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Culture of *Gigartina skottsbergii* (Rhodophyta) in southern Chile. A pilot scale approach

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Abstract

In the last 10 years studies on the management and exploitation of Chilean carrageenophytes have proliferated in response to the increasing development of the local processing industry. One of the most important sources of raw material for Chilean carrageenan, *Gigartina skottsbergii* Setchell et Gardner, was the subject of an intensive study to design a commercial cultivation technique which could be an alternative to wild harvest. In this context this pilot study reports the first successful attempt to culture *G. skottsbergii* from spores to harvestable plants. A three-step farming approach was developed: (i) seeding of spores onto scallop shells followed by a two-month nursery period in a greenhouse (until the development of initial upright thalli from the discoid crust occurred), (ii) outplanting juvenile plants on shells in the sea on a long-line system (until thalli attained 3–4 cm diameter) and (iii) detachment of fronds from the shells, fixing of individuals to vertical ropes and growth until commercial size was reached. Additional experiments to compare bottom and suspended growth, cultivation by fragmentation and whole fronds and meristematic activity of different zones of the fronds were performed. This study shows the technical feasibility of culturing *G. skottsbergii* from spores, complemented with growth of vegetative fragments, in order to optimize the management of the culture. In the future, therefore, it may be possible to replace the heavy exploitation of wild beds in southern Chile with farming activities.

Introduction

Recently, several studies on wild populations of *Gigartina skottsbergii* Setchell et Gardner have been performed in order to acquire a basic knowledge on yearly abundance patterns, reproductive phenology and recruitment of young thalli (Zamorano & Westermeier, 1996; Avila et al., 1997, 1999a; Westermeier et al., 1999; Marín et al., 2002). Buschmann et al. (1999, 2001) suggested that *G. skottsbergii* was being overexploited and consequently that the development of culture technologies was urgently required to support the local carrageenan industry. In fact, Avila et al. (2003) reported that the heavy exploitation of natural beds was reaching as far south as Seno Año Nuevo (55°25′S; 69°00′ W). Such harvest displacement to southerly sites (approximately 1500 km south from traditionally harvested sites in Chiloe during the 1990s) was the logical consequence of an increasing *G. skottsbergii* shortage in previously productive beds of Chiloe. Since 2002 to late 2003, exploited beds have shown serious symptoms of depletion. Consequently, a continued decline in standing stocks could be a serious risk for both local and overseas carrageenan industries.

Preliminary experiments done by Buschmann et al. (1999) recorded that the growth of germlings seeded in the laboratory on Petri dishes and transplanted to outdoor tanks reached 1–2 mm in 3 months. Buschmann et al. (2001) reported that the growth of germlings seeded in the laboratory on ceramic plates and later transplanted to the field reached about 5 mm^2 after 5 months in the sea. Growth during 6 months, of 16 fragments of fronds excised from immature wild fronds

was reported by Buschmann et al. (1999) and growth of 20 whole young fronds during 12 months was reported by Buschmann et al. (2001). Both experiments used the same methods: fastening the fragments and fronds to ropes settled at 20 cm above the bottom. In addition, Correa et al. (1999) suggested from *in vitro* experiments that mass cultures by vegetative propagation techniques could be a promising tool for establishing future farms.

A greenhouse nursery method for *G. skottsbergii* mass culture consisting of: (a) settlement of spores on natural and artificial substrata; (b) survival of germlings under indoor conditions and then outplanting into the sea, was developed by Avila et al. (2003). Germlings of about 60 to 110 mm^2 were obtained in 15 months. The method consisted of seeding of spores on different types of substrata followed by the development of germlings, for two months in a greenhouse. Plants were then transplanted to suspended systems at different depths in the Bay of Hueihue (41°54′S; 73 °31′W in Chiloé Island). The best depth for early growth of *G. skottsbergii* in the environmental conditions at the Bay of Hueihue was shown to be 3–6 m (see Zamorano & Westermeier, 1996, for general abiotic conditions of Ancud near the study area).

This paper presents for the first time the results of a pilot study of 31 months on commercial culture techniques of *G. skottsbergii,* to produce kappa-carrageenan gametophytes, at the Bay of Hueihue. The culture was based on a tetraspore-seeding method using scallop shells as substrata (Avila et al., 2003). Cultivation was followed by the growth of thalli on suspended cultures in the sea until plants reached of commercial size, complementing the results of Avila et al. (2003). In addition, growth of germlings on bottom and suspended systems, and both whole thalli and fragments were compared. Finally, meristematic activity on different parts of young plants was assessed.

Material and methods

Mass culture starting from spores

All mature sporophytes used as reproducers were collected at the Bay of Ancud (41°52′S; 73°31′W) and at Calbuco Channel (41°45′S; 73°05′W). The spore-seeding method on scallop shells was used (Avila et al., 2003). Scallop shells were one of the best substrata reported and were easily available from farms of scallops near the site of study. Spore seeding, using only tetraspores to produce kappa-carrageenan gametophytes, was done at the greenhouse facilities of the Maricultural Station at the licensed site of the Instituto de Fomento Pesquero in the Bay of Hueihue (41°54′S; 73°31′W). After two months, once the basal crusts developed their uprights of gametophyte fronds, the germlings were transplanted to the sea.

Variations of the floating structures described in Romo et al. (2001b) were used in this study. Cylindrical floats of PVC which separated the lines of the 100 m double long-line and the small 2 L buoys were replaced by 9 polystyrene 200 L buoys for better flotation. The shells, perforated in the centre, with germlings growing in the upper side of the shells, were arranged in sets of ten shells fixed to 1 m polypropylene ropes of 3 mm diameter (Figure 1A). Knots adjusted on and under each shell secured them to the ropes; shells were separated by 10 cm. A total of 1370 shells disposed in 137 ropes, were placed at 6 m depth, one of the most suitable depths for growth reported by Avila et al. (2003). Three metres depth was also good for germling growth but in the present study this depth was not used due to excessive fouling that developed on shells in previous

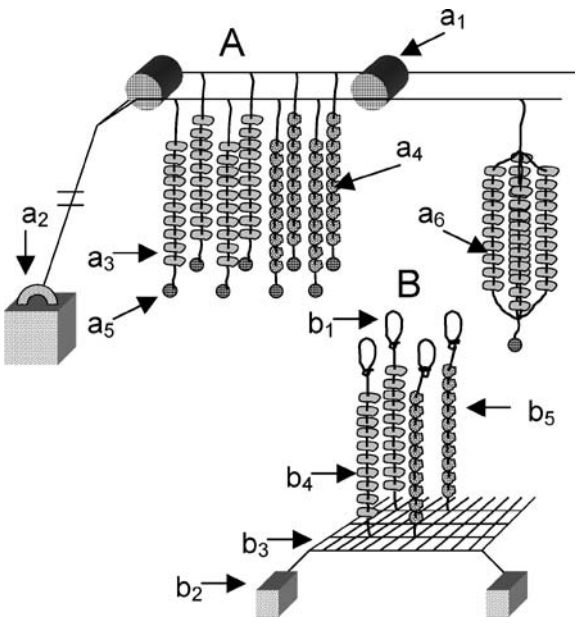

Figure 1. (A) Floating system for *G. skottsbergii* cultivation: a_1 = buoys; a_2 = concrete anchor; a_3 = fronds; a_4 = shells with young thalli; a_5 = sinker; a_6 = four ropes with fronds in a vertical rope. (B) Bottom system, b_1 = buoys; b_2 = concrete anchor; b_3 = net; b_4 = fronds; b_5 = shells with young thalli.

trials. Growth was estimated monthly by measuring frond width, which corresponds to the major diameter of the ellipsoidal thallus. Monthly cleaning to remove fouling and possible grazers was done by using high pressure jets of seawater on the shells. Once the fronds attained 3 to 4 cm diameter they were detached from shells and individually attached to 2 mm diameter polypropylene ropes, at intervals of 10 cm. The attachment was done by means of a 2 mm hole perforated with a cork borer and 10 fronds were threaded in the rope. As in the shells, each young frond was secured to the rope by knots on and under the blade. Once the fronds attained more than 10 cm diameter the 2 mm ropes were replaced by 3 mm ropes and attached with cable-ties on and under each frond until commercial size was reached. In order to optimize the capacity of the floating long-line a minimum of two and maximum of five 1 m ropes with adult fronds were attached to the main vertical ropes (Figure 1A).

Bottom and suspended growth

An experiment to assess the growth of early gametophytes of *G. skottsbergii* growing on shells attached to ropes anchored to the bottom, and also in suspended conditions at 6 m constant depth, was conducted from April to December (autumn to late spring) 2002. The experiment was established in order: (i) to evaluate the performance of both cultivation systems in a site exposed to strong tidal currents like in Chiloe Island and neighbouring areas occur and (ii) to evaluate differences in growth of *G. skottsbergii* in both conditions. A 30 cm stretched-mesh fishing net of 9 m^2 was attached to the bottom of the Bay of Hueihue in a site where there are fluctuating depths from 3 to 9 m (7 m is the maximal tidal amplitude during spring tides). A total of 3610 shells (361 ropes with 10 shells per rope) with thalli of about 0.1 mm initial diameter was placed in ropes maintained in vertical rows by means of 2 L plastic buoys (Figure 1B). Two sets of 8 ropes were identified in suspended and bottom systems, respectively, for monthly measurements of the width of 160 gametophyte germlings growing on shells. While the bottom system was subject to fluctuating depth according to the daily tidal rhythm, the suspended system maintained the plants on the shells at a constant 6 m depth. The Mann Whitney test (Zar, 1999) was used to test whether differences in frond growth in both systems were significantly different.

Whole thalli and fragment growth

An experiment was established to compare growth of whole thalli with growth of fragments. Growth of 10 triangular fragments of young blades per rope on 10 ropes (cutting 2–3 triangular pieces from the basal-hapterial region to the margin of each blade) was compared with ten young fronds per rope on 10 ropes. Ropes were suspended at 6 m depth. As wet weight was different for fragments and whole fronds, monthly growth measurements were converted to daily growth rates (DGR) assuming exponential growth according to Hansen (1980):

$$\text{Daily growth rate } (\%/\text{day}) = 100\,(\text{Ln}(W_\text{f}/W_\text{i}))/t,$$

where Ln: natural logarithm; W_f: final wet weight; W_i: initial wet weight and t the time interval (in days) between final and initial measurements. Differences in growth were analyzed at the end of the experiment by the Mann-Whitney test. Some comparisons in the Discussion section were made as monthly growth rate, so the variable t in the formulae in that case corresponded to a monthly interval.

Meristematic activity in different parts of the fronds

In order to investigate zones with faster growth in the fronds and to harvest only old unproductive areas of the thallus with slow growth, the response of different zones of a frond was assessed using a modification of a perforation technique (Norris & Kim, 1972) using a cork borer. Norris & Kim made many perforations at 2–3 mm apart on each frond while we made only 11 perforations (Figure 2). Growth causes the separation of holes in proportion to the amount of growth that has occurred (Figure 2). Growth was assessed during 70 days in two groups, for each of 10 young gametophyte thalli selected among the seeded plants growing in the suspended culture. The size of plants was about 10–15 cm diameter and neither cystocarps nor spermatangia were present. One group had perforations and the other group had none. They were hung from the long-line system at 6 m depth in the same way as described above. Differences in growth rate among marginal, basal zone and whole fronds were analyzed by the Kruskal-Wallis test (Zar, 1999).

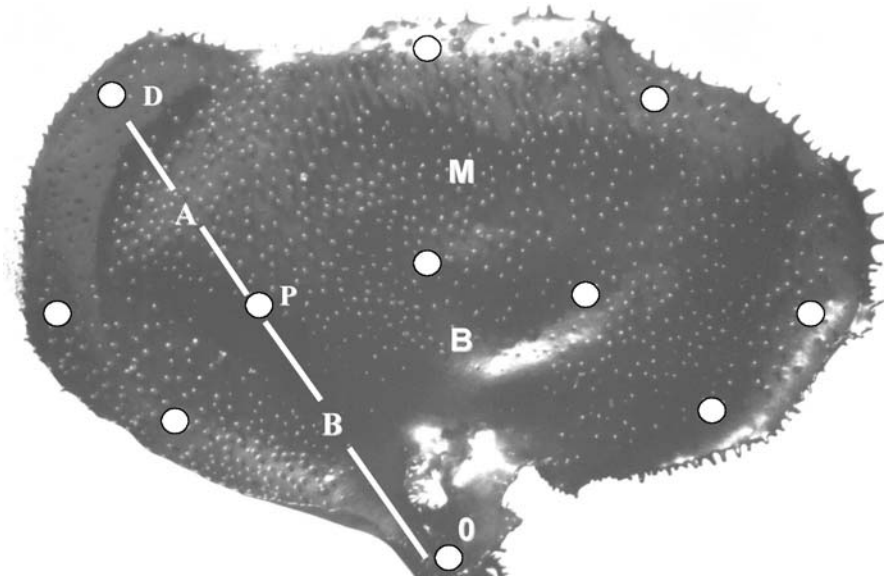

Figure 2. Perforated *G, skottsbergii* frond for measurement of growth in different zones of the thalli. (D) distal point; (P) central point; (0) basal point; (M) marginal zone and (B) basal zone.

Results

Mass culture starting from spores

After the second month in the greenhouse, the germlings were 0.08 to 0.15 mm tall on the shells and were outplanted into the sea at 6 m depth in vertical rows of ten shells per rope. In general, the germlings displayed a very slow growth, especially during the initial 14 months growing in the floating system, when the monthly growth could be measured in millimetres of growth of frond diameter (Figure 3A). Figure 3B shows the continuation of growth during the next months and year reaching about 60 cm width in April 2004. The minimum time to reach a harvestable size of 20–30 cm diameter can be considered to be 26–27 months. Fouling by *Polysiphonia* sp., *Antithamnionell*a sp. and undetermined hydrozoans was the major problem when the plants were growing on the shells, but periodical cleaning, using high pressure jets of seawater on the shells, eliminated the fouling. When female fronds developed cystocarps from October and November 2004 until the end of the study, heavy settlement of young commercial mussels, *Aulacomya ater* Molina and *Choromytilus chorus* Molina, occurred on cystocarps as well as on the hapteral area of all fronds, especially during summer.

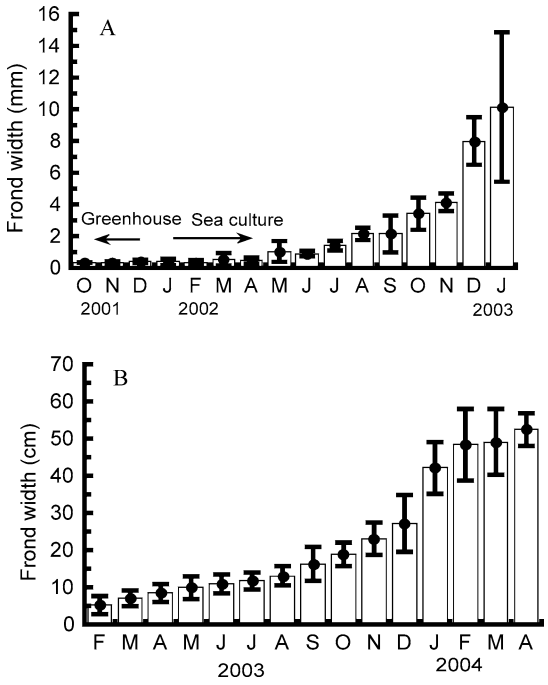

Figure 3. Culture of *G. skottsbergii* suspended at 6 m depth. (A) Growth of very young thalli (frond size is measured in mm width ± S.D.). (B) Growth of young to adult thalli (frond size is measured in cm ± S.D.).

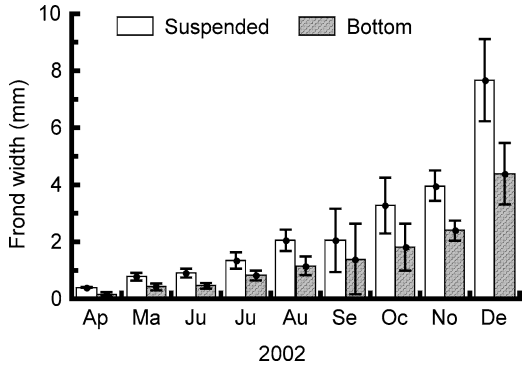

Figure 4. Growth (width ± S.D.) in *G. skottsbergii* suspended at 6 m depth and on the bottom.

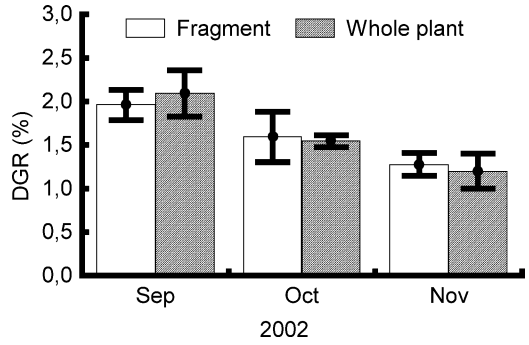

Figure 5. Daily growth rate (± S.D.) of *G. skottsbergii* fragments and whole fronds in the floating system.

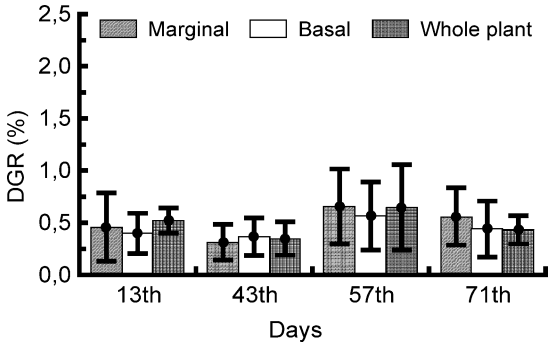

Figure 6. Daily growth rate (± S.D.) of marginal and basal zones of the *G. skottsbergii* frond compared with whole fronds. Thalli were growing at 6 m depth in suspended conditions.

Bottom and suspended growth

There was a significant difference between cultivation methods: the suspended culture of germling gametophytes had significantly greater growth than the bottom culture ($p < 0.05$; Mann-Whitney test, Figure 4). Heavy invasion of drifting *Ulva* sp. on and among the ropes and shells of the bottom system continually occurred. Despite the monthly cleaning by divers, *Ulva* sp. repeatedly invaded the experimental site in few days after each cleaning. The bottom experiment was terminated in November 2002 due to the strong interference by *Ulva* sp. and the severe entangling of the vertical ropes by drifting algae.

Whole thalli and fragmens growth

This experiment, designed to test differences between growth of whole fronds and fragments of young fronds, did not show significant differences between treatments ($p > 0.05$; Mann-Whitney test, Figure 5). Fragments showed total healing along the cut edges before or at the end of the second week of experimentation.

Meristematic activity in different zones of the frond

Growth of marginal and basal zones of the fronds and whole fronds did not differ significantly ($p > 0.05$; Kruskal-Wallis test; Figure 6). The wounds produced by the cork borer healed rapidly and neither promoted nor inhibited the growth around the wounded hole.

Discussion

Mass culture starting from spores

Gigartina skottsbergii belongs to the group of species that must be cultivated in a multi-step sequence (Santelices, 1999). The cultivation process should consider a spore-seeding stage in indoor facilities followed by outplanting to the sea where growth may continue until harvest. This procedure contrasts with the cultivation of *Eucheuma* and *Gracilaria* which can easily be propagated by thallus fragmentation. Our study and Avila et al. (2003) showed that a relatively long time must be spent to obtain visible plants growing on shells during the first year in the sea (Figure 3A). Results reported by Avila et al. (2003) from experiments started on July 1999 (winter) showed 80 mm^2 (about 10 mm width) as the final size at 3 m depth after 13 months in the sea. In the present study, started in October 2001 (mid spring) the mean width recorded on young fronds was also about 100 mm during the same

period of time, but in this case the fronds were suspended at 6 m depth in the sea. (Figure 3A). After the following 13–15 months the plants reached commercial size.

Usually shells supported a great number of *G. skottsbergii* germlings, but the majority of them remained dormant, covered by several larger 3–4 cm width dominant fronds. The removal of these fronds in order to grow them independently attached to ropes could allow triggering of the growth of the uncovered germlings on the shells.

Growth rates of *G. skottsbergii,* of between 0.5 to 2.5% monthly, computed in several experiments in this study, are low compared, for instance, to the growth of *Sarcothalia crispata* (Bory) Leister, another blade-shaped commercial Gigartinaceae in cultures at the same site (Avila et al., 1999b) and in Bay of Coliumo, in central Chile (Romo et al., 2001a). These studies concluded that *S. crispata* was ready for harvest in eight to ten months after spore seeding onto artificial substrates. One of the more striking morphological features of *G. skottsbergii,* in contrast to *S. crispata,* is the extreme blade thickness of *G. skottsbergii* which attains about 400 μm in youngest fronds to almost 600 μm in old fronds. On the contrary, mature *S. crispata* is about 80 μm thick. Extremely low photosynthetic rates and high metabolic expenses required to grow and maintain a thick non photosynthetic medullar tissue could be the cause for low growth rates of *G. skottsbergii*. On the other hand, thickness of the fronds permitted the fronds to be re-attached to vertical ropes for suspended culture and subsequently to grow independently from shells.

Bottom and suspended growth

It could be assumed that disturbances produced by heavy and repeated invasions of drifting *Ulva* sp. (forming a thick carpet above the bottom system and entangling the net and vertical ropes) could be one of the causal factors for low growth of bottom fronds. For example, the canopy provided by large fronds of *Ulva* sp. reduced the availability of light for photosynthesis. Although biomass of *Ulva*. sp. was not monitored, the persistent invasion of *Ulva* sp. on the bottom stand of *G. skottsbergii*, despite the monthly clearance of drifted *Ulva* sp. by divers, reinforced our idea that *Ulva* sp. could retard the normal growth of *G. skottsbergii.* After 9 months and several repairs to entangled ropes, the bottom method was discarded for use in commercial farming. Notwithstanding, and considering the worst conditions that affected the bottom system at the Bay of Hueihue, the growth of bottom germlings from 0.5 mm in April to 4.5 mm diameter in December, 2002 (Figure 4) represented about 0.8 daily growth rate similar to the results of several indoor, short-term experiments reported by Buschmann et al. (2004). In that study the highest specific growth rate with a mean value of about 1% day^{-1} corresponded to free-floating and attached fronds in experiments done under semi-controlled conditions in six 2-litre containers during 4 weeks.

Suspended cultivation in the Bay of Hueihue seems to be clearly better for growth than the bottom conditions at Ancud tested by Buschmann et al. (1999). That experiment consisted of "16 fragments excised from immature fronds fastened to 8 m nylon cord" which in 6 months grew from 30 cm in July to 40 cm length in December (Buschmann et al., 1999a, p. 433). Interpolating the data in Figure 8A and assuming exponential growth (see Materials and Methods in the present contribution) calculations revealed a 4.8% monthly growth rate. This was compared with our data on suspended culture in the same period of the year in which the cultivated plants grew from 14 cm width in July to 30 cm width in December 2003 (Figure 3B). The computed growth of suspended plants at Hueihue was 12.7% monthly growth rate, about twice as high as the growth at Ancud. It was not possible to repeat the comparison on data of young fronds, growing on the bottom at Calbuco, reported by Bushmann et al., 2001 (see Figure 7, p 262), because while in the text it is reported that juveniles increased from 100 to 390 cm^2 in 8 months, the graph in their Figure 7 indicates that growth really occurred during 12 months of experimentation (September to August).

Whole thalli and fragment growth

The non-significant difference in growth between whole fronds and fragments confirmed for the first time that fragments could grow in the sea with the same growth rate as intact thalli. This had been suggested by Correa et al. (1999, p. 326). They stated that "vegetative propagation is possible not only because the early stages in the process can be successfully manipulated *in vitro*, but because those plantlets can develop normally in both tank systems and in the sea, growing at the same rates as wild plants (unpublished)". Surprisingly, the same research group commented later that Westermeier et al. (unpublished data)

said that, "vegetative fragments and subsequent regeneration resulted in plants with an elongated habit and a growth potential higher than non fragmented fronds" (see Buschmann et al., 2001, p. 262). At present our findings are solving this discrepancy and, at least in field-suspended conditions, both fragments and whole fronds grew at similar daily growth rates.

Meristematic activity in different parts of the frond

The fact that growth of marginal and basal zones of the fronds did not differ significantly from whole fronds confirms the statement of Norris and Kim (1972) that in the genus *Gigartina* most growth of the young and adult blade is by a diffuse intercalary meristem that is formed as the upright thallus expands to form a blade.

A practical application of these results is: (i) similar rate of growth of fragments and whole fronds and (ii) confirmation of the uniform activity of meristems in different parts of the fronds. These results suggest that opportune fragmentation of adult fronds, for further growth, should prevent their excessive weight which could cause premature detachment from the ropes. This procedure could allow fronds to grow to their maximum size before the harvest. The diffuse intercalary pattern of growth differs from the apical-to-basal gradient in growth rate exhibited by *Eucheuma* or *Kappaphycus*. For about 30 years it was known that farmers usually tie young pieces of thalli with good growth potential onto lines and that they harvest the unproductive old pieces (Doty, 1980). Young fragments function as "seeds" but in the case of *G. skottsbergii,* our findings suggest that the young fragments function as little plants, with uniform meristematic proprieties, that should be harvested once mature. Another farming strategy has been proposed by McNeill et al. (2003) for *Gigartina atropurpurea* from New Zealand. This species shows regenerative activity whereby prunings taken near the base of the frond develop a new frond with higher yield than wild un-pruned plants used as controls. On the contrary, *G. skottsbergii* did not show this pattern of growth.

Finally, growth management of young fronds of *G.skottsbergii*, selected from wild harvests, can be done by attaching them to ropes to grow as described above. This procedure will allow extra growth, after three or more months, in suspended culture, by increasing the yield of young, wild harvested fronds. Young thalli can be considered to be thin plants, less than 20 cm wide, and without signs of gametophytic maturation (for instance plants from October-November 2002 in this study): in three additional months they can reach a commercial size of 40 cm width and either be mature or non-reproductive. This management can complement the farming production of spore-propagated plants. This research proved that it is technically possible to farm *G. skottsbergii* but it is necessary to search for new approaches to improve its growth during the first year and to assess its economic feasibility.

Acknowledgements

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