strains of *K. alvarezii* were exponential (Fig. 4) during the month of highest growth (June), with the highest regression coefficient for the

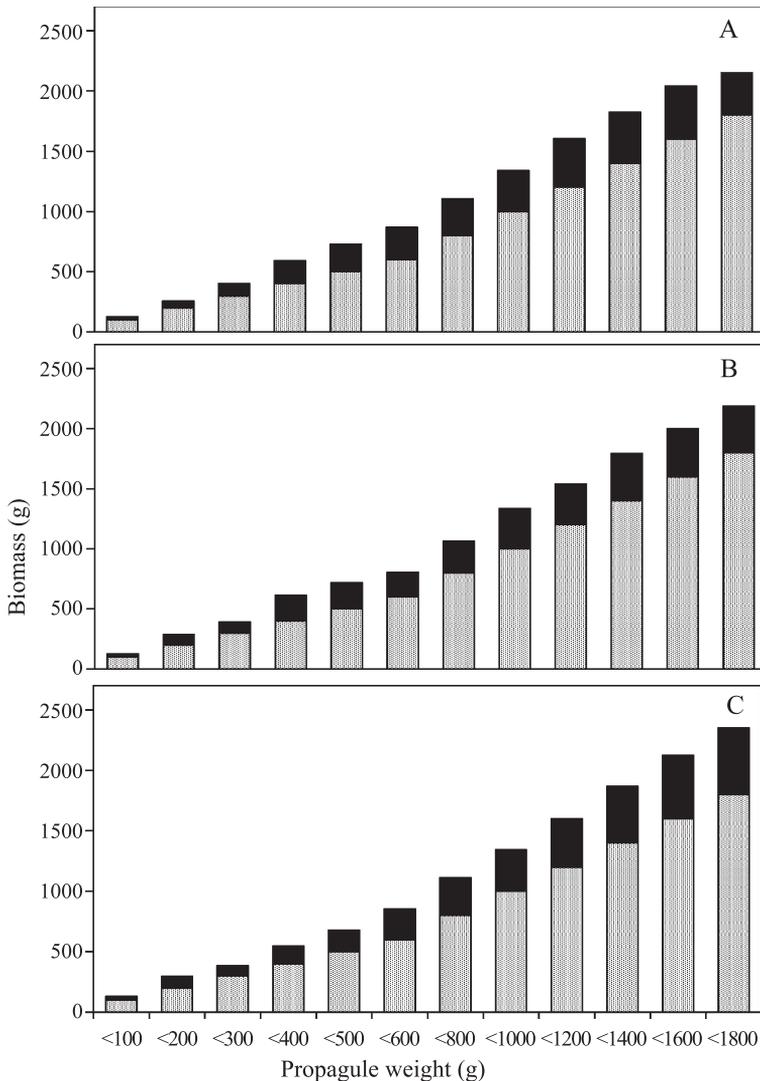


Fig. 6. *K. alvarezii* biomass increase in relation to propagule weight. (A) Brown, (B) green, and (C) red color strain. Initial weight of inoculum (▨); net biomass yield after 15 days in cultivation (■).

red strain (Fig. 4C). A 10-fold increase in weight was obtained after 30 days in cultivation for all the colored strains. As propagule weight increased the growth rate was reduced (Fig. 5). *K. alvarezii* growth rates calculated from the equation, assuming a standard propagule weight of 100 g, were 3.9% day<sup>-1</sup> for the red (Fig. 5C), 4.8% day<sup>-1</sup> for the green (Fig. 5B), and 4.9% day<sup>-1</sup> for the brown strain (Fig. 5A). Biomass yield (% weight gained) (Fig. 6) fluctuated as propagule weight increased and averaged 33.2±8.4% for the green (Fig. 6B) 35.4±6.0% for the red (Fig. 6C), and 36.3±10.8% for the brown strain (Fig. 6A). The highest biomass yield (48.5%) was obtained for propagules of the red strain weighing 200 g, whereas a propagule weight of 400 g gave the highest biomass yield for the brown and green strains (47.7 and 53%, respectively). The lowest biomass yields were obtained for propagules weighing ≤1800 g, and a great proportion of propagules weighing ≥2000 g were lost due to breakage.

Temperature was negatively correlated with daily growth rate showing that the period of minimal growth corresponds to the highest water temperatures (Table 1). On the other hand, dissolved reactive phosphate was positive correlated with daily growth rate ( $P<0.05$ ). Multiple stepwise regression identified temperature as the only environmental factor that explained 96% of the observed variation in *K. alvarezii* growth rate ( $R^2=0.96$ ).

No evidence of grazing activity was observed throughout the study period, although herbivore fish of the family Sparidae (*Archosargus rhomboidalis* Linnaeus, 1758; and *Lagodon rhomboides* Linnaeus, 1766) were commonly observed in the farmed area. The presence of the sea urchin *Arbacia punctulata* Lamarck was noted at densities >7 individuals m<sup>-2</sup>, particularly during June and July; however, no evidence of damage or grazing was noted in farmed plants.

### 3.3. Carrageenan yield and viscosity analysis

Carrageenan yield and viscosity for color strains are shown in Table 2. The highest carrageenan yield was obtained for the green strain (40.7%), whereas the average carrageenan yields for the red strain were 32.7±3.9% and 37.5±1.1% for the brown. The carrageenan extracted from the green strain also had the highest viscosity with a mean value of 161.1±20.1 cP, while the carrageenan from the brown and red strains showed lower viscosities (132.6±7.4 and 133.5±11.7 cP, respectively). However, no significant

Table 1

Correlation coefficients between daily growth rate of *K. alvarezii* (% day<sup>-1</sup>) and main environmental factors at Dzilam

	Daily growth rate	Irradiance	Temperature	DRP	DIN
Daily growth rate	–	–0.12	–0.99*	0.89*	0.27
Irradiance		–	–0.15	–0.30	–0.55
Temperature			–	–0.92*	–0.32
DRP				–	0.48
DIN					–

Irradiance (μmol photon m<sup>-2</sup> s<sup>-1</sup>), temperature (°C) and nutrients: DRP and DIN (μmol L<sup>-1</sup>).

\* Indicates significance at  $P<0.05$ .

Table 2

*K. alvarezii* growth rate (% day<sup>-1</sup>), carrageenan yield (%) and viscosity (cP) reported in the literature for the different color strains cultured experimentally in open sea

Specie	Strain	Growth rate	Yield	Viscosity	Reference
<i>K. alvarezii</i>	Brown	0.1–8.4	–	–	Dawes et al. (1994)
	Green	0.2–6.3	–	–	
	Brown	0.13–8.12	27.6–42.5	890	Ohno et al. (1994)
	Green	0.2–7.3	–	–	
	Brown	1.1–3.4	7.5–8.5	23–34.5	Hurtado (1995)
	Green	0.9–3.2	4.7–4.8	28–34.5	
	Red	1.2–3.8	10.8–11.6	24–25.5	
	Brown	4–11	18.8–24.6	52.5–71.4	Ohno et al. (1996)
	Brown	0.2–4.2	–	–	Hurtado et al. (2001)
	Green	0.9–3.9	–	–	
	Brown	3.6–8.9	25–35	–	Paula and Pereira (2003)
	Brown	2.0–7.1	36.8–38.8 (1.1)	122.2–147.2 (12.8)	This study
	Green	2.0–8.1	33.7–40.7 (3.6)	121.6–186.8 (34.8)	
	Red	2.6–6.5	30.3–37.2 (3.9)	143.3–110.0 (20.4)	

Standard deviation in parentheses.

differences in either carrageenan yield or viscosity were found between the three color strains ( $P > 0.05$ ).

#### 4. Discussion

*Kappaphycus alvarezii* can be grown in the tropical waters of the Yucatán peninsula, Mexico, during the dry and part of the wet season. The three color strains of *K. alvarezii* showed maximum growth rates (6.5–8.1% day<sup>-1</sup>) similar to the results obtained in subtropical waters of Brazil (Paula and Pereira, 2003). In Brazil the brown strain of *K. alvarezii* grew between 3.6% and 8.9% day<sup>-1</sup>. Nevertheless, our results were higher than those obtained in Panagatan Cays, Philippines, for the brown and green strains that ranged from 2.3% to 4.2% day<sup>-1</sup> (Hurtado et al., 2001). From April to June the highest growth rates (above 5.5% day<sup>-1</sup>) were obtained. Glenn and Doty (1990) reported average growth rates for *K. alvarezii* at 5.06% day<sup>-1</sup> in Hawaii. In Dzilam, from July to September (wet season) *K. alvarezii* growth ranged from 2.0% to 2.7% day<sup>-1</sup> for the green, 2.5% to 3.3% day<sup>-1</sup> for the brown and 2.6% to 3.1% day<sup>-1</sup> for the red strain. These growth rates are comparable with the results obtained by Adnan and Porse (1987) and Mollion and Braud (1993) at 2.5–3.5% and 3.0–4.0% day<sup>-1</sup>, respectively (as *Euचेuma cottonii*). However, our results were slightly lower than those reported by Luxton et al. (1987) in the south Pacific Islands (3.5–3.7%).

There was no significant difference in the growth rates between the three color strains, which is similar to the results for green and brown strains cultured in northern Bohol (Trono and Ohno, 1989). In contrast, Dawes et al. (1994) and Hurtado (1995) reported slightly higher growth rates for the green strain when compared to brown or red in laboratory and field studies. In the former study, ecotypic differentiation and variation in photosynthetic efficiency were not exhibited by *K. alvarezii*. More recently, it has been

shown that differences in the pigment content for these cultivars had no effect on either photosynthesis or growth (Aguirre von Wobeser et al., 2001).

According to the site fertility concept, seaweed growth is regulated by a complex interaction of irradiance, temperature, nutrients and water movement (Santelices, 1999). Some of these factors may interact regulating the growth of the target species and that a major decline of one factor (e.g. nutrients) could be compensated by another factor (e.g. water movement). Thus, in environments with low or erratic nutrient supply, surge ammonium uptake has been described for *Kappaphycus alvarezii* as a strategy to avoid nitrogen limitation of growth (Dy and Yap, 2001).

Temperature, light intensity and nutrients were believed to be the most important factors affecting *Kappaphycus* growth (Glenn and Doty, 1990). However, these authors reported a low correlation between growth and the environmental factors studied. In another study, Glenn and Doty (1992) demonstrated that water motion accounted for 81–98% of the variation in growth rate, although they also found an inverse significant relationship between maximum temperature and growth. Further, they report that photosynthetic temperature response for *K. alvarezii* increased up to 32 °C, then sharply declined.

In the present study, temperature was the main environmental factor affecting the growth rates of *K. alvarezii* in Yucatán. Ohno et al. (1994) showed that *K. alvarezii* grew well only during the warm weather season (20–30 °C) in the subtropical waters of Tosa Bay, Japan. Similar results have been found for the subtropical waters of Brazil, where a positive correlation with temperature was observed with the growth of the brown strain of *K. alvarezii* (Paula and Pereira, 2003). The seasonal variation of seawater temperature at Dzilam (28–31 °C), when compared to Kaneohe Bay, Hawaii (21–28 °C; Glenn and Doty, 1990), and Ubatuba Bay, Brazil (17–33 °C; Paula and Pereira, 2003), may explain the differences in growth between subtropical and tropical areas.

The temperature range, where *K. alvarezii* is farmed, indicates that rapid growth and high biomass production occur during months characterized by warmer temperatures, i.e. 25–30 °C as reported by Trono and Ohno (1989). In the shallow waters of the experimental farm at Dzilam environmental conditions may change very rapidly over time, such as water temperature. Reduction in *K. alvarezii* growth during the wet season could be attributed to increase temperature. Further, temperature explained 96% of the variation in growth rate at Dzilam. The effect of temperature during the wet season could be compensated by increasing water motion or water depth to avoid any detrimental effect on growth rate.

In Dzilam, growth rates of *K. alvarezii* decreased by 34% (green strain), 45% (brown strain) and 47% (red strain) during warmer months when compared to the best growing months. In Vietnam *K. alvarezii* growth rates decreased 63% during August, the warmest month (Ohno et al., 1996). In Fiji, high growth rates occur during the southeasterly trade winds (April to December), while growth slows in January to March due to elevated water temperatures and a change in the wind patterns (Prakash, 1990). In the Philippines the highest growth was observed during September to February, whereas growth rates during the southwest monsoon (July to August) were minimal, mainly due to strong wave action, lower temperatures and low salinity brought by monsoon rains (Hurtado et al., 2001).

Biomass losses of cultivated material have been attributed to herbivore grazing or plant breakage. Biomass losses of 50% due to grazing have been reported in Hawaii (Russell, 1983); however, no grazing was evident in our study. Either the net used to confine the cultured area served as a barrier for some herbivore fishes or these fishes may not graze this exotic seaweed due to its deterrent chemical compounds (Cetrulo and Hay, 2000). Although a high density of sea urchins was noted ( $>7$  individuals  $m^{-2}$ ), none were found feeding within cultured plants, most probably because of the distance between monolines and sea bottom ( $\sim 70$  cm).

On the other hand, breakage ratio has been related to the size of the thalli and roughness of the sea, reaching above 10% during windy weather (Mollion and Braud, 1993). In our study, propagules weighing  $\leq 1800$  g only grew between  $2.1\%$   $day^{-1}$  for the green and  $2.7\%$   $day^{-1}$  for the red, whereas biomass gain was only 19.4% for the brown and 26% for the red strain under calm weather conditions; however, during strong wind and currents greater losses may occur. If cultivation of *K. alvarezii* is intended during the cold season in the Yucatán coast (October to February) other cultivation methods should be employed. In future studies, net tubing method such as the one used in the Philippines during adverse weather conditions could be tried to evaluate growth performance of the different color strains during cold season.

The information on carrageenan content of *K. alvarezii* remains limited, despite the great importance of this genus in the phycocolloid industry. The extraction methods used by authors make carrageenan yield comparison quite difficult. In this study, the use of CTAB allowed the precipitation of pure carrageenan without precipitation of the floridean starch fraction. This fraction has been shown to account for  $\sim 14\%$  of the polysaccharide content in other carrageenophytes (Chopin et al., 1991). Thus caution should be employed when reviewing carrageenan yields in publications based on ethanol precipitation as they are probably overestimates because of co-precipitation with floridean starch. This could be the reason why our yields are lower than those reported in the Philippines (54.6%) and Indonesia (45%), the traditional sources of biomass for  $\kappa$ -carrageenan extraction (Trono and Ohno, 1989). Nevertheless, our values are within the range of the industrial requirements for this specie ( $\sim 38\%$ ) (Table 2). In addition, the higher viscosities obtained in the present study when compared to those reported by Hurtado (1995) may be attributed to the growing conditions in the area.

The introduction of *K. alvarezii* has been the subject of much discussion and many countries have adopted a very cautious attitude to the introduction of this species. Nevertheless, all the experimental evidence available today indicates that the introduction of this specie has no deleterious effect on the natural biota of the countries involved (McHugh, 2001; Paula et al., 2002). However, it is important that quarantine protocols must be adopted and monitor procedures established in order to minimize the risks of such introduction as proposed by Zemke-White (2002) and Ask et al. (2003).

In the Yucatán coast, the feasibility of cultivating *K. alvarezii* has been demonstrated in this study. As reported by Ask and Azanza (2002) growth rates above  $3.5\%$   $day^{-1}$  are considered adequate for commercial farming. During April to July the best growing conditions were observed, with growth rates above  $4\%$   $day^{-1}$ . This period coincided with the closed season of the main fisheries in the area (i.e. octopus, lobster, grouper). Therefore, *Kappaphycus* cultivation could be proposed as an alternative livelihood for fishermen

during this season. However, experimental testing of potentially useful farming areas, cultivation technologies and routines are needed before farms are expanded to full scale.

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