J Appl Phycol
DOI 10.1007/s10811-014-0316-4

The effects of different light intensities on the culture of *Gigartina skottsbergii* (Rhodophyta, Gigartinales) tetrasporophytes and gametophytes in the Magellan region, Chile

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Received: 21 December 2013 / Revised and accepted: 8 April 2014

Abstract *Gigartina skottsbergii* is a red seaweed used as raw material for extracting carrageenans, constituting an important economic resource for Chile. In 2009, extraction in the Magellan region reached 15,064 t. The growing demand has adversely affected the sustainability of natural beds, creating an interest in the culture of this resource. In order to provide information relevant to the culture and regeneration of this seaweed, the present study addresses the effects of different light intensities on the growth of *G. skottsbergii* gametophytes and tetrasporophytes during the early stages of development. Mature reproductive fronds were induced to release spores in the laboratory by a drying process. Gametophytes cultured at different light intensities showed an increase in diameter, which reached 519.13±108.95 μm with 4 μmol photons m^{-2} s^{-1}, while tetrasporophytes showed a greater increase in diameter, reaching 714.11±116.45 μm with 8 μmol photons m^{-2} s^{-1}. Results indicate that both stages of the reproductive cycle are influenced by different light intensities within a limited range. Therefore, both phases require different and specific ranges of light intensity.

Keywords *Gigartina skottsbergii* · Culture · Light intensity · Development · Magellan region

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Introduction

In Chile, benthic marine seaweeds constitute an important economic resource (Ramírez 1995). They are harvested and exported mostly as raw material for agar, carrageenan, and alginate production (Ávila and Seguel 1993). Therefore, phycocolloid extraction and seaweed culture in Chile is increasing and is regarded as one of the country's most productive activities (Werlinger et al. 2008). The order Gigartinales is strongly represented in the list of the most suitable natural resources for the extraction of phycocolloids, in particular, *Mazzaella laminarioides*, *Iridaea chordata*, *Sarcothalia crispata*, and *Gigartina skottsbergii*.

G. skottsbergii Setchell & N.L. Gardner, commonly known as "Luga Roja," presents a three-phase isomorphic life cycle (Kim 1976; Bird et al. 1977). From this life cycle, it has been determined that natural beds of "Luga Roja" have biomass and density fluctuations (Piriz and Cerezo 1991).

The species *G. skottsbergii* is endemic to the southernmost region of South America, and its geographical distribution ranges from Valdivia to Tierra del Fuego, with accounts of this species in Valdivia and Valparaiso (Ramírez and Santelices 1991). This seaweed is also located on the southern coast of Argentina and sub-Antarctic islands (FAO 1985; Piriz and Cerezo 1991) and along the coast of the Antarctic Peninsula (Piriz 1996). It is a resource used as raw material in the extraction of carrageenan (Westermeier et al. 1999; Marin et al. 2002). In 1998, large-scale landings of "Luga Roja" began in the Magellan region (55° 20′ S; 66° 41′ W), used mainly as fresh raw material for carrageenan extraction. In 2009, exploitation in the region reached 15,064 t, and in 2010, landings reached 20,000 t. At a national level, commercialization of seaweeds and their by-products generates annually the amount of approximately US$ 22 million (Mansilla et al. 2004). At present, high domestic and international demand has resulted in exponential growth year after year,

Published online: 02 May 2014

adversely affecting the sustainability of the resource in wild beds (Westermeier et al. 1999). Consequently, exploitation pressure has now shifted to the south, mainly to the XI (43° to 49° S) and XII (50° to 57° S) regions (Romo et al. 2001). Exploitation (decrease in productivity of wild "Luga" beds) has caused a social and economic impact in the Magellan region, similar to the situation that occurred with *S. crispata* or "Luga Negra." Increased efforts have been made to extract *G. skottsbergii* in recent years, and while landing volumes have been maintained or have declined, extraction from natural beds located in areas difficult to reach has increased (Ávila and Seguel 1993; Ávila et al. 1996). As a result, cultivation techniques have been developed in the X region, to recover wild beds and to meet market needs (Romo et al. 2006). In the Magellan region, natural beds have also been exploited, and today, it is not known whether the reproductive behavior or physiological needs of these seaweeds during development differ from those identified in the study on *G. skottsbergii* in the X region. Field and laboratory experiments have shown that seaweed growing from spores can be an effective method of mass production (Alveal et al. 1995, 1997; Candia et al. 1993). In this case, environmental factors are vital to the development of future seedlings. Light intensity is one of the major factors that influence growth, pigment concentrations, and photosynthetic rate in red seaweed (Necchi 2005). Laboratory research to identify more suitable environmental culture conditions for the "Luga Roja" in the Magellan region will have an important social, economic, and ecological impact on a regional level. Innovations acquired at this stage will provide the basis for establishing the physiological requirements and enable the future production of this seaweed by artisan fishermen in the region. In order to provide information that will permit the culture and regeneration of this species, this research addresses the effects of different light intensities on the growth of *G. skottsbergii* gametophytes and tetrasporophytes in its early stages of development.

Materials and methods

Tetrasporophytic and cystocarpic fronds of *G. skottsbergii* were selected randomly from two natural beds: Punta Santa Ana (53° 37′ S–70° 52′ W) and Punta Santa Maria (53° 21′ S–70° 27′ W) during the autumn and winter months of 2012 (Fig. 1). Fronds were in an obvious state of maturity, thus increasing the chances of obtaining a successful sporulation and greater spore viability. Selected fronds were rinsed under pressure in drinking water to remove epiphytes and organic material in general, thus avoiding future contamination.

Clean fronds were subjected to stress by drying for about 1 h to obtain spores. After the drying process, fronds were immersed in seawater (average 34 psu), previously filtered at 0.4 μm and sterilized in an autoclave. This procedure was performed until a spore suspension in seawater was obtained, which presented a reddish color. The suspension should possess an approximate density of about 50 spores per ocular field at×100 (Romo et al. 2001).

The photoperiod used was 12:12 h light–dark cycle. Standard Bayfolan dissolved in seawater at 34 psu in a concentration of 0.1 mL L^{-1} was used as a culture medium nutrient source (Mansilla et al. 2008a). The culture medium was renewed weekly. The carpospores and tetraspores were cultured simultaneously under different light intensities at 2, 4, and 8 μmol photons m^{-2} s^{-1} light photosynthetic active radiation (PAR), which was monitored by a PMA2200 Solar Light Radiometer Photometer. For each light intensity, three plastic containers of 11×4 cm^2, with a volume of 500 mL each, were available, with glass slides previously sterilized in the autoclave at 120 °C for 20 min (Alveal et al. 1995) and placed at the bottom of each container to optimize the spore fixation. The temperature of the culture chamber was maintained at 8 °C, corresponding to the spring–summer temperature in the Strait of Magellan.

The diameter of 30 spores per container was measured and images were recorded on a CX31 Olympus photographic microscope, with Micrometrics Premium software. The duration of the test was 2 months, until the formation of upright seedlings. Daily growth rate (DGR) was calculated according to the Hansen (1980)): DGR (%): 100 (ln (N_t / N_0)) / t, where DGR (%): growth rate in percentage, N_t: length or final diameter, N_0: length or initial diameter, and t: time (days).

Data analysis

Data from DGR was arcsine-transformed (Zar 1999). To evaluate the effect of various light intensities on *G. skottsbergii* seedling growth over time, repeated measures ANOVA was used. The sphericity assumption was checked through the Mauchley test, and if this test was violated, the adjusted probabilities of Greenhouse–Geisser and Huynh–Feldt were provided. Data analysis was performed with Statistica 7.1 software. All conclusions were based on a 95 % confidence level ($p<0.05$).

Results

Gametophytes

G. skottsbergii gametophytes cultured at different light intensities showed an increase in diameter growth of the basal disc during the culture period; the largest increase in disc diameter was 519.13±108.95 μm, with 4 μmol photons m^{-2} s^{-1} up to day 64. At 8 μmol photons m^{-2} s^{-1}, the largest increase in disc diameter was 279.8±54.6 μm, and at 2 μmol photons m^{-2} s^{-1},

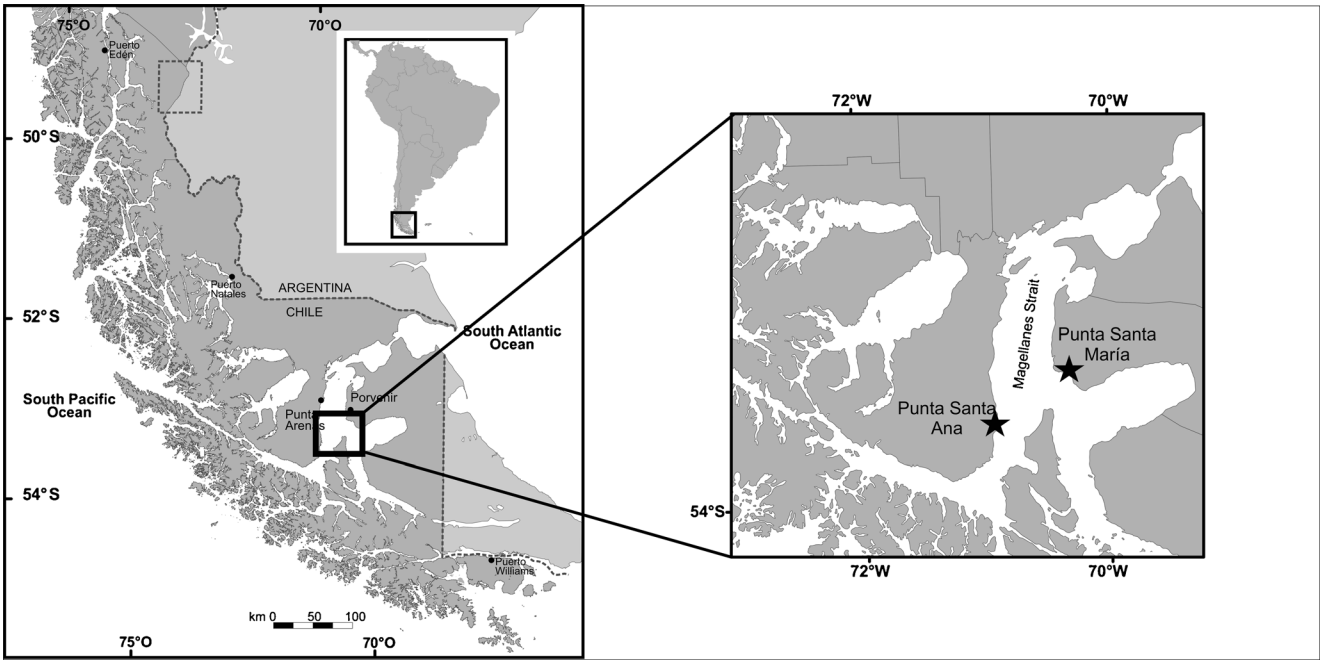

Fig. 1 Location of the two collection sites of mature *G. skottsbergii* reproductive material. Santa Ana Point (53° 37′ S–70° 52′ W) and Santa María Point (53° 21′ S–70° 27′ W)

the largest increase in disc diameter was 249.0±55.6 μm, showing a lower growth under these conditions (Fig. 2a). From day 15 onward, significant differences ($p<0.05$) were observed, between all three treatments.

The growth rate of gametophyte basal discs during culture reached a maximum value on day 15, for all treatments, and then decreased gradually up to day 64 (Fig. 2b). While the treatment exposed to 2 μmol photons m^{-2} s^{-1} reached a maximum growth rate of 12.60±1.07 % day^{-1} on day 15 of culture, at the end of the cultivation period, this treatment showed a DGR of 0.56±0.38 % day^{-1}, being the lowest in the study. Treatment exposed to 4 μmol photons m^{-2} s^{-1} also reached its maximum DGR on day 15, with 14.54±1.28 % day^{-1} decreasing gradually to 3.52±1.28 % day^{-1} on day 64. This treatment achieved the highest growth rate during the study period. Finally, although the treatment exposed to 8 μmol photons m^{-2} s^{-1} had the highest growth rate on day 15, 10.36±2.00 % day^{-1}, it was the lowest of the three treatments on day 64; this treatment showed a DGR of 2.79±1.19 % day^{-1} (Fig. 2b).

Tetrasporophytes

G. skottsbergii tetrasporophytes cultured at different light intensities showed an increase in growth of the basal disc diameter during the culture period; the largest increase in diameter was 714.11±116.45 μm, with a light intensity of 8 μmol photons m^{-2} s^{-1} at day 64. While in the treatment exposed to 4 μmol photons m^{-2} s^{-1}, the diameter reached 390.83±77.67 μm on the final day of culture. The lowest growth was obtained in the treatment exposed to 2 μmol photons m^{-2} s^{-1} showing a maximum basal disc size of 293±50.78 μm at the end of the experiment. In Fig. 2c, significant differences can be observed from day 22 onward.

During the culture period, it was observed that the basal disc growth rate of tetrasporophytes was highest on days 15 and 22 of culture in all treatments, decreasing over time, up to day 64. Maximum growth rate (18.60±0.94 % day^{-1}) was reached during day 8, in the treatment exposed to 8 μmol photons m^{-2} s^{-1} (Fig. 2d). Subsequently, the growth rate decreased up to day 64 (2.05±0.26 % day^{-1}). Significant differences were observed from day 8 to day 22 with a $p<0.05$ by ANOVA analysis (Tables 1 and 2). From day 29 onward, no significant differences between treatments were observed.

Discussion

The early stages of seaweed development are well adapted to low light intensities because, in the reproductive stage, spores are mainly released in winter, and they must grow under the canopy of adult seaweeds (Gómez and Wiencke 1996; Hanelt et al. 1997). In the case of *G. skottsbergii*, it was observed that growth is significantly lower in the case of gametophytes at light intensities of 2 and 8 μmol photons m^{-2} s^{-1}. In some cases, seedlings in these treatments showed pigmentation loss and dead cells; Tasende and Fraga (1992) have described similar circumstances for *Chondrus crispus* culture. The

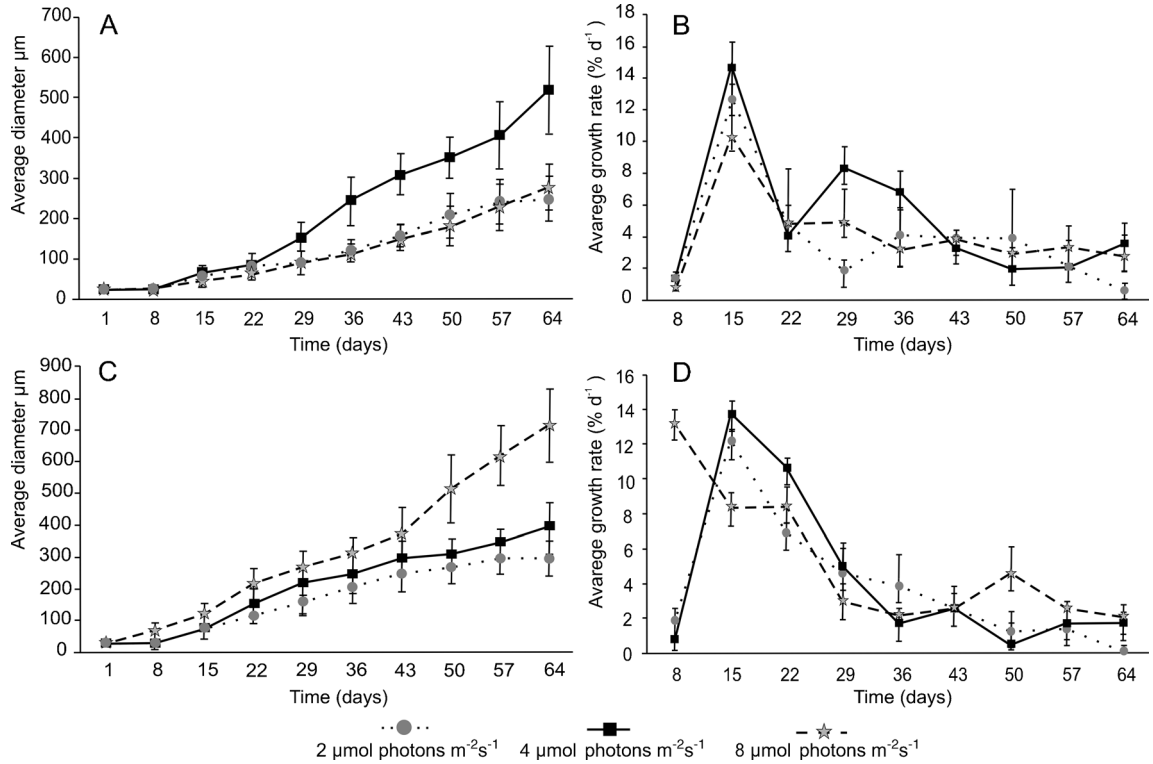

Fig. 2 Effects of light intensity. **a** Gametophyte average diameter (µm); **b** gametophytes' growth rate (% day^{-1}); **c** tetrasporophyte average diameter (µm); and **d** tetrasporophyte growth rate (% day^{-1}). Each data point is the mean of three replicates (n=3 ± standard deviation)

treatment at 4 µmol photons m^{-2} s^{-1} showed that this light intensity is the most appropriate for initial tetraspore culture. It was observed that initial growth rates were high in all treatments, but over time they decreased. This observation is consistent with the fact that spores initially have a high rate of cell division to form the individual fastening disc until it reaches a certain diameter, then growth is directed toward the formation of the erect thallus. The treatment of 2 µmol photons m^{-2} s^{-1} showed no greater growth than the treatment of 4 µmol photons m^{-2} s^{-1} at the end of the treatment, but remained stable during the culture period without significant mortalities. This indicates that their development stage was influenced by light intensity and the range at which they can be exposed is limited. These results agree with those of Ugarte and Santelices (1992), who found significant differences in the development of *M. laminarioides* tetraspores under different light intensities. In contrast, Ávila et al. (1999) found no differences in growth between *G. skottsbergii* carpospores and tetraspores in the northern distribution zone of the species (41° 52′ S; 73° 51′ W). These authors used 6, 18, and 25 µmol photons m^{-2} s^{-1}; in this case, all treatments produced similar results in spore growth. Other studies in *Iridaea* species indicate that the optimal range of photon flux densities may be different in the early development stages (Hannach and Santelices 1985) (Figs. 3 and 4).

The carpospores cultivated at different light intensities showed the highest growth and DGR at 8 µmol photons m^{-2} s^{-1}; these spores showed a strong red pigmentation. This is possibly due to a high concentration of phycoerythrin which could influence the photosynthetic rate, consequently compromising their development (Van der Meer and Bird 1977; Van Deer Meer 1979; Costa and Plastino 2001). The other two experiments carried out with 2 and 4 µmol photons m^{-2} s^{-1} showed significantly lower growth and DGR at

Table 1 Statistical analysis of growth rates of *G. skottsbergii* gametophytes

	SS	Degree of freedom	MS	*F*	*p*
Intercept	49,876,866	1	49,876,866	45,800.01	0.001
Light intensity	3,875,870	2	1,937,935	1,779.53	0.001
Error	94,744	87	1,089		
Time	19,890,599	9	2,210,067	2,495.93	0.001
Time * light intensity	2,751,669	18	152,870	172.64	0.001
Error	693,321	783	885		

Table 2 Statistical analysis of growth rates of *G. skottsbergii* tetrasporophytes

	SS	Degree of freedom	MS	*F*	*p*
Intercept	21,274,880	1	21,274,880	28,338.07	0.001
Light intensity	1,830,744	2	915,372	1,219.27	0.001
Error	65,315	87	751		
Time	10,765,236	9	1,196,137	1,791.08	0.001
Time * light intensity	1,481,922	18	82,329	123.28	0.001
Error	522,912	783	668		

2 μmol photons m^{-2} s^{-1}. Furthermore, mortality occurred during the last weeks of culture, due to pigment loss and seedling necrosis. In some cases, it was not possible to erect the thalli, perhaps due to changes in light intensity and spectral quality, which are important factors in the photosynthetic and growth rate response of seaweeds (Falkowski and La Roche 1991). Observation in this experiment showed that this phase requires a higher light intensity than gametophytes grown in similar conditions, demonstrating that both phases have different light intensity requirements in this latitude. This is not consistent with observations made by Buschmann et al. (2004) for the same species in the northern distribution zone of the species. With regard to this point, literature has stated that different developmental stages require different degrees of light (Henkel 1952; Boalch 1961; Fries 1963) and also that seaweeds of the same species, but from different populations or different regions, will present different behavior (Gantt 1990).

Buschmann et al. (2004) mentioned that *G. skottsbergii* was found in environments that presented irradiances ranging from 1 to 40 μmol photons m^{-2} s^{-1}, while in the sub-Antarctic (55° 20′ S; 66° 41′ W) and Antarctica regions, these values can reach half, or less, of those described above (Gómez 2001). Species have been collected at 4 m depth and found frequently under the *Macrocystis pyrifera* (kelp) canopy (Ojeda and Santelices 1984; Vásquez 1993; Ríos et al. 2007; Mansilla and Ávila 2011). Other studies suggest that different developmental stages of red seaweeds require different temperatures, light intensities, and photoperiods (Henkel 1952; Boalch 1961). Moreover, differences exist among individuals from different populations of the same species, or among species inhabiting the same region, but in areas with different ecological conditions (Gantt 1990). This is consistent with observations made during the present study, that different reproductive phases of the same

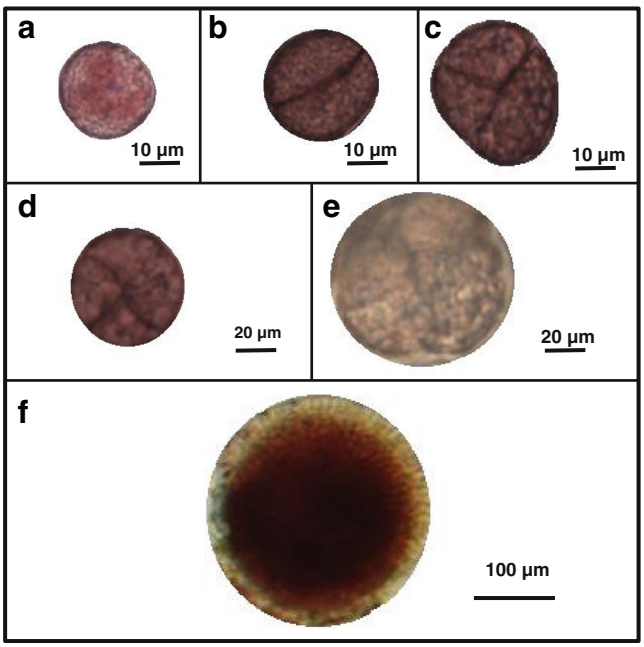

Fig. 3 Development of gametophytes of *G. skottsbergii*. **a** Tetraspores, day 1 of cultivation; **b** seedling development during the first week of cultivation; **c**–**e** development of the seedling up to day 50; and **f** erect seedling formation up to day 57

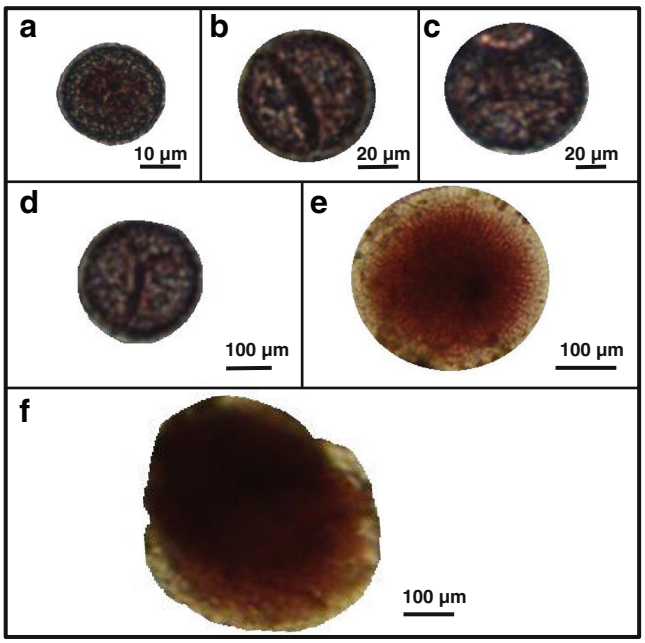

Fig. 4 *G. skottsbergii* tetrasporophyte development. **a** Carpospores, day 1 of cultivation; **b** first divisions in the seedling formation during the first week of cultivation; **c**–**e** development of the seedling up to day 50; and **f** erect seedling formation up to day 57

seaweed require different conditions for cultivation. Finally, regarding the size of tetraspores and carpospores analyzed during the initial phase of the experiment, they are consistent with those described by other authors (Ávila et al. 1999; Buschmann et al. 2004; Mansilla et al. 2008b). This study presents the first results relevant to the culture of the carrageenan species, *G. skottsbergii*, in the Magellan region of Chile. As a result, it represents an important contribution to the future culture and repopulation of this resource of considerable commercial interest in the sub-Antarctic region of Chile.

Acknowledgments Financial support to MA and AM is acknowledged from FONDEF and CONICYT, Chile (Project AQ08I1011). The facilities and equipment required were provided by the Laboratory of Sub-Antarctic Macroalgae (LMAS) University of Magallanes. AM acknowledges the Millennium Scientific Initiative (Grant P05-002 ICM, Chile) and the Basal Financing Program of CONICYT (Grant PFB-23, Chile). SR thanks the Masters Scholarship provided by the Institute of Ecology and Biodiversity (Chile) (code ICM P05-002). The collaboration of Susan Angus in the improvement of the English language of the manuscript is also acknowledged.

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