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Kappaphycus alvarezii (Rhodophyta, Areschougiaceae) cultivated in subtropical waters in Southern Brazil

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Abstract Four strains of *Kappaphycus alvarezii* were cultivated in the subtropical waters of Florianópolis, Santa Catarina State, Brazil (27°29′19″ S/48°32′28″ W), from February 2009 to February 2010. Seaweeds were cultivated on floating raft near of mussel farms. Salinity ranged from 29 to 36 psu and temperature from 17.1 to 28.5°C. Higher growth rates (5.12–4.29% day^{-1}) were measured in summer and autumn, showing a positive correlation between growth rate and water temperature. Lower growth rates (0.54–0.32% day^{-1}) occurred in winter, resulted mainly by biomass loss. Significant differences were observed among the strains in spring and the brown tetrasporophytic strain was the only one which failed to recover, being excluded of the experiments. The effect of cultivation periods (36, 42, and 97 days) on carrageenan yield, gel strength, and viscosity were analyzed. Carrageenan yields were higher for plants kept 42 days in the sea (28%), against 25% for 36 and 97 days. There were no significant differences in carrageenan yield among the strains analyzed. Viscosity increased with the increase of cultivation period, while gel strength seemed to vary at random. Tetrasporangia and cystocarps were not observed, and lost fragments did not attach outside the raft. In general, dissolved inorganic nitrogen concentration decreased around the cultivation area as compared to the mussel farm. Results show that cultivation of *K. alvarezii* is technically feasible in subtropical waters and can be associated with local mussel farms, mitigating the eutrophication and, eventually, increasing the economic return of the farmers.

Keywords Aquaculture · Brazil · Carrageenan · *Kappaphycus alvarezii*

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Introduction

The production of *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) in 2007 reached ca. 1,400 tonnes, yielding US $132,000 (FAO FishStat Plus Database 2010). This species is the main source of *kappa* carrageenan, a hydrocolloid utilized worldwide as a thickening agent in several industries. The success of commercial farming in the Philippines, one of the main producers together with Indonesia, stimulated the introduction of the species in several countries (Ask and Azanza 2002).

Documented introduction of *K. alvarezii* in Brazil took place in 1995 on the coast of São Paulo State (23°27′85″S/45°02′49″W) to evaluate the potential of its commercial cultivation (Paula et al. 1999). Commercial cultivation south of Rio de Janeiro began in 1998 at Ilha Grande Bay (23°09′17″S/44°20′08″W). In 2003, a commercial farm was established at Sepetiba Bay (22°57′06″S/43°54′26″W) (Castelar et al. 2009).

After more than 10 years of research and experimental cultivation (Paula et al. 2002; Paula and Pereira 2003; Bulboa and Paula 2005; Hayashi et al. 2007a; Hayashi et al. 2007b), formal authorization to farm the species was granted in 2008 by the Brazilian Government from the northern littoral of São Paulo to the southern littoral of Rio de Janeiro States.

Data from the Brazilian Ministry of Development, Industry and Foreign Trade show importation values of dry *K. alvarezii* and *Eucheuma denticulatum* of approximately 1,800 tonnes from ASEAN (Association of Southeast Asian Nations) countries, in addition to 1,400 tonnes of carrageenan in 2009, indicating that Brazil is still dependent of seaweed and carrageenan importation.

K. alvarezii cultivation in Brazil is just starting and should expand in the near future. With the aim of expanding the allowed farming area, the species was introduced in Florianópolis, Santa Catarina in 2008 under the auspices of the Brazilian Environmental Institute (IBAMA). Santa Catarina was chosen as a promising area for this species experimental cultivation because has the highest mariculture development and social structure towards the production of marine organisms, mainly mollusks, in Brazil. Previous studies (Ohno et al. 1994; Areces 1995; Ask 2006) have shown that the species does not thrive in temperatures below 20°C, which is the case in Florianópolis during the winter. Even so, we decided to test the viability of its cultivation in the region to bring additional revenue to mollusk farmers and to remediate the eutrophication brought by this activity. This work presents the first results to test the viability to farm this species in the region.

Material and methods

Sea cultivation

Four strains of *K. alvarezii* (brown, green, and red tetrasporophytes and brown female gametophyte), which originated from the experimental cultivation at Ubatuba, SP, Brazil, were kept under quarantine for one year using inland 0.5 m^3 tanks at the Universidade Federal de Santa Catarina. Subsequently, the algae were transplanted to the sea and cultivated on floating rafts at Ponta do Sambaqui (27°29′18.65″S/48°32′17.96″W), Florianópolis, SC. The raft was anchored on the fine sediment of the bay, and its basic structure consisted of three squares, each 3×4 m. In each square, eight tubular nets with seedlings were attached 0.3 m apart just below the water surface. A fishing net was installed below the tubular nets to minimize the dispersion of detached seedlings.

The experiments were conducted from February 2009 to February 2010. There were four tubular nets for each strain ($n=4$) which were weighed monthly. The tubular nets were inoculated with seedlings in a density of 1.15 kg m^{-1}, according to preliminary experiments. Mean growth rate (GR) was calculated according to Lignell and Pedersén (1989): GR (% day^{-1})=$[(W_t/W_i)^{1/n}-1]\times 100$, where W_i= initial wet weight and W_t=wet weight after t days.

Daily temperature was recorded with an Optic StowAway Tidbit Logger (TBI32-20–50) installed under the raft. Salinity was recorded weekly with a refractometer.

Effects of cultivation periods

Three cultivation periods were tested from February to July 2009: 36 days, 42 days and 97 days. These periods were related with the growth performance in different temperatures, and were chosen with the aim to evaluate its effects in the carrageenan yield, viscosity and gel strength. Growth rates were calculated according to Lignell and Pedersén (1989) described before, and net production (NP) was estimated as NP (kg m^{-2})=$(W_f-W_i)\times A^{-1}$, where W_i=initial wet weight, W_f=wet weight on the day of harvesting and A=cultivation area (m^2).

After each cultivation period, plants were harvested and samples were cleaned, air dried and then dried in an oven at 35 and 60°C, until 25–35% moisture had been reached, for carrageenan analyses.

Carrageenan analyses

Carrageenan data refers to samples from the three cultivation periods tested. Alkali-modified carrageenan was produced by alkali transformation in 6% KOH for 2 h at 80°C. The material was then washed overnight and the carrageenan extracted in distilled water at 60°C for 4 h, under agitation. The digestion product was filtered under low pressure and precipitated in 85% isopropanol with 0.2% KCl solution. The coagulum was recovered and dried in an oven at 60°C for 12 h (Hayashi et al. 2007b).

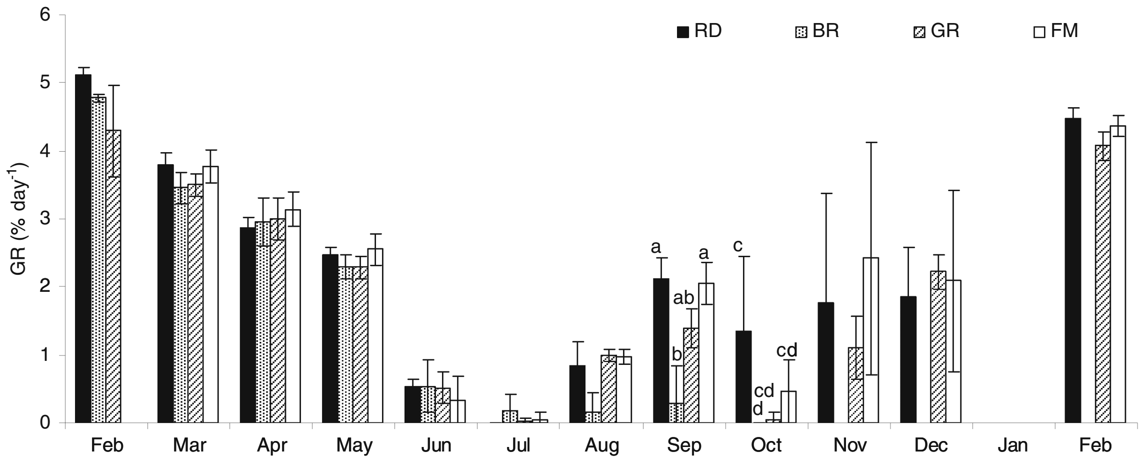

Fig. 1 Growth rates (GR% day^{-1}) of red (*RD*), brown (*BR*), and green (*GR*) tetrasporophytes and brown female (FM) gametophyte of *K. alvarezii* cultivated from February 2009 to February 2010. Values presented as average (n=4); vertical bars show the confidence intervals. *Letters* represent significant differences among the strains, according to Unequal *N* a posteriori test, considering $p<0.05$

Viscosity was analyzed in 1.5% carrageenan solution with a Brookfield Viscosimeter at 75°C and 30 rpm. Gel strength was measured in a 1.2% alkali-modified carrageenan with 0.3% KCl at room temperature, using the Texture Analyser TAXT plus.

Environmental monitoring

The intertidal area in the vicinity of the experimental cultivation was monitored every two weeks by visual scan to look for any attached specimens of *K. alvarezii*. After each harvest, samples of each strain were randomly selected and observed under a stereomicroscope to look for reproductive structures.

Water samples were collected monthly in three points of the bay, during the three cultivation periods tested (from February to July 2009): inside the seaweed raft, inside the mollusk farm located nearby and offshore, but still within the bay (considered the control point). The raft was located 330 m from the coastline and 100 m from the center of the mussel farm. For each point, three water samples were collected for determination of the dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP), according to Grasshoff et al. (1983)

Statistical analyses

Statistical analyses were determined using unifactorial ANOVA and either the Unequal N or Fisher LSD *a posteriori* test. Correlation coefficients were calculated using simple linear models (Pearson's). All statistical analyses were performed using Statistica™ (Release 6.0) at the $p<0.05$ level.

Table 1 Average, maximum and minimum temperature (°C) and monthly average salinity (psu) collected in the experimental cultivation of *Kappaphycus alvarezii* during 1 year

	Average temperature (°C)	Minimum temperature (°C)	Maximum temperature (°C)	Average salinity (psu)
February/09	27.18	25.4	28.5	33.8
March/09	26.98	25.4	28.5	32.0
April/09	24.49	21.6	27.2	32.1
May/09	22.46	19.7	23.5	34.0
June/09	17.99	17.3	19.5	35.2
July/09	17.74	15.7	19.3	35.2
August/09	18.07	15.5	20.2	35.0
September/09	19.75	16.8	20.8	35.0
October/09	20.81	17.6	23.4	31.0
November/09	25.53	24.0	26.9	33.9
December/09	26.52	24.4	30.1	33.3
January/10	27.23	26.1	28.4	33.5
February/10	28.03	26.4	29.8	35.0

Table 2 Correlation coefficients between average, maximum and minimum temperature (°C), average salinity (psu) and growth rates (GR) collected in the experimental cultivation of *Kappaphycus alvarezii* cultivated for 1 year

	Average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Average salinity (psu)	GR (% day^{-1})
Average Temperature (°C)		0.9711*	0.9216*	−0.4862	0.8951*
Maximum Temperature (°C)			0.8953*	−0.4509	0.8476*
Minimum Temperature (°C)				−0.4563	0.8266*
Average Salinity (psu)					−0.3943

*$p<0.05$, significant differences according to Spearman *R*

Results

Sea cultivation

Growth rates ranged from 0% to 5.12%day^{-1} during the experimental period. The highest growth rates were observed in February 2009 and February 2010 (between 4.07 and 5.12%day^{-1}) and the lowest growth rates were observed in June and July (Fig. 1). In this period, plants were brittle, and a considerable amount of branches were found in the fish net located under the raft. As it was not possible to identify all strains retained in the net, they were not considered for growth analyses.

Significant differences were observed among the strains in September and October. Brown tetrasporophytic strain (BR) showed significant lower growth rates and failed to recover, being excluded of the experiments from October. The growth rates observed in February 2009 were similar to the ones observed in February 2010 (Fig. 1).

Lowest temperatures were registered between July and August, and highest temperatures between December and February (Table 1). Positive correlation was observed between growth rates and temperatures. No correlation was observed between growth rates and salinity (Table 2).

Effects of cultivation periods

Mean growth rates in different cultivation periods ranged from 1.17±0.10% day^{-1} to 5.12±0.12% day^{-1}. Plants cultivated in 36 days showed growth rates significantly higher when compared with the rates observed in other cultivation periods, while plants cultivated in 97 days showed signifi-

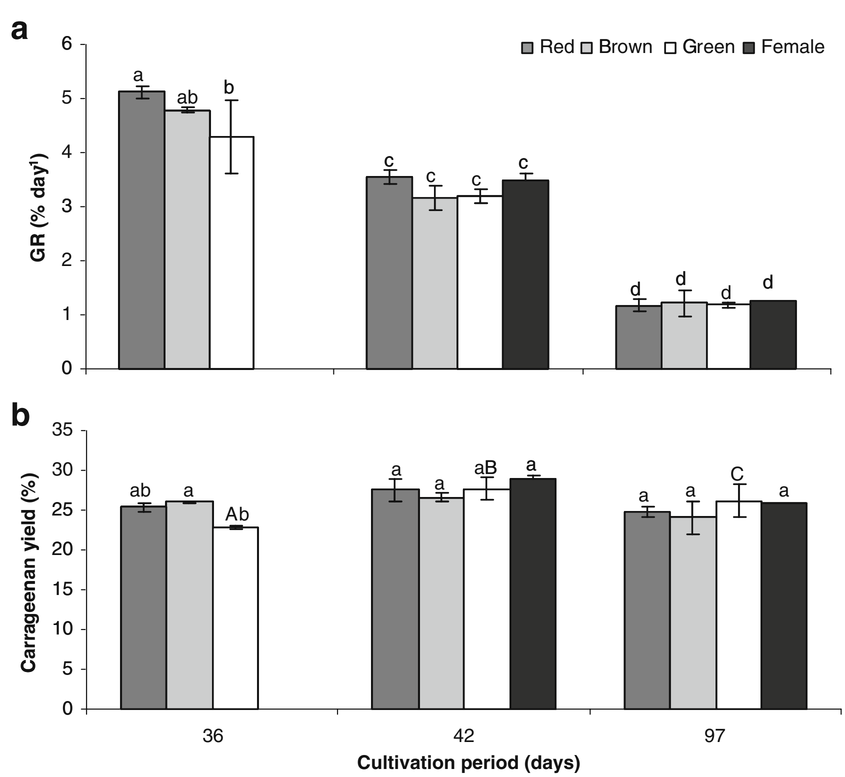

Fig. 2 **a** Growth rate (GR% day^{-1}) and **b** alkali-modified carrageenan yield of red (*RD*), brown (*BR*), and green (*GR*) tetrasporophytes and brown female (FM) gametophyte of *K. alvarezii* cultivated in three different cultivation periods (36, 42 and 97 days). Values presented as average ($n=4$); vertical bars show the confidence intervals. *Small letters* represent significant difference among the strains, and *uppercase letters* represent significant differences among the cultivation periods, according to Fisher LSD a posteriori test, considering $p<0.05$

Table 3 Alkali-transformed carrageenan viscosity and gel strength of red (RD), brown (BR) and green (GR) tetrasporophytes and brown female (FM) gametophyte of *Kappaphycus alvarezii* cultivated in different periods

Strains	Viscosity (mPas)			Gel strength (g cm^{-2})		
	36days	42days	97days	36days	42days	97days
RD	479±7	639±17	nd	1,398	1,462	1,124
BR	610±21	602±28	nd	1,095	1,683	936
GR	321±10	4625±431	nd	1,958	1,713	1,010
FM		3532±225	nd		1,700	1,579

Values presented as average±confidence intervals ($p<0.05$)

nd no data

cantly lower growth rates (Fig. 2a). Net productivity was 13.22, 9.22, and 7.00 kg m^{-2} for 36, 42, and 97 days of cultivation period, respectively.

Analyses of carrageenan

Yields of alkali-modified carrageenan varied from 28.8±1.0% (brown female gametophyte cultivated for 42 days) to 22.93±0.2% (green tetrasporophyte cultivated for 36 days). Higher carrageenan yields were obtained from samples of the 42-day cultivation period, although significant differences were observed only for the green tetrasporophyte. In general, there were no significant differences in carrageenan yield among the strains analyzed (Fig. 2b). Viscosity increased with the duration of the cultivation period, while gel strength values were more variable (Table 3). Samples from strains cultivated over 97 days presented such high viscosity that it was not possible to measure with our method.

Environmental monitoring

We found no reproductive structures or specimens attached to the rocky shore nearby or growing on the sediment throughout the course of the experiment.

Except for February and July, the concentration of DIN inside the seaweed pilot farm was significantly lower than the concentration inside mussel farms or offshore. DIP concentrations were significantly lower inside the pilot farm in April

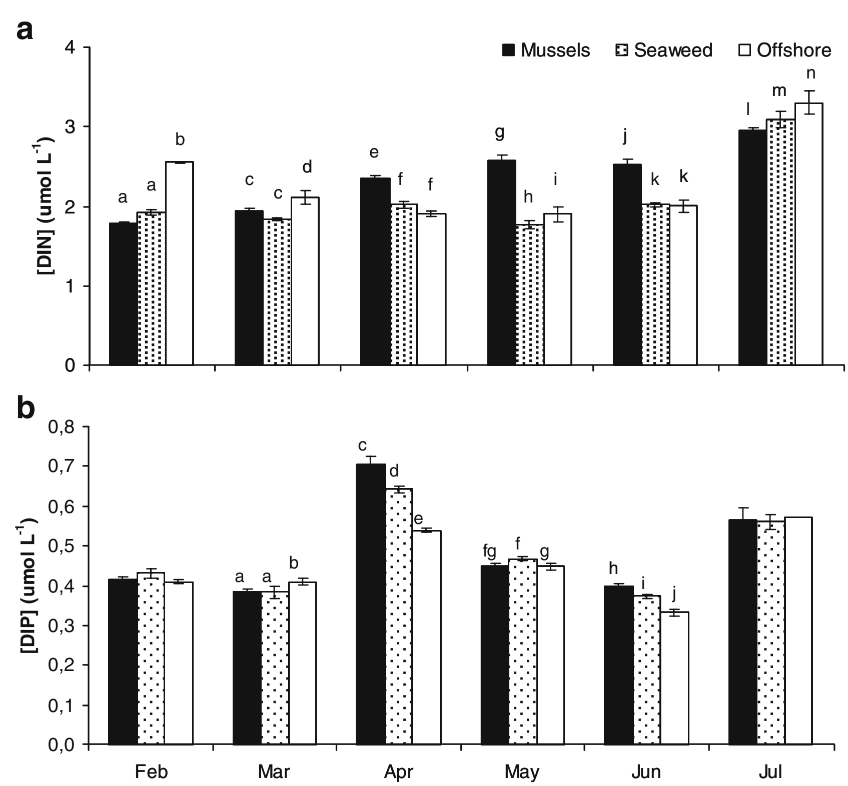

Fig. 3 **a** Dissolved inorganic nitrogen (*DIN*) and **b** dissolved inorganic phosphorus (*DIP*) of water samples collected in three different points: inside mussels farms (mussels), inside *Kappaphycus* cultivation (seaweed) and offshore from February to July 2009. Values presented as average± confidence intervals and letters represent significant differences, according to Fisher a posteriori test, considering $p<0.05$

and June when compared to the mussels farms; in other months, they were similar (Fig. 3).

Discussion

It is well known that *K. alvarezii* grows in water temperatures between 20°C and 32°C (e.g., Areces 1995; McHugh 2003; Ask 2006), and the introduction of the species in the subtropical water of Santa Catarina brought some concern about its survival in winter, a period when the water temperature can reach 16°C. Our results showed that this species could survive the local winter, although the growth rates were reduced. It was observed that none of the strains stopped growing. The utilization of tubular nets was fundamental for *K. alvarezii* farming in Florianópolis because of strong currents once the traditional tie–tie method did not work.

The higher growth rates observed in February 2009 and 2010 were slightly lower than those observed in São Paulo (Paula and Pereira 2003; Bulboa and Paula 2005; Hayashi et al. 2007a), and in Mexico (Muñoz et al. 2004). From April to May, growth rates were similar to those observed in commercial cultivation of the Philippines, India, and Vietnan (Hurtado et al. 2008; Subba Rao et al. 2008; Hung et al. 2009). From June, growth rates decreased, mainly due to the breakage of the thalli, although all biomass lost were accumulated in the fishing net under the raft. Significant differences among the strains were observed only in September and October, where they began to recover from the stress caused by the low temperatures. The brown tetrasporophyte was the only strain that could not recover.

From September to November, strong currents and winds, and some episodes of extra-tropical typhoons caused great lost of biomass and all strains developed ice-ice symptoms. Because of this, the experiments needed to be interrupted in January to recover all biomass and continue in the next month.

Significant positive correlation were observed between growth rates and temperature as already observed by Paula et al. (2002) and Hayashi et al. (2007a) in São Paulo, Brazil, Muñoz et al. (2004) in Mexico, and Hung et al. (2009) in Vietnam.

The cultivation period also reflected the effects of temperature in growth rates and net productivity. However, it seems that it had no influence on carrageenan yield, being similar to those obtained in São Paulo and other places (Hayashi et al. 2007a,b; Hung et al. 2009). Viscosity of the gel increased with the cultivation period as reported before (Hayashi et al. 2007b). The high viscosity could be related to a high content of *iota* carrageenan, which was not analyzed in the present study, or a response to strong currents (Hayashi et al. 2007b).

Corroborating results obtained in São Paulo (Paula and Pereira 2003) and Rio de Janeiro (Castelar et al. 2009), we found neither tetrasporangia nor cystocarps. As we did not find this species growing outside the farm it seems that its bioinvasive potential in this region is negligible.

Studies to evaluate the potential of *K. alvarezii* as a biofilter, co-cultivated with oysters (Qian et al. 1996) and fishes (Rodrigueza and Montaño 2007; Hayashi et al. 2008) were made early, showing that seaweeds can remove approximately 80% ammonium and 26% phosphate. The reduction of DIN inside the raft, as compared to the mussel farm, for most of the experimental period, as observed in the present study, could be considered an extra benefit and contribute to alleviate occasional blooms of harmful micro-algae in the region.

These findings represent the very first confirmation that the cultivation of *K. alvarezii* in the subtropical waters of Santa Catarina is technically feasible, particularly between spring, summer and autumn which is the optimal period for seaweed growth. We recommend that the seaweeds farming can be located near mussel farming, which could be improve the water quality, alleviate the effects of micro-algae blooms and be an alternative income to the producers.

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