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Experimental indoor cultivation of the carrageenophytic red alga *Gigartina skottsbergii*

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Abstract

Exploitation of carrageenophytic seaweeds in Chile has increased significantly over the past few years. *Gigartina skottsbergii* is one of the species exploited and the harvesting pressure could have negative consequences on the sustainability of this resource. Although demand for raw material of *G. skottsbergii* continues to increase, basic knowledge needed to cultivate this unique carrageenophyte is lacking. In this study, we determined the light, temperature, and nutrient requirements for juvenile *G. skottsbergii* cultivation, as well as the culture system and stocking densities required to optimize its indoor cultivation. An optimal temperature range of 10–14 °C combined with dim light conditions are recommended. Nutrients do not need to be constantly added and ammonium instead of nitrate should be used, whereas phosphate must be also added. Floating cultures provide an adequate alternative, where containers support loads of 13–14 g (fresh weight) L^{-1}. Only in very precise combination of factors, the specific growth rate can be over 2% per day.

Keywords: Chile; Cultivation methods; *Gigartina skottsbergii*; Growth; Harvesting; Indoor tanks; Irradiance; Temperature

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1. Introduction

Seaweed exploitation started in Chile in the mid-1940s with the export of *Gelidium* spp. Thereafter, the export of dried *Gracilaria* to Japan began to increase steadily up to mid-1980s, and resulted in overexploitation of the major natural stands in northern, central and southern Chile (Santelices and Ugarte, 1987). Subsequently, cultivation and processing of *Gracilaria* began and by 1996 over 90% of the 120,000 wet tons harvested was produced by farming (Buschmann et al., 1995; Santelices, 1996). During the 1990s, more species were incorporated into the list of harvested algae, triggering the need to develop cultivation methods for several brown and red seaweeds (Buschmann et al., 1999a, 2001a). One of these species, *Gigartina skottsbergii*, is used as raw material to produce carrageenan, and is therefore in great demand. The steady increase in the quantities harvested is already having negative consequences on the sustainability of this resource in several wild stands (Westermeier et al., 1999).

In spite of the persistent increase of the demand for raw material of *G. skottsbergii* by the carrageenan industry in Chile, basic knowledge needed to cultivate this unique carrageenophyte is lacking. In fact, the limited information available is mainly related to taxonomy (Kim, 1976), phenology and standing stock assessment (Zamorano and Westermeier, 1996; Piriz, 1996), and chemical composition (Palermo et al., 1984; Cerezo, 1986; Noseda, 1989; Matulewics et al., 1990; Piriz and Cerezo, 1991; Schnettler et al., 1995). More recently, a series of studies have begun to address aspects related to ecology and reproductive biology (Westermeier et al., 1999), ultrastructure and laboratory cultivation (Correa et al., in preparation), and development of methods for vegetative propagation (Buschmann et al., 1999b, 2001b; Correa et al., 1999). However, no information is yet available in relation to culture conditions for juveniles (Buschmann et al., 2001a). In this context, knowledge on optimal temperature, and light and nutrient requirements, is of primary importance to cultivate any alga in tank systems (Hanisak, 1987; McLachlan, 1991; Craigie et al., 1999). Cultivation in indoor tanks is considered as an intermediate stage, where the "seed" plants are produced at high densities in order to provide the inoculum for transplantation to the sea.

In this study, we determined the light, temperature, and nutrient requirements for cultivating juveniles of *G. skottsbergii*. The culture system and stocking densities used for indoor cultivation of this species are discussed to establish the basis for nursery development.

2. Materials and methods

2.1. Study site

All experimental material consisted of juvenile fronds (macroscopically immature) collected in Ancud (41°46′ S, 73°54′ W) at a depth of 6–7 m. Experiments were carried out in the field station (CEACIMA) belonging to the Universidad de Los Lagos, 30 km southeast of Puerto Montt, where all fronds were transported within 12 h of collection (Fig. 1).

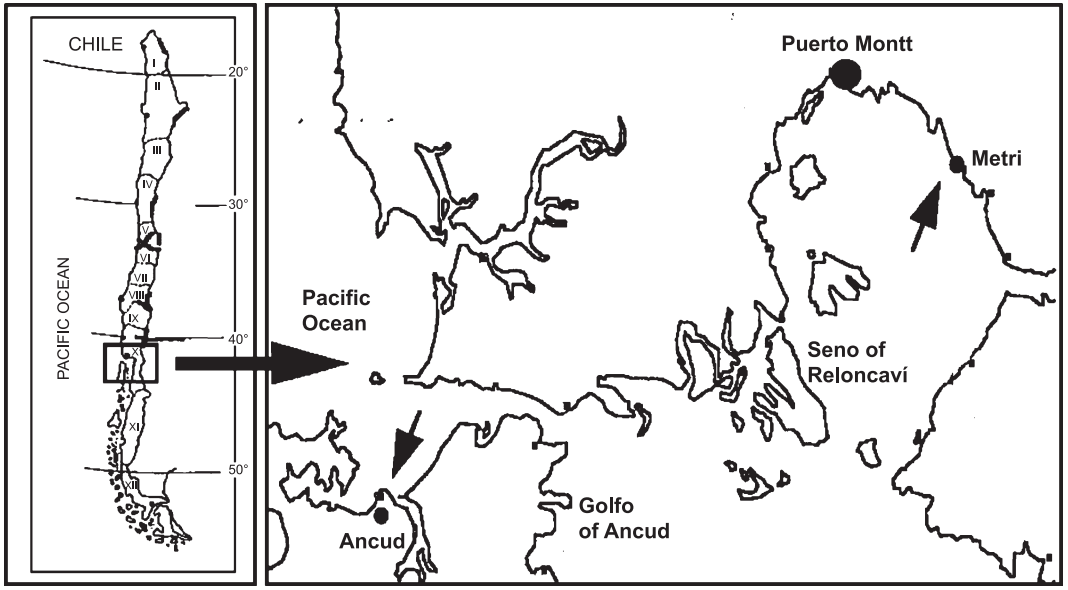

Fig. 1. Map of the collection sites (arrows) for *G. skottsbergii* in Ancud and the Marine Science and Aquaculture Station located in Metri.

2.2. Effects of photon flux density

The effect of photon flux density on photosynthesis rate in *G. skottsbergii* was measured in the laboratory using thallus discs (1 cm diameter) obtained from seven plants cut <24 h after collection. These samples were placed in closed chambers connected to Clark type-O_2 electrode (DW-3 electrode chamber, Hansatech Instruments). The oxygen evolution was first monitored at darkness for 15 min, and increasing irradiance exposure afterwards: 3, 13, 31, 60, 103, 162, 239, 336, and 448 μmol photons m^{-2} s^{-1}, for 5 min each. Saturated photosynthetic rate (P_{max}) was calculated from mean photosynthetic rates at higher light intensity (336 and 448 μmol photons m^{-2} s^{-1}). The photosynthetic efficiency (α) was estimated from the photosynthetic linear slope region given at zero and lower irradiances (0, 3, 13, and 31 μmol photons m^{-2} s^{-1}). Compensation (I_c) and saturation (I_k) points were estimated using P_{max} and respiration rate (R) according to: $I_c=P_{max}/\alpha$ and $I_k=R/\alpha$ (after Henley, 1993).

2.3. Effects of temperature

Manipulating temperature in a continuous supply water culture system is expensive and for this reason a correlative protocol for studying its effect was implemented. Eighteen 2-L transparent plastic containers were used in this experiment. Fronds with an initial average fresh weight of 0.75 g were placed in each container, set up with a continuous flow of 1 L min^{-1} of filtered (50 μm) seawater, photon flux density of 50–55 μmol photon m^{-2} s^{-1} and a photoperiod of 12:12 h (L:D). During the study period, pH varied between 8.0 and 8.4 and nutrient concentrations were 0.4–0.6 mg L^{-1} nitrate, 0.036–0.042 for nitrite, and 0.053–0.067 phosphate; ammonium was below detection limit. Fresh weight of each frond was monitored during 2 months at weekly intervals and the specific growth rate [SGR=(ln final weight−ln initial weight)/time (days)100] was calculated independently for each week. The specific growth rate was correlated with water temperature by following the continuous water flow controlling the water temperature daily with a digital thermometer (0.1 °C accuracy).

2.4. Effects of nutrient addition

To test the effect of two different sources of nitrogen on the growth performance of *G. skottsbergii*, pro-analysis 1.0 mM NH_4Cl and $NaNO_3$ were used to prepare culture medium (after McLachlan, 1973). Phosphate concentration (50 μM) and source were not modified and followed the standard protocol described by McLachlan (1973).Filtered seawater (50 μm) was replaced at weekly intervals in all the containers. The experimental design considered nine 40-L plastic containers (60×40×20 cm), with three of them assigned as controls (without nitrogen addition), three with ammonium addition and three with nitrate addition. The experiment was run at 50–55 μmol photons m^{-2} s^{-1} and at a photoperiod of 12–12 h. In each container, six *Gigartina* fronds were fastened to a nylon rope as a way of maintaining them on the bottom. During the experimental period (4 months), temperature varied between 9 and 13 °C, pH between 8.0 and 8.3, and salinity between 30‰ and 34‰. Fresh weight was measured initially and after 16 weeks of cultivation using a digital balance of 0.01 g accuracy and the SGR was calculated as described above.

A total of 24 tanks, 40-L each, were used to characterize the optimum nitrogen-to-phosphorus ratio required by *G. skottsbergii*. Six *Gigartina* fronds were kept on the bottom of each container by fastening them to a nylon rope. All tanks were maintained at 50–55 μmol photons m^{-2} s^{-1} and at a photoperiod of 12:12 h (L:D). Treatments, run in triplicate, considered the addition of phosphate (50 μM NaH_2PO_4; McLachlan, 1973) and four regimes of ammonium addition (added as NH_4Cl), including half of the medium concentration ($N_{1/2}$), full culture concentration (1.0 mM; N_p), and 50% higher than the culture medium ($N_{1.5}$). Simultaneously, another set of plants was prepared to be cultivated under constant concentration of ammonium (1.0 mM) and various regimes of phosphate addition (added as), including half strength ($P_{1/2}$), standard concentration (50 μM; P_p) and 50% higher than the standard medium ($P_{1.5}$). Two sets of triplicate tanks were used to control for no addition of ammonium (No) and no addition of phosphate (Po). Finally, controls with no addition of nutrients (i.e., plain filtered seawater) were also included in the design. During the experimental period, temperature varied between 10 and 12 °C, pH between 8.1 and 8.6, and salinity between 34‰ and 35‰. Fresh weight was measured at the beginning of the experiment and after 2 weeks of cultivation using a digital balance of 0.01 g accuracy, the SGR was calculated as described above.

2.5. Culture system

To test the feasibility of using free-floating plants as an alternative to cultivate *G. skottsbergii*, six 2-L plastic containers provided with continuous aeration during the day and a water flow of 1 L min^{-1}, were installed in a culture room with a photon flux density of 50–55 μmol photons m^{-2} s^{-1} and a photoperiod of 12:12 h (L:D). During the experimental period, temperature varied between 9.5 and 14.0 °C, pH between 8.0 and 8.3, and salinity between 27‰ and 34‰. Frond surface was determined at the beginning of the experiment and after 4 weeks of cultivation using a digital plan meter. SGR was calculated as described above.

2.6. Stocking density

To define the biomass that should be used, nine 1-L containers were set up to hold three stocking densities: 0.75, 6.7, and 13.3 g L^{-1}. The culture conditions were 50–55 μmol photons m^{-2} s^{-1}, photoperiod of 12:12 h (L:D), continued aeration during day hours and a water flow of 1 L min^{-1}. During the experimental period, the temperature varied between 11 and 16 °C, pH between 8.0 and 8.6, and salinity between 31‰ and 36‰. Fresh weight and SGR were determined as described above and the biomass production calculated as the fresh weight increase per liter per week.

3. Results

The photosynthetic rate showed that light saturation is reached at 60 μmol photons m^{-2} s^{-1}, reaching a P_{max} of 22.4 mL O_2 g^{-1} fresh weight h^{-1} and the compensation point is ca. 19 μmol photons m^{-2} s^{-1} at 11.5 °C (Fig. 2). These results allowed to calculate the

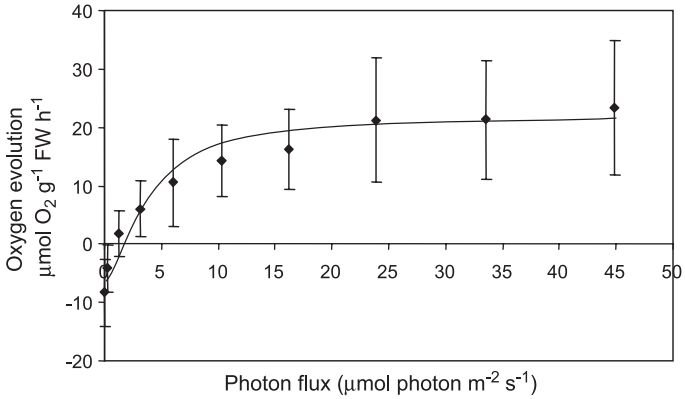

Fig. 2. Mean (±1 S.E.) net photosynthetic rate of *G. skottsbergii* at different photon flux densities.

photosynthetic efficiency (α=0.43). Cultivated free-floating fronds reached a mean fresh weight of 1.83 g in 7 weeks, with a daily mean growth rate of 1.82% at a photon flux density of 50 µmol photons m^{-2} s^{-1} (Fig. 3A). *G. skottsbergii* displayed its maximum growth rates at temperatures between 10 and 12 °C (Fig. 3B), whereas higher temperatures resulted in negative growth rates. The growth rate is significantly correlated with temperature through a second order polynomium (SGR=−0.444temp^2+10.437temp−59.92; r^2=0.503; P<0.05).

Nitrogen source also had significant effects (F=6.613; P<0.002) on the growth of *G. skottsbergii* (Fig. 4). Specific growth rate dropped significantly in the presence of nitrate additions. However, the addition of ammonium did not result in a significant growth enhancement as compared to controls.

The experiment with different N/P ratios demonstrated that by maintaining the phosphate source at the concentration defined in the Provasoli medium, the addition of ammonium at different concentrations produced a significant effect (F=7.94; P<0.05) on the growth rate

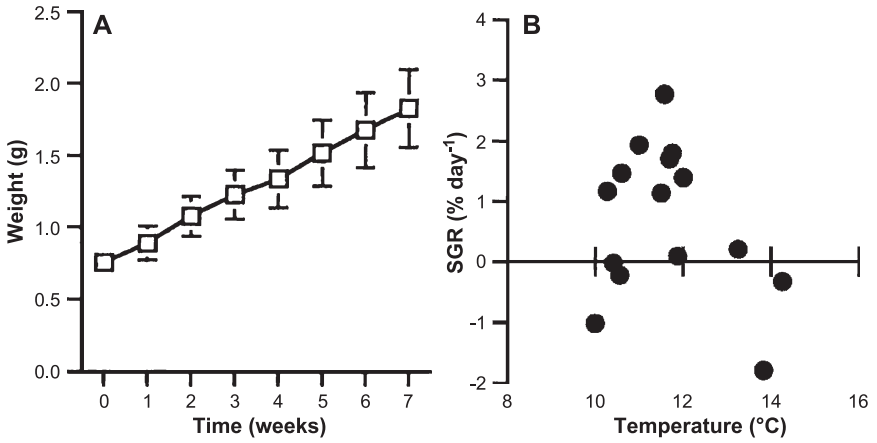

Fig. 3. Changes in fresh weight (mean±1 S.E.) of *G. skottsbergii* (A) and relationship between daily specific growth rate (SGR) and water temperature (B).

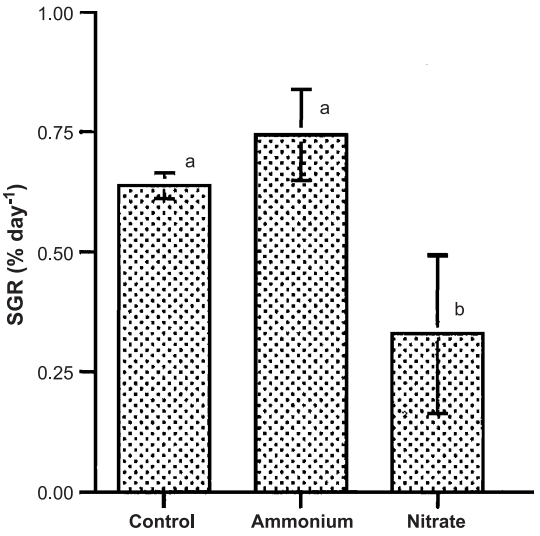

Fig. 4. Mean (±1 S.E.) specific growth rate (SGR) of *G. skottsbergii* incubated with different sources of nitrogen. Control treatment consists of plain seawater.

of *G. skottsbergii* (Fig. 5A). Half-strength ammonium treatment produced the best growth response of the alga in culture tanks, but only when phosphorus was increased with respect to the nitrogen source. On the other hand, when the concentration of nitrogen remained constant, the addition of phosphate also produced a significant (F=8.32; P<0.05) effect on the growth rate of *G. skottsbergii* (Fig. 5B). In this case, the best result was obtained when the level of phosphate was increased by 50% above the standard Provasoli medium.

Free-floating fronds of *G. skottsbergii* showed specific growth rates of ca. 1% day^{-1}, and were not significantly different (F=0.096; P≥0.767), from those recorded in the attached fronds (Fig. 6). Nevertheless, after a 16-week cultivation period, free-floating fronds seemed healthier than attached fronds, suggesting that suspended culture of this species produces a better response in the long term. Furthermore, free-floating fronds produced from spores showed specific growth rates of ca. 0.7–0.8% day^{-1}, and survival rate of over 80% during a 38-week culture period, where they reached a blade-like normal morphology (Fig. 7).

Finally, stocking density affected only marginally (F=6.399; P≤0.042) the specific growth rate of *G. skottsbergii*, with a trend toward a reduction from 0.8% day^{-1} at 0.75 g L^{-1} to less than 0.5% day^{-1} when the biomass reached over 13.3 g L^{-1} (Fig. 8A). The production of biomass, however, was significantly higher (ANOVA, F=12.88; P≤0.011), at the highest stocking density tested (Fig. 8B).

4. Discussion

Optimal growth in *G. skottsbergii* was observed at temperatures between 10 and 12 °C. This temperature optimum is much higher than the values reported for Antarctic *G.*

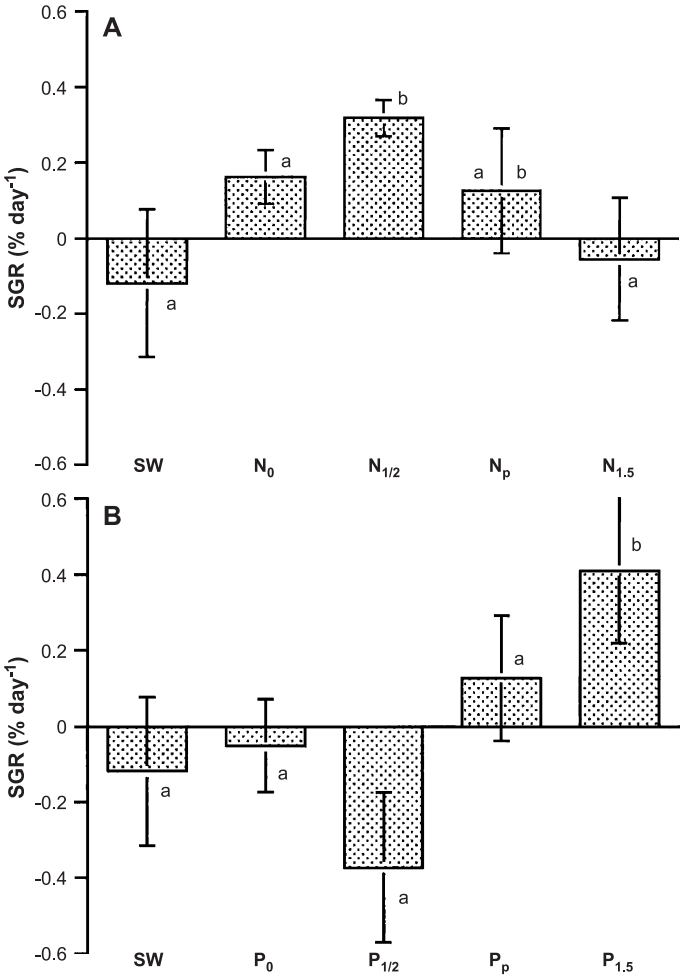

Fig. 5. Mean (±1 S.E.) specific growth rate (SGR) of *G. skottsbergii* incubated under different N/P ratios. (A) Concentration of phosphorus as in standard culture medium, varying the nitrogen concentration (H_2O=filtered seawater control; N_0=no nitrogen addition; $N_{1/2}$=half concentration of that in standard medium; N_p=full standard medium; and $N_{1.5}$=50% increment of the nitrogen concentration). (B) Concentration of nitrogen as in standard culture medium, varying the concentration of phosphorus (H_2O=filtered seawater control; P_0=no phosphorus addition; $P_{1/2}$=half concentration of that in standard culture medium; P_p=standard medium concentration; and $P_{1.5}$=50% increment of phosphorus concentration). Bars with the same letter do not differ significantly (Tukey test; $P>0.05$).

skottsbergii by Bischoff-Bäsmann and Wienecke (1996), but similar to the results obtained for sporelings from Ancud by Correa et al. (in preparation). Our data also coincide with the temperature that is commonly found in the area where our experimental material was collected. Based on the above, we hypothesized that the clear differences in responses to temperature when comparing plants from the Antarctic with those from Ancud is the result of local adaptation, in this case of the Ancud population, to environmental conditions. This

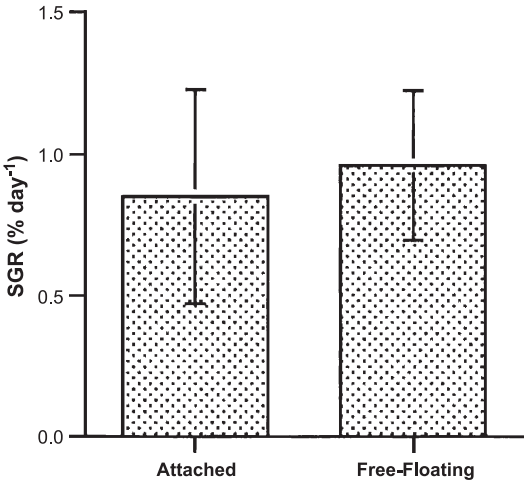

Fig. 6. Mean (±1 S.E.) specific growth rate (SGR) of *G. skottsbergii* in a free-floating and bottom-attached culture condition.

view is supported by recent data obtained using neutral molecular markers, where a clear genetic differentiation of *G. skottsbergii* populations that, although separated by relatively short geographic distances, appeared as quite distant genetic entities (Faugeron, personal communication). Such strong genetic differentiation is likely the result of local genetic drift (driven by local specific conditions) being more important than the homogenizing effect of gene flow, a situation that has been detected in other red algae (Soza and García-Reina, 1992; Faugeron et al., 2001; Zuccarello et al., 2001).

Nitrogen can greatly enhance growth and production in seaweed mariculture (e.g., Lapointe and Ryther, 1979; Fujita and Goldman, 1985; Hanisak, 1987; Chopin and Yarish, 1998). Studies have demonstrated that some red algae, such as species of *Gracilaria*, grow

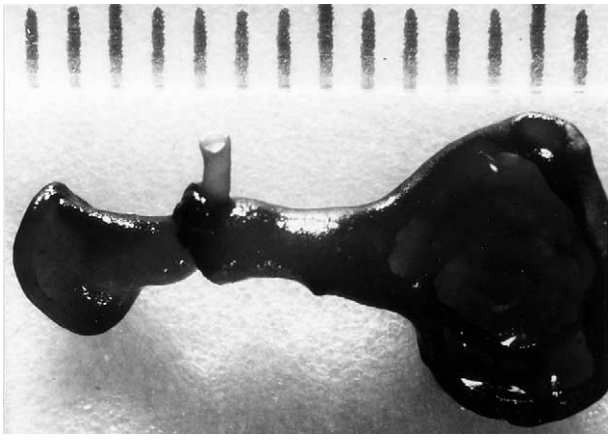

Fig. 7. Photograph showing a free-floating *G. skottsbergii* plant obtained through the cultivation of tetraspores under laboratory conditions. Scale in mm.

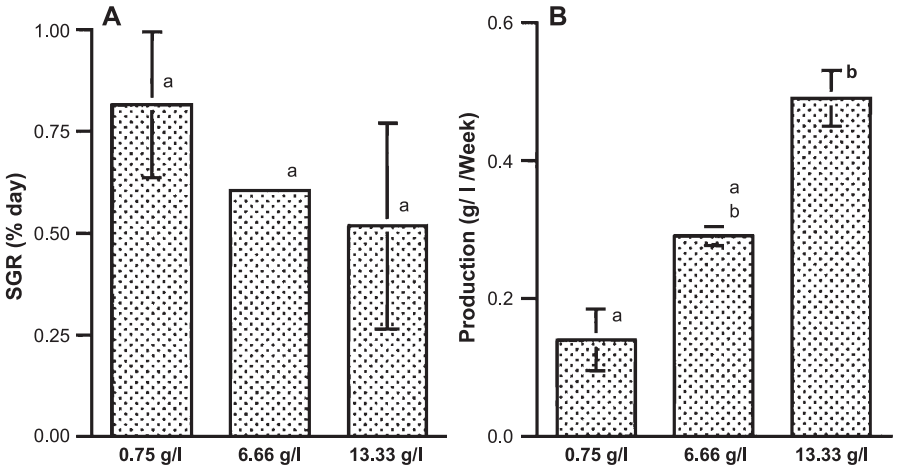

Fig. 8. Mean (±1 S.E.) specific growth rate (SGR) (A) and mean (±1 S.E.) biomass production (B) of *G. skottsbergii* at three stocking densities.

better under conditions of high ammonium (Buschmann et al., 1994; Smit et al., 1997), whereas other species, like *Chondrus crispus* and *Soliera chordalis*, perform better when nitrate is added (Craigie, 1990; Brown, 1995). In this study, we found higher growth rates of *G. skottsbergii* associated with the addition of ammonium, rather than nitrate. In an effort to understand the nutrient physiology of seaweeds, many studies have focused on the uptake kinetics of ammonium and nitrogen (e.g., Haines and Wheeler, 1978; Friedlander and Dawes, 1985; Lewis and Hanisak, 1996). In this context, four important characteristics of nitrogen absorption could be useful to interpret our results. First, ammonium depressed the uptake rate of nitrate (e.g., Thomas and Harrison, 1987; O'Brien and Wheeler, 1987; Smit, 1998). Second, ammonium demonstrates biphasic uptake kinetics with a strong diffusive component (e.g., D'Elia and DeBoer, 1978; Fujita, 1985; Friedlander and Dawes, 1985; Smit, 1998). Third, seaweeds with a low surface area/volume ratio should also have lower rates of nutrient uptake (Rosemberg and Ramus, 1984; Wallentinus, 1984; Hanisak et al., 1990). Finally, past conditions of nutrient availability influence the uptake performance of algae (e.g., Fujita, 1985; Peckol et al., 1994; Pedersen, 1994). The possibility of a surge uptake of ammonium enables its assimilation at a lower energetic cost, as described by Lobban and Harrison (1994) and could be of great advantage for a thick-bladed seaweed like *G. skottsbergii*. The significant surge uptake capacity for ammonium could provide considerable advantages in environments with fluctuating concentrations of nitrogen, temperature, and light conditions as are those typically found in the coastal waters of southern Chile (see Westermeier et al., 1999).

Phosphate, on the other hand, can be a limiting factor for seaweed biomass and gel production (Chopin et al., 1990a,b; 1996). In general, a 10:1 proportion of N/P is used to obtain the best growth responses in seaweeds (e.g., Friedlander and Levy, 1995). In the case of *Gracilaria cornea*, it has been shown that at ratios of 10:0 the alga presented lower growth rates than at 10:1 ratios, whereas a negative effect of P was detected when 10:10 ratios were tested (Navarro-Angulo and Robledo, 1999). The experiments using different

N/P ratios demonstrated that, in general, *G. skottsbergii* requires a proportionally higher addition of phosphate than ammonium. It is not known whether this is due to different uptake rates or to some other unidentified physiological factor. Thus, further experiments related to nitrogen and phosphorus uptake would seem important to improve our understanding of the growth responses of *G. skottsbergii* found in this study.

Photon flux density in the natural *G. skottsbergii* stands of southern Chile fluctuates between 1 and 40 μmol photons m^{-2} s^{-1}, mainly because of the high concentration of suspended material present most of the time (Buschmann, unpublished data). For this reason, it is not surprising that *G. skottsbergii* showed a low compensation point and that a maximum photosynthetic rate was achieved at a relatively low photon flux density, as described for other subtidal red algae (Mathieson and Norall, 1975; Gomez, 2001). Nevertheless, the saturation point of *G. skottsbergii* in the most southern end of Chile and the Antarctic is half of the values found in our study (Gomez, 2001). From an aquaculture point of view, this seems favorable as it implies lower energy costs. However, *G. skottsbergii* showed a low growth rate potential (1–1.5% day^{-1}) as compared to other temperate carrageenophytes (Kain and Norton, 1990). Daily growth rate of *C. crispus* can reach values of 2–4%, which are still lower than those reported for tropical and subtropical carrageenophytes (Chopin et al., 1999). For this reason, achieving a commercially sustainable profit seems difficult under today's conditions. Nevertheless, conditions for vegetative propagation combined with defined nursery protocols are becoming available (Buschmann et al., 2001b), and indicate that propagation of selected strains can be initiated. Example of the strain selection in related algae is the case of *G. exasperata* where selected individuals displayed growth rates 50% higher than their wild counterparts (Waaland, 1979). On the other hand, significant improvements in the growth of *G. skottsbergii* have been obtained solely by manipulating the form of thalli fragmentation (Buschmann et al., 2001b). Nevertheless, management strategies for wild populations of this carrageenophyte must not be left aside as could be the most important mode to sustain the further carrageenan production in Chile (Buschmann et al., 2001a).

Traditionally, fronds with flat blade morphology have been cultivated in bottom culture systems (Santelices, 1999). Culturing *G. skottsbergii* in floating systems opens the possibility of using the entire water column (i.e., not only the bottom), increasing both the stocking biomass and the associated biomass production (e.g., Bidwell et al., 1985; Buschmann et al., 1994). Furthermore, our results showed that *G. skottsbergii* can be cultured at 12–14 g L^{-1}, which suggests that on-shore cultivation is technically feasible.

5. Conclusions

At this stage, the basic knowledge accumulated for cultivating *G. skottsbergii* in Chile indicates that culture facilities must be established in localities where temperature does not exceed 14–15 °C. For indoor cultivation (i.e., nursery), dim light conditions are recommended and nutrients do not need to be constantly added. Ammonium should be preferred over nitrate, and phosphate should be added in a proportion higher than the recommended or the Provasoli medium. Cultivation of floating juveniles can be used with loads of at least 13–14 g L^{-1}.

Considering the above, further studies are still needed on order to establish sound culture protocols for both nursery and outdoor tank cultures. We highlight the need to define the optimum concentration and frequency of ammonium addition, as well as salinity effects on growth and how these environmental factors modify the quality of the carrageenans obtained from the alga.

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