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Article *in* Journal of Applied Phycology · January 2012

DOI: 10.1007/s10811-010-9640-5

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J Appl Phycol (2012) 24:11–20
DOI 10.1007/s10811-010-9640-5

A pilot-scale study of the vegetative propagation and suspended cultivation of the carrageenophyte alga *Gigartina skottsbergii* in southern Chile

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Received: 23 June 2010 / Revised and accepted: 13 December 2010 / Published online: 7 January 2011

Abstract Different propagation techniques for cultivation of vegetative *Gigartina skottsbergii* fronds were tested using a system of suspended ropes, to which inoculants were attached. Our results showed that triangular fragments obtained from the circular *G. skottsbergii* thalli produced harvestable frond of 800 cm^2 after 8 months. In contrast, inoculants of intact juvenile fronds of comparable size needed at least 10 months to reach the same size. A control experiment with spores developing on an artificial substrate showed that 20 months were needed to reach a surface of 500 cm^2, confirming the superiority of our fragment culture system. A pilot study demonstrated that with a density of six fronds m^{-1} of farming line, the proposed system can be economically interesting for local fishermen.

Keywords Carrageenophyte · Cultivation · *Gigartina skottsbergii* · Regeneration · Rhodophyta · Southern Chile

Introduction

Wild stocks of carrageenophytes in Chile, mainly species belonging to the genera *Sarcothalia*, *Mazzaella*, and *Gigartina*, have been under increased harvesting pressure in the past years. This activity resulted in an erratic raise of the biomass harvested from 2000, which reached 61,209 wet tons in 2005, as compared with 72,413 tons harvested in 2008 (Sernapesca 2008) Further evidence of over-exploitation of wild populations, particularly in the southern hemisphere, has been presented in a series of reports on stock assessment in Southern Chile (41° to 53° S) (Westermeier et al. 1995, 1996, 1997; Avila et al. 2003) and the Falkland Island, South Atlantic (Westermeier and Patiño 1999).

Various attempts have been made to cultivate some of the species concerned. For example, Navarro and Westermeier (1995) optimized the conditions to enhance carpospore germination of *Sarcothalia crispata* in the laboratory and field. Later, Westermeier and Sigel (1997) and Westermeier et al. (1999) found that *Gigartina skottsbergii* Setchell and Gardner produced spores which germinate only in autumn and winter, and that enhanced germination could be achieved by combining a temperature of 5°C, with a photon flux density of 48 μmol photons m^{-2} s^{-1} and a neutral photoperiod (12:12). Additional information is also available regarding the success of transplantation of spores obtained in the laboratory to both in-door culture and open sea systems (Westermeier et al. 1999; Avila et al. 2003). Our results

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indicated that a high mortality of plants (over 95%) occurs only during the early developmental stages of *G. skottsbergii* in the field. This reduces the temporal window for massive inoculation of substrata required to develop a commercial farm. Alternatively, these studies reported a high capacity of thalli regeneration after propagule release, through intercalary meristem tissue, recovering the loss (Westermeier et al. 1999). As a consequence, it was possible to use vegetative fragmentation of the fronds.

Vegetative propagation of *G. skottsbergii* has been suggested as a potential way to cultivate this species as an alternative to cultivation from spores. Healing and regeneration of frond fragments and rhizoids can be enhanced by manipulation of light and temperature under laboratory conditions (Westermeier et al. 1999; Correa et al. 1999; Buschmann et al. 1999; Romo et al. 2006; Hernández-González et al. 2007). Further information on the growth of recruits and juvenile fronds of this alga under field conditions indicated that a commercially interesting frond size is reached after only 2 years in the sea (Westermeier et al. 1999).

The difficulties to obtain large quantities of spores throughout the year, the low growth rate of the early developmental stages of this alga (Westermeier and Sigel 1997; Avila et al. 2003), and the over-exploitation of wild stocks of *G. skottsbergii* has caused significant problems. Therefore, vegetative propagation appears as an attractive alternative to develop an economically interesting cultivation model. In the present study we evaluate the use of vegetative fragments instead of whole fronds as starting point for *G. skottsbergi* cultivation, and specify the first steps towards the establishment of a commercial farm. In addition, we assess the best system proposed in the present study from an economic point of view. The resulting analysis constitutes the first cost projections reported for the application of commercial mariculture in *G. skottsbergii*, and will be useful to local fishermen.

Material and methods

This study was carried out in a sheltered environment in southern Chile, located near Calbuco (41°43′ S; 73°05′ W; Fig. 1). This area has a rainy winter season with water temperatures of 9–10°C and maximum summer temperatures of 15–16°C. The salinity fluctuates during the year very poorly, around 31 PSU. Different experimental trails were set at 12–13 m depth as described below.

Half-trimmed fronds fastened to ropes A total of 15 fragments (26 cm^2 area on average) were obtained by trimming whole individual fronds of *G. skottsbergii* into half (Fig. 2a). Whole uncut fronds of the same surface (juveniles) area were used for the control (Fig. 2b). Fragments and whole fronds were fastened to nylon ropes at 30-cm intervals. Three replicate ropes, with five fragments or whole fronds each, were included in each treatment (total ropes=6, total algal units=30) and individual ropes were held at 30 cm from the bottom. The increase in frond surface area (cm^2) was monitored at monthly intervals by scuba divers, measuring the maximum diameter and width of each frond. The increase in fresh biomass was estimated by untwisting the rope and harvesting the fronds randomly. Thallus was re-inoculated instead of using the sampling procedure.

Cutting method: rectangular ships fastened to ropes A total of 16 rectangular thallus fragments (strips of 30×3 cm) were excised from vegetative fronds. The marginal meristems at both ends of the stripes remained intact (Fig. 2c). The fragments were fastened at 30-cm intervals, and the nylon rope was held 30 cm from the bottom (Fig. 2d). As a control experiment, 20 whole fronds with an average surface area of 110 cm^2 were fastened to a nylon rope at 30-cm intervals. Concrete blocks were used to keep the ropes at 30 cm from the bottom. Young whole fronds attached to rocks, which had the same initial surface area, were tagged and used as controls. Changes in length, width and weight of each fragment were monitored at monthly intervals by scuba divers.

Cutting method: triangular segments fastened to ropes Since preliminary experiments demonstrated that *G. skottsbergii* survives cutting, and growth is not impaired, we attempted to improve our cutting methods. Considering *G. skottsbergii* has an elliptical morphology, the cuts were made from the center forming triangular segments, leaving a portion of the meristematic section (Fig. 2e). A total of 800 fragments were fastened at 50-cm intervals to a nylon rope as described above and installed in the sea at 13 m. The experiment started in February (summer inoculation) and June (winter inoculation), and frond surface area and fresh weight were determined at monthly intervals.

Fronds on natural and artificial substrata Management of natural population and areas rotation has demonstrated previously a high effectiveness in suitability of *G. skottsbergii* fisheries, where the recruitment occurs similar to natural populations (Westermeier et al. 1999). In this case, spore-based cultivation would representative of growth on natural population, trough traditional management of *G. skottsbergii*. Five concrete blocks (30 cm^2) were installed at the study site at the beginning of the reproductive period of *G. skottsbergii* (May) to follow recruitment and growth. Once recruits were visible, eight individuals were tagged and used as experimental individuals. A total of 16 recruits settled on natural

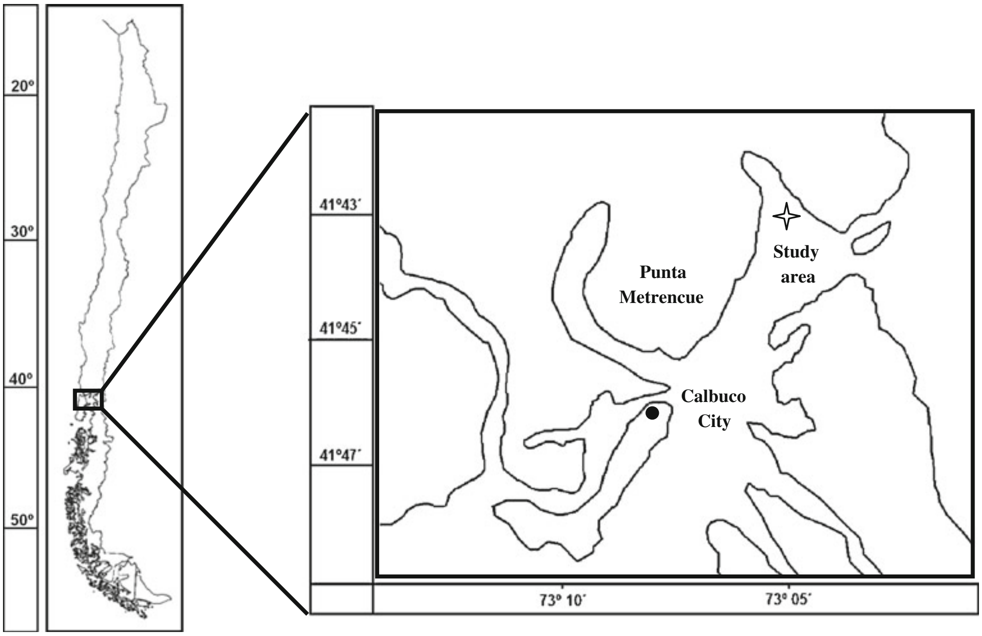


Fig. 1 Study area, Calbuco, X region, Chile

rocks were also tagged and used as controls. The increase in frond area was monitored at monthly intervals by scuba divers.

Growth rates and culture system comparison Growth rate in each culture system is expressed as relative growth rate (RGR; % month^{-1}), and was calculated according Evans (1992):

$$\mathrm{RGR}\left(\%\,\mathrm{month}^{-1}\right) = 100\,\frac{(\ln sf - \ln si)}{\Delta t}$$

Where ln S_f is the natural logarithm of final surface, ln *Si* is natural logarithm of initial surface and Δ*t* is experimental time period (months). To estimate RGR of natural and artificial substrata treatments, we considered initial surface of frond at 10 months of culture. The RGR of all treatments were analyzed by one-way analysis of variance (ANOVA) test.

Economic analysis A 5-year economic analysis was performed taking in consideration our production results of triangular segments and local market of *G. skottsbergii*. A net present value (NPV) and internal rate of return (IRR) was calculated to evaluate the economic feasibility of *G. skotsbergii* cultivation over two culture season: winter and summer. Pilot-scale culture was obtained by seedling 3,000 m^2 in each season. Investment, costs and incomes were extrapolates to commercial mariculture of 1 ha.

Results

Frond area in control individuals (intact fronds) showed a steady increase from October 1996 to April 1997, followed by a sharp decrease in frond surface, which lasted until July 1997 (Fig. 3). At this point, control fronds had had shrunk by about 35%. From July onward, frond surface resumed a steady increase, and by June 1998 reached an average value of 33 cm^2, 27% higher than at the beginning of the experiments. Trimmed fronds, in contrast, showed an immediate, although smaller, decrease in area, with mean values of 23 cm^2 in December 1996 (Fig. 3). Subsequently, these fronds responded similarly to the controls, including a steady increase during the first part of 1997, and a sharp decrease in the following winter. They ended up with a slow but steady increase in area by June 1998 (Fig. 3). At this stage, surface area of the half-trimmed frond was nearly 38% larger than at the beginning of the experiment (Fig. 3).

Our results showed that whole fronds, growing on ropes, had a net surface area increase of 525 cm^2 over the monitoring period (13 months). This represented a sixfold increase of the original area (Fig. 4). The control whole fronds, attached to the rocky bottom, on the other hand, increased 14 times their initial surface area. The performance of frond fragments was poorer: after a steady increase in surface until May 1998, they showed a sharp decline in June and July, reaching a final surface area only slightly larger than at the beginning of the experiment (Fig. 4).

Triangular segments showed a steady, but lower increment in their frond surface area during the summer and winter in suspended culture (Fig. 5a,b). However, in spring these fragments significantly increased their surface area, reaching over 700 cm^2 in October. This was significantly higher than in whole fronds, which reached a total of 600 cm^2. The fresh weight of triangular fronds reached 130 g m^{-1} line in the same period (February–October),

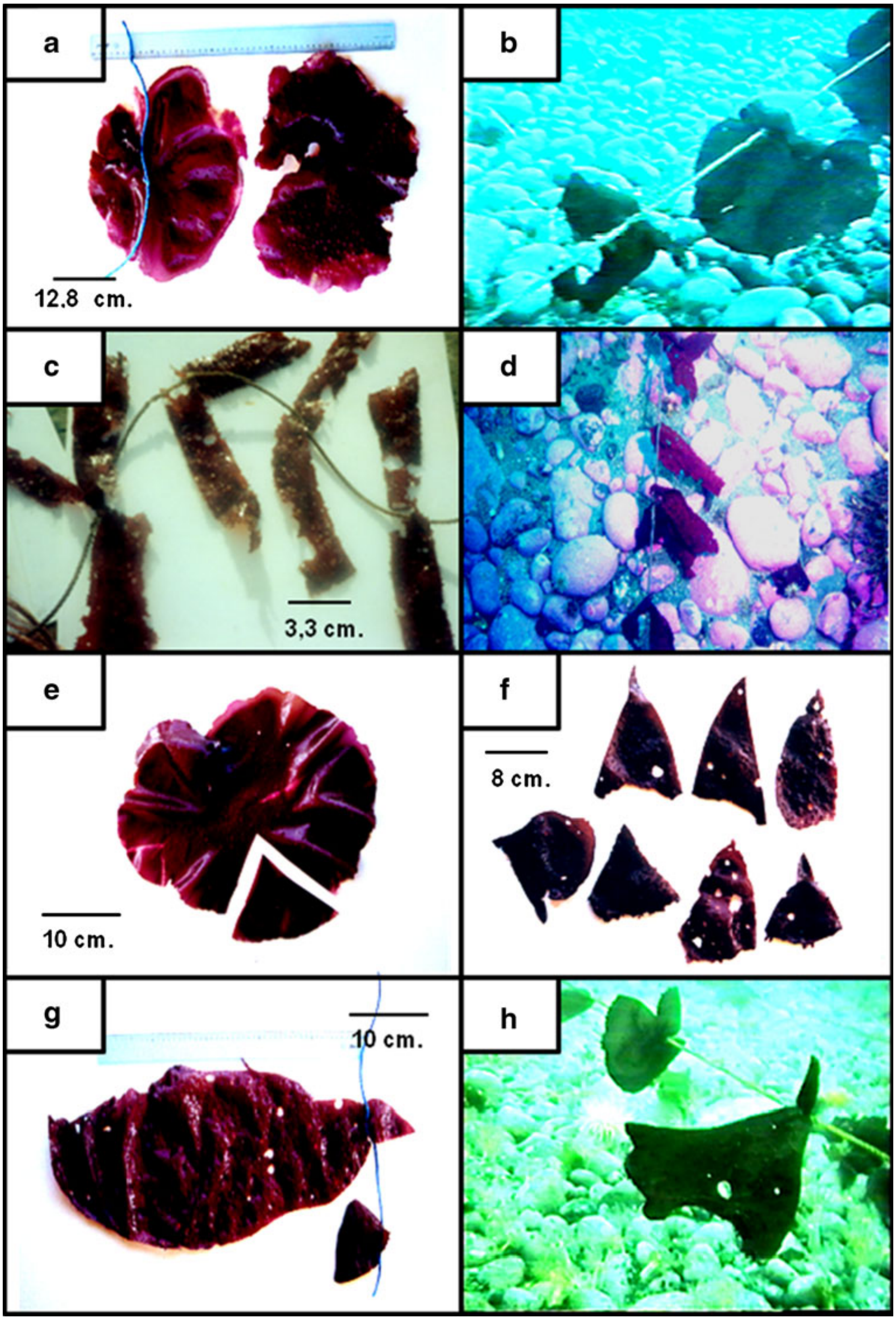


Fig. 2 Morphology of *Gigartina skottsbergii* fragments used in suspended cultivation. Half-trimmed fronds **a**; whole fronds **b**; rectangular ships **c**–**d**; triangular segments **e**–**f**; elongated morphology **g**–**h**

which was significantly higher than the whole fronds that reached, on average only 93 g m^{-1} line (Fig. 5c, d). Finally, the triangular segments reached between 800–1,000 cm^2 and 150–180 g m^{-1} over 10 months.

The growth responses of *G. skottsbergii* were an overall similar on both individuals settled on artificial and natural substrata (Fig. 6). No significant growth was recorded from December 1996 to July 1997. This “resting period” was followed by a steady and fast growth of individuals attached to both natural and artificial substrata. This active period, characterized by consistently higher surface area values of those individuals attached to our artificial substratum, concluded with almost identical mean values of frond surface area (Fig. 6).

No significant differences were found in the initial surface thalli (ANOVA, $p>0, 05$). The estimated growth

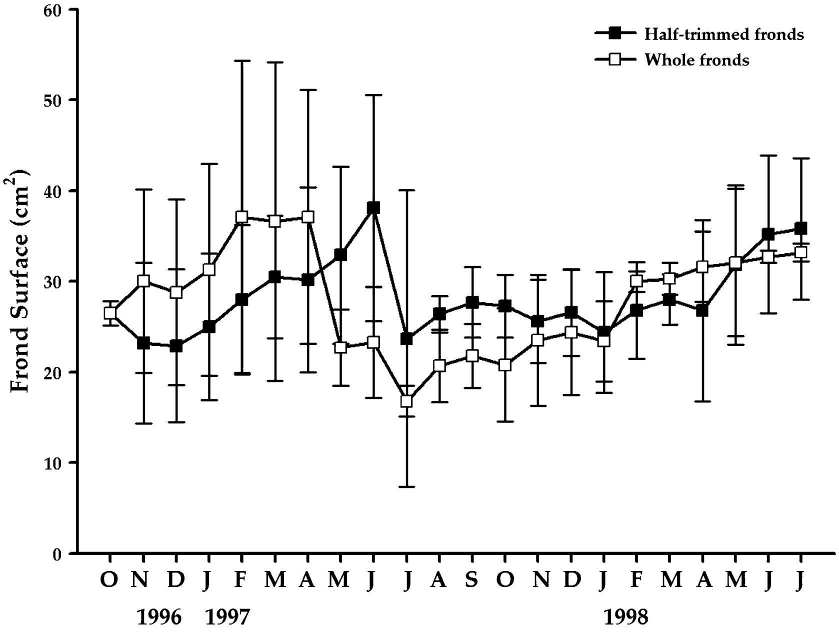


Fig. 3 Monthly variations in the area of half-trimmed and intact fronds (mean ± SD)

rates for *G. skottsbergii* under different experimental conditions indicated that the best perfomance was recorded in juvenile individuals growing on the artificial substrata (Fig. 7). High growth rates were also observed in suspended whole fronds as well as in control plants. Relatively low growth rates were recorded when the plants were cut, either in half or into strips (rectangular ships). In contrast, triangular fragments presented the highest growth rates including those seen in whole fronds (Fig. 7).

Five-year economic data for *G. skottsbergii* culture is shown in Tables 1 and 2. The initial investment and total cost as a function of final production (total harvest per year), and final income was obtained from the product between sales price on beach (Sernapesca 2008) and final production. Pilot-scale trials were characterized by a simple system of culture with low physical investment. This system involved an anchor system and ropes of polipropilene, where the main supports were four 1,000 kg anchors in corners and three horizontal basal long lines of 18 mm of diameter, without floating system (Fig. 8a, b). Culture lines were installed perpendicular to basal lines, and triangular segments were inoculated in the core of rope. Total cost

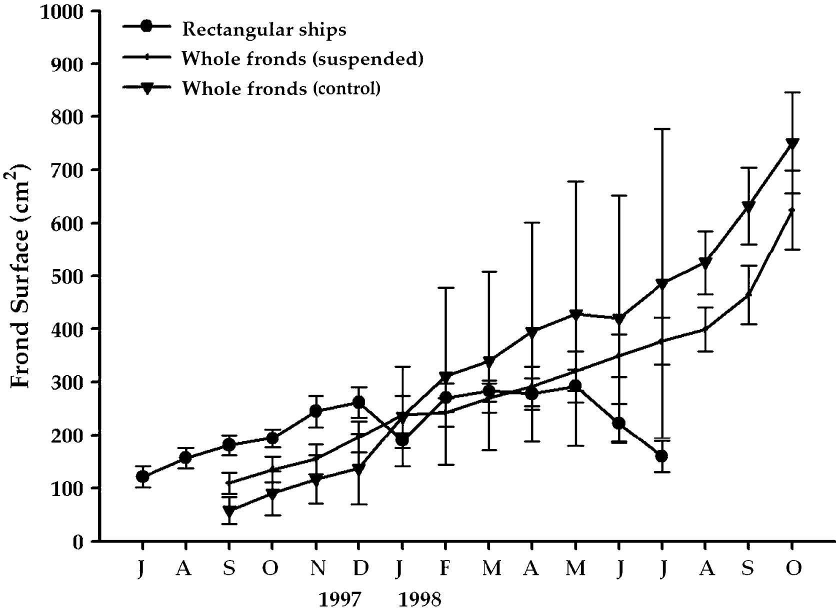


Fig. 4 Monthly variations in the area of rectangular ships and whole fronds growing on suspended ropes. Control fronds grew attached to rocks (mean ± SD)

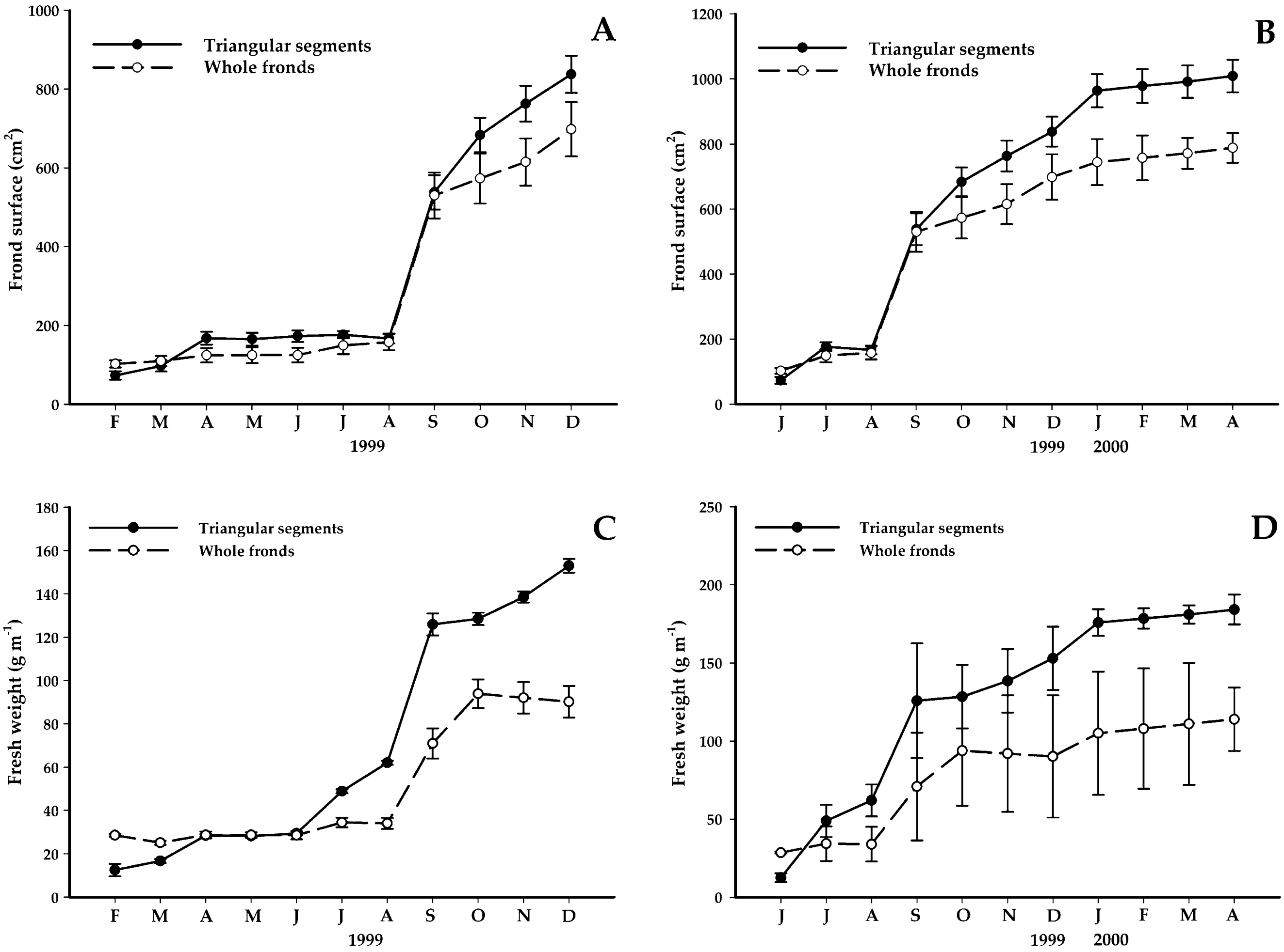
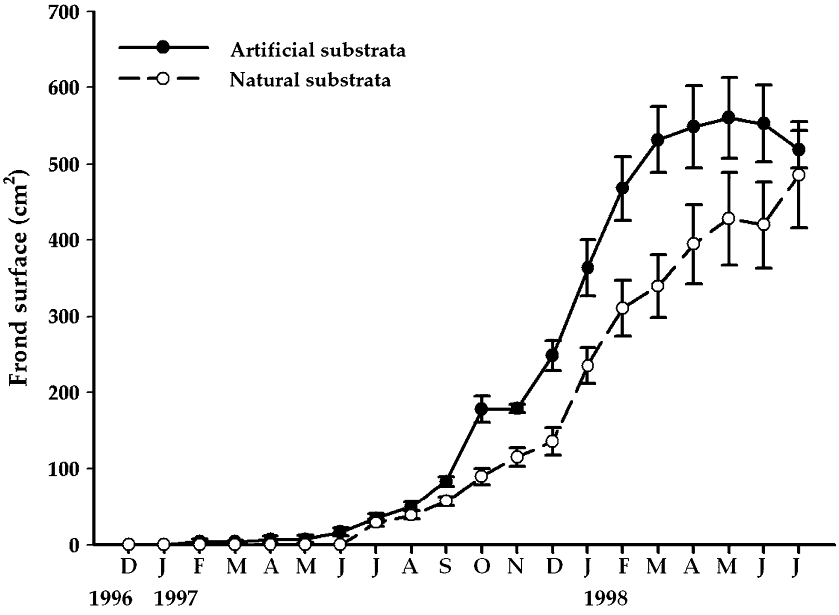


Fig. 5 Monthly variations in the area of whole fronds (mean ± SD) and biomass (fresh weight) of triangular segments growing on suspended ropes. **a**, **c** Summer inoculation; **b**, **d** winter inoculation

Fig. 6 Monthly variations in the area of fronds attached to artificial (concrete blocks) and natural substrata (mean ± SD)



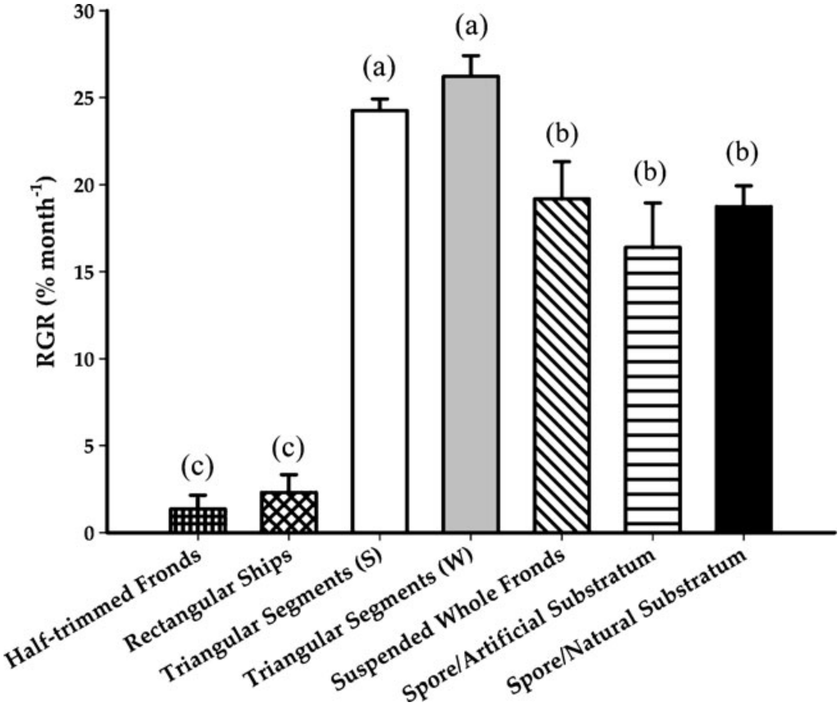


Fig. 7 Relative growth rates in *Gigartina skottsbergii* in different treatments. *S* summer inoculation, *W* winter inoculation

was relatively stable independent of seedling season or total biomass (US $1,000–1,200 ha^{-1} year^{-1}), where costs associated to infrastructure maintenance were the highest (Table 1).

NPV and IRR obtained by economic analysis showed that the winter cultures generated higher returns than the summer cultures, and seedling at six fronds m^{-1} will recover the initial investment, considering annual costs, availability of fronds and economical returns (Table 2). Furthermore, annual incomes were high also, fluctuating between US $1,600 and $3,000 ha^{-1} year^{-1} in summer seedling and US $2,100–3,500 ha^{-1} year^{-1} in winter cultures.

Table 1 Initial investment and annual costs for hypothetical commercial mariculture of *Gigartina skottsbergii*

	Summer seedling			Winter seedling	
Investment	Unit price (US $)	Required units	Total (US $)	Required units	Total (US $)
Long-line ropes					
Basal ropes[a] (18 mm)	142.9	1.5	214.35	1.5	214.35
Culture ropes[a] (4 mm)	6.5	150	975	150	975
Rings[a] (8 mm)	16.3	1	16.3	1	16.3
Anchor system					
Basal anchors (1,000 kg)	163.3	4	653.2	4	653.2
Secondary anchors (250 kg)	30.6	21	642.6	21	642.6
Total investment			2,501.45		2,501.45
Annual costs					
Triangular segments[b] (US $)	0.00155	180,000	279	180.000	279
Operation costs (US $ ha^{-1} year^{-1})	–	–	450.7	–	450.7
Harvest costs[c] (US $ kg^{-1})	0.03	4,794–9,180	143.8–275.4	6,390–10,440	191.7–313.2
Aquaculture taxes	–	–	175.4		175.4
Total costs			1,048.9–1,180.5		1,096.8–1,218.3

[a] Reel of 220 m

[b] Originated from 30 g whole frond (six segments per frond)

[c] Variable cost of 2 days of work (in function of total biomass)

Table 2 Economic data of *Gigartina skottsbergii* suspended culture

	Summer seedling	Winter seedling
Seedling density (no. of fronds m^{-1})	6	6
Long-lines (100 m) per hectare	300	300
Beach sales price (US \$ kg^{-1})	0.34	0.34
Highest production[a] (kg ha^{-1} $year^{-1}$)	9,180	10,440
Lowest production[b] (kg ha^{-1} $year^{-1}$)	4,794	6,390
Initial investment (US \$)	2,501.45	2,501.45
Highest Production costs[a] (US \$ $year^{-1}$)	1,180.50	1,218.30
Lowest production costs[b] (US \$ $year^{-1}$)	1,048.92	1,096.80
Highest income[a] (US \$ $year^{-1}$)	3,091.22	3,515.51
Lowest income[b] (US \$ $year^{-1}$)	1,614.31	2,151.73
Depreciation (US \$ $year^{-1}$)	500.29	500.29
NPV (12% (US \$))	737.89	2,410.53
IRR (%)	27.49	55.13

Net present value (NPV) is represented as a function of seedling density

[a] Data used in first year

[b] Data used in second third, fourth, and fifth year of analysis

Discussion

Suspended-culture of vegetative thalli tended to be the best system in our study. Triangular segments of *G. skottsbergii* had the highest growth rate (ANOVA, $p<0.05$), averaging between 24.36% and 26.22% per month, followed by the control, suspended whole fronds. Our experiments with fronds cut into two and its control showed erratic growths

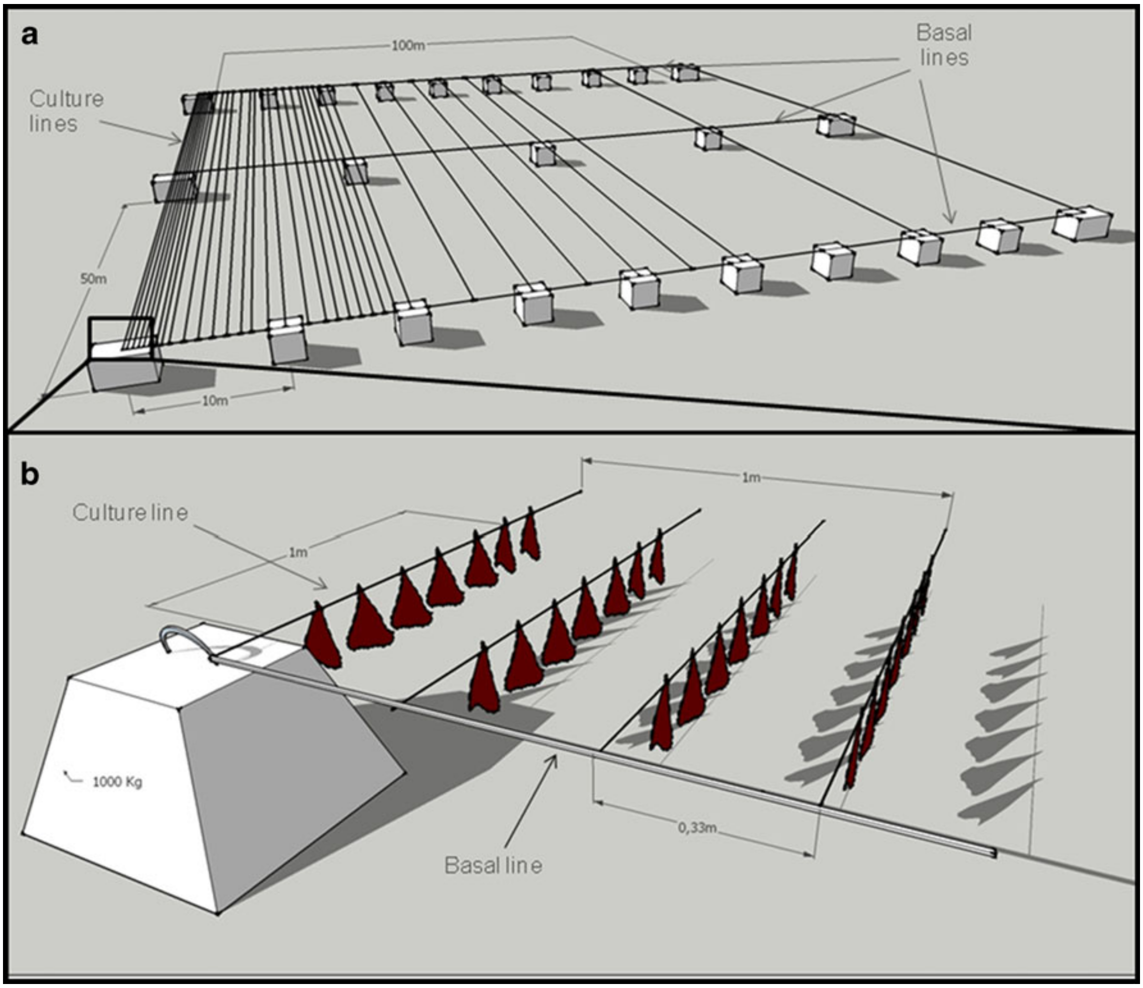


Fig. 8 Pilot module of *Gigartina skottsbergii* culture in 1 ha of sea bottom. **a** Full view of basic installation; **b** magnification of culture lines

and a slight recovery, which are explained by high herbivory by the echinoids *Loxechinus albus*, *Pseudoechinus magellanicus*, and *Arbacia dufresni*, which were inhabitants of the study area. The spore seeding systems tested showed similar growth rates, independent of the substrata used. However, the RGR was considerably higher, since it did not include the first 10 months of culture. At 19 months of culture, these systems are the longest of all treatments.

In general, *G. skottsbergii* has relatively low growth rates (Westermeier et al. 2001). In similar studies, Romo et al. (2006) compared the triangular segments and whole fronds performance in suspended culture. That research group did not detect statistical difference between both treatments, probably because of the relatively short duration of the trial (ca. 3 months), was not enough to demonstrate any difference. Moreover, their performance results were slightly higher than the data presented here (between 1% and 2% per day), but showed a decreasing tendency in subsequent months. In the first months of regeneration, the growth potential is the highest of total culture time, which could be the main cause of differences with our results. Also, in cultures of major temporal scale, the difference in growth between triangular segments and whole fronds is more evident, decreasing the RGR in both treatments but finally showing the differential growth detected in our study.

Although in previous studies in Ancud, our plants attached to artificial substrata showed the highest growth rates, their permanence is probably limited by the mechanical effect of waves on the fronds (Westermeier et al. 1999). Similarly, Navarro and Westermeier (1995) found maximal growth of *Sarcothalia crispata* in a suspended culture at sites protected from wind and swells.

The beginning of our experiments corresponds to the winter months for the suspended systems and for the artificial substrata on the bottom, and therefore coincided with the reproductive season of *G. skottsbergii* (Westermeier et al. 1999). Using the fragment method, would be recommended that culture initiation should start in winter, allowing the regenerative process before the growing period starts in spring.

The growth of triangular segments of *G. skottsbergii* fastened to ropes enhanced the production of biomass. The fronds obtained were elongated and differed from the normal elliptical morphology of *G. skottsbergii* (Fig. 2e–h). We postulate that this elongated morph is better adapted to our rope system, since a higher number of fragments can be installed per meter of rope. This issue is fundamental to improve the production potential of *G. skottsbergii* considering that it is a slow growing species. However, our results suggest that *G. skottsbergii* could also be cultivated in the same manner as other carrageenophyte seaweeds such as *Eucheuma* and *Kappaphycus*. As indicated in a previous study (Westermeier et al. 1999), we further suggest that the use of vegetative propagation techniques seems to be an alternative for the restoration of *G. skottsbergii* natural populations or mariculture programs, considering the possibility to produce gametophytes or tetrasporophytes separately, in function to lambda or kappa requirements of carrageen industry.

From technical and economical approaches, and unlike other systems developed with *G. skottsbergii*, our system does not need floating system, saving 30% of initial investment and fully exploiting the surface of sea bottom, where conditions of growth are optimal. Cultivation systems with floating buoys need a minimal space between culture lines of 5 m, where craft may pass during harvesting work. A possible design of culture with the features abovementioned is not rentable, which is the main reason of the current context, where extraction from natural population supported by management techniques is preferred. The possibility of producing relatively high biomass when fresh *G. skottsbergii* is scarce (i.e., winter months), would be interesting also, resolving several of the main problems of this activity: the seasonality of standing stock and its availability in nature.

The results of our study suggest that cultivation of *G. skottsbergii* via vegetative propagation is feasible if the following combination is used: artificial substrata to collect spores and suspended culture for biomass production as well as inoculant recovery. The best cutting method presently known is to use triangle fragments, which showed higher growth rates than whole fronds.

Acknowledgments This work was funded by FONDECYT 1951203 and FONDAP Subprograma Algas grants. The authors thank the strong field support from Dr. Westermeier's students and Dr. Dieter Müller for helpful corrections, and two anonymous reviewers for constructive criticism that helped improved the manuscript.

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