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Growth of the commercial carrageenophyte *Sarcothalia crispata* (Rhodophyta, Gigartinales) on suspended culture in central Chile**

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Abstract

Laboratory and field studies on growth of *Sarcothalia crispata*, which is one of the most important carrageenophytes of Chile, were made in to assess its viability and growth in a system of suspended culture. *In vitro* experiments on the survival of the germlings and on the effect of temperature and irradiance on its growth allowed to determine the best temperature and irradiance for seeding polypropylene culture ropes in indoors conditions. The growth of plants developed on ropes at three depths in Coliumo Bay (central Chile) supports the idea that it is feasible biologically to manage the resource via aquaculture. Fouling and epiphytes were the main problem at the experimental site. The results suggest that maintaining the cultures in deeper water during summer could minimize this problem.

Introduction

Since about 1960, *Sarcothalia crispata* (Bory) Leister (ex *Iridaea ciliata* Kütz.) or '*luga-luga*' has been traditionally harvested in central Chile to be exported as raw material for carrageenan manufacture in European and American factories. Only during the last ten years has this species, together with *Gigartina skotssbergii* Setchell and Gardner and secondarily *Chondracanthus chamissoii* (C. Agardh) Kütz. and probably *Mazzaella laminarioides* (Bory) Fredericq, been used by the Chilean carrageenan factories. The rapid development of the Chilean carrageenan industry (from 26 t in 1989 to 1834 t in 1998, Servicio Nacional de Pesca, 1998), and the increasing world carrageenan production by the Philippines, North American, French and Danish industries have exerted a heavy harvest pressure on *Sarcothalia* and on the other mentioned carrageenophytes. Bixler (1996) estimated that current the annual world demand of 20,000 t of carrageenan requires about 80,000 t of dried red seaweed annually. About 15,000 t of carrageenophytes currently come from Chile, 40,000 t come from Philippines, 15,000 t from Indonesia and about 10,000 t come from other areas.

Unlike in the Philippines, the main supplier of carrageenophytes, which sustains its production on the mass culture of *Kappaphycus* and *Eucheuma*, the Chilean production of raw materials is entirely based on the productivity of wild populations and no legal harvest regulations exist.

Sarcothalia crispata is a typical subtidal endemic species from cold temperate to cold waters of the Pacific coast of South America. Its latitudinal distribution is from Valparaíso (33° S) to the Magellan Strait and Tierra del Fuego (54° S: Ramírez & Santelices, 1991; Avila et al., 1996), but its area of exploitation

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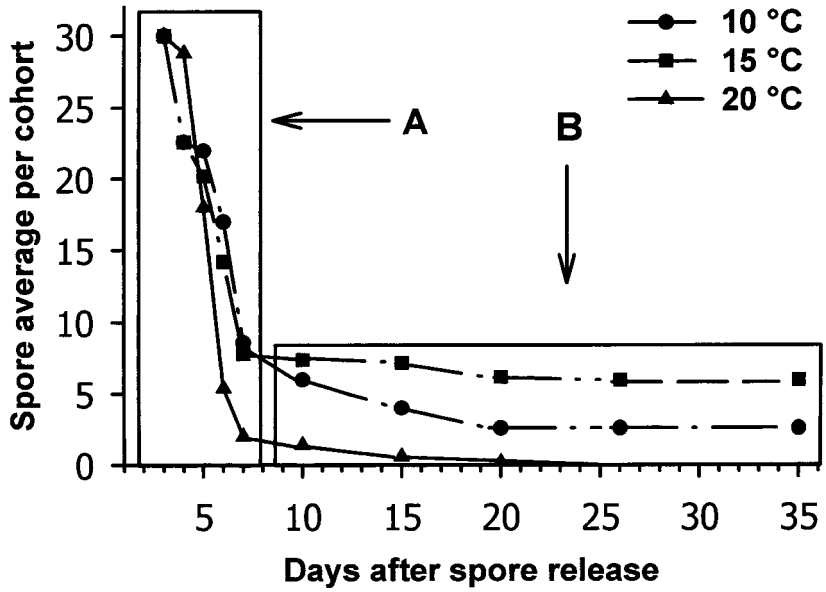

Figure 1. Survivorship curve for 3 sets of 5 cohorts for each temperature during the early development of *Sarcothalia* germlings. Initial number of attached spores per cohort = 30.

is centered from Bay of Concepcion (37° S) to the Archipelago of Chiloe (44° S).

The purpose of this study is to understand the seeding and growth performance of this species in suspended systems of culture in the northern area of its distribution, with the aim to contribute to its domestication and to achieve a more sustainable management of this resource.

Materials and methods

Survival of spores and germling

Survival measurements in relation to sporeling incubation, at different temperatures (10 °, 15 ° and 20 °C), were carried out over 36 days following the procedures of Romo and Alveal (1995). Carefully cleaned 1–2 cm^2 pieces of mature and partially dehydrated tetrasporangial thalli were allowed to release the spores, during 3 h, in a beaker containing 200 mL of 0.45 μm filtered and sterile seawater. The beaker was gently shaken during spore release in order to prevent the premature attachment of the spores. Once a pale rose colored spore suspension developed, 5 mL aliquots containing spores were disposed in 15 petri dishes containing 20 mL sterile seawater and germanium dioxide to suppress diatom development (McLachlan, 1975). Five replicates for each temperature were left undisturbed during 48 h to permit the settlement of spores. Uniform conditions of 60 μmol photon $m^{-2}s^{-1}$ irradiance and 12 h light: 12 h dark photoperiod were maintained. Once the attachment of spores occurred, a cohort of 30 spores per dish was defined by isolating 30 attached spores in an area of about 4–6 mm^2 on the bottom of the dishes and detaching the surplus ones with a sterile needle so each cohort could be easily identified. Five replicates for each temperature were incubated during 36 days in full strength Provasoli enriched seawater (McLachlan, 1975). In all cases the medium was changed weekly. During the first week, the number of survivors was daily counted under microscope and followed by intervals of 5–10 days during the rest of the experiment.

In vitro *effect of temperature on germling growth*

A new culture of tetraspores was initiated by seeding 10 petri dishes. Five dishes were incubated at 10 °C and other five at 15 °C for 15 days until their survival was stabilized. Irradiance was maintained at 60 μmol photon $m^{-2}s^{-1}$ and all other environmental conditions were maintained as in the survivorship experiment. After 15 days of development, the initial diameters of the discoid crust germlings were measured under microscope. Thirty-five days after spore

release, growth was assessed by measuring the final diameter of 40 randomly sampled germlings for each temperature condition (8 germling measurements per dish).

In vitro *effect of irradiance on germling growth*

Eight petri dishes were seeded and used in the irradiance experiment. The irradiance was adjusted at 5, 24, 60 and 120 μmol photon $m^{-2}s^{-1}$ provided by 40 W, cool white, fluorescent lamps. The temperature was 15 °C and the remaining environmental conditions were as explained above. The initial incubation lasted 20 days until survival stabilization was observed. For each irradiance level, the incubation was done in duplicate (two dishes for each irradiance condition). Initial germling diameter was recorded 20 days after spore release. The experiment lasted the subsequent 20 days when the final 40 diameter measurements for each irradiance condition were done as explained above.

Field growth measurements

Tetraspores from cleaned mature fronds were seeded on 50 m of 3 mm (diameter) polypropylene ropes placed on PVC frames following the procedure described for *Gracilaria* by Alveal et al. (1994) and Alveal et al. (1997). The spores attached to the rope were incubated in a walk-in culture room inside a 25-L glass aquarium at 15 °C and under 60 μmol photon $m^{-2}s^{-1}$ (the best temperature and irradiation conditions found in the previous experiments) and aerated with a laboratory compressor. Other experimental conditions were maintained as described for the exploratory experiments. After 30 days of incubation, the ropes were cut in 1 m length pieces and disposed hanging from the main line of a floating system used for mussel culture in the licensed marine area of the University of Concepcion. The cultivation line was similar to the 'single line floating system' described by Tseng (1986) and used for commercial cultivation of *Laminaria* in China. In April 1996, three sets of 12 seeded ropes were located at 0.5 m 2.5 m and 3.5 m depth (4.5 m is the maximal depth in the site during low tide). In September, after 5 months of growth, six ropes from each depth were randomly sampled and the plants were harvested to measure the wet weight. Finally, in January 1997 the remaining 18 ropes were removed from the floating system and harvested to measure the wet weight of *Sarcothalia* as well as epiphytes and fouling. The standing crop of *Sarcothalia* and fouling were expressed as g per meter of rope.

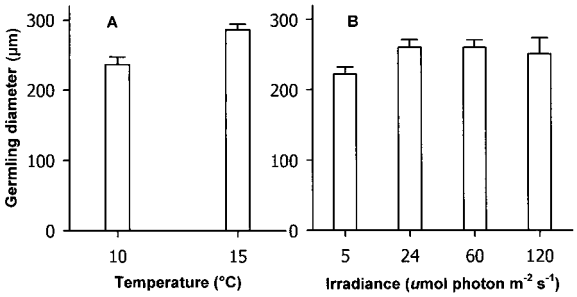

Figure 2. Growth of *Sarcothalia* germlings after incubation for 20 days: A) Effect of temperature; B) Effect of photon flux density. (mean ± SD, n = 40).

Analysis of variance was applied to the *in vitro* growth versus irradiation data and a Student t test to the *in vitro* growth versus temperature data. Field growth data were analyzed by a Kruskal Wallis test and *in vitro* survivorship by means of Mann-Whitney test (Zar, 1984).

Results

Effect of the temperature on germling survival

There was heavy mortality of spores and sporelings during the first week at all three tested temperatures. The sporelings at 20 °C showed about 93% mortality and those at 15 °C and 10 °C showed 73% at the end of the week (Figure 1A). During the following 25 days of incubation, survival stabilized at levels of about 6 spores for the cohorts incubated at 15 °C (20%), 2.5 spores for the cohorts maintained at 10°C (8%) while all those growing at 20 °C died before 20 days of incubation (Figure 1B). After 35 days of incubation the survival at 15 °C and at 10 °C was significantly different (p <0.05; Mann-Withney test).

In vitro *growth measurements: Effect of temperature*

The best temperature conditions in the *in vitro* short term experiment was 15 °C (Figure 2A) which approximately corresponds to the mean summer temperature in shallow waters of Coliumo Bay (15.9 °C) registered by routine measurements done in the Marine Station. The average diameter attained by the crusts was 285.9 μm, which was significantly different to the average of 236.2 μm measured for the germlings grown at 10 °C ($p \ll 0.001$, Student t test). Despite their

smaller size, the germlings maintained at 10 °C appeared in good condition, being normal in color and morphology.

In vitro *growth measurements: Effect of irradiance*

Suitable condition for growth of germlings occurred in a wide range of irradiance from 24 to 120 μmol photon $m^{-2}s^{-1}$ (Figure 2B). The average diameter ranged from 250.8 to 260.2 μm which was significantly different from the final average diameter of 222.1 μm attained by germlings growing at 5 μmol photon m^{-2} s^{-1}($p \ll 0.05$; ANOVA). However, at 120 μmol photon $m^{-2}s^{-1}$ a large proportion of the discoid germlings showed a certain colorless aspect in its marginal cells. As indicated by the previous experiments the best conditions for a fast development of tetraspores were approximately 15 °C and 60 μmol photon $m^{-2}s^{-1}$.

Growth of Sarcothalia *in field culture*

After 5 months of growth in the field (April to September 1996, which correspond to autumn and winter) some of the largest fronds reached a length of 7 to 15 cm, looked healthy and had minimal fouling (excepting juvenile *Polysiphonia* sp. and ulvoids). After 5 months, the standing crop did not show significant differences among plants at the three depths (Figure 3; $p \gg 0.05$, Kruskal Wallis test). In January 1997, after 9 months of growth in the field, the plants at 3.5 m depth showed significantly higher standing crop than plants grown at 0.5 m and 2.5 m depth (Figure 3; $p < 0.05$, Kruskal Wallis test). The largest fronds at the three depths reached a length of 35 to 40 cm, but the oldest ones from the 0.5 and 2.5 m depth became clearly senescent, red-greenish and were severely attacked by epiphytes such as *Ceramium,* benthic diatoms and hydrozoa. On the other hand, plants grown at 3.5 m were healthier in appearance. Significant differences were found in the standing crop at the three depths, due to remarkably less fouling and epiphyte invasion in deeper waters (Figure 4: $p \ll 0.05$, Kruskal Wallis test). The most important fouling organisms on the ropes which could become aggressive competitors for *Sarcothalia* were *Polysiphonia absscissa* Hooker *f.* and Harvey, *Ceramium* sp. *Ulva nematoidea* Bory, *Ectocarpus siliculosus* (Dillwyn) Lyngbye, several species of benthic-colonial diatoms, hydrozoa and juvenile mussels.

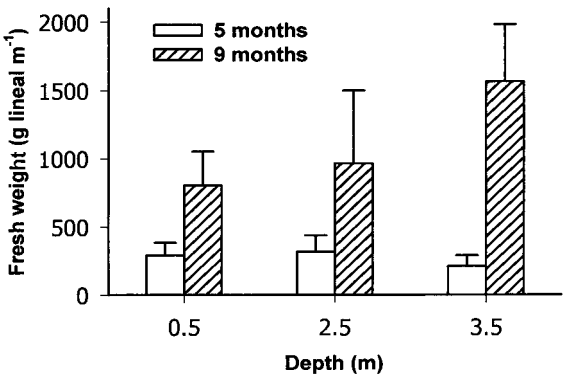

Figure 3. Effect of depth on growth of adult plants of *Sarcothalia* on ropes after 5 and 9 months in the field. (mean ± SD, n = 12).

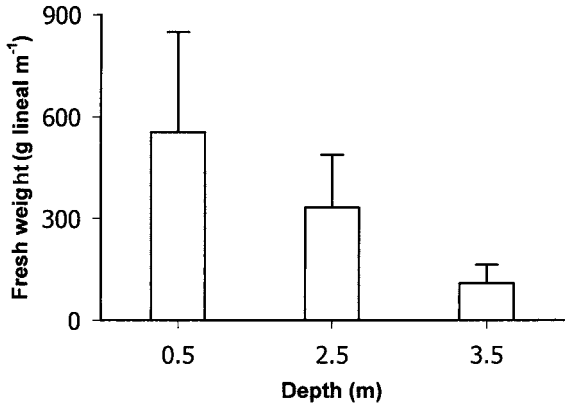

Figure 4. Fouling and epiphyte development on *Sarcothalia* maintained at three depths during 9 months. (mean ± SD, n = 12).

Discussion

The use of floating techniques to culture carrageenophytes appears technically feasible and one of the more promising candidates for commercial cultivation could be *Sarcothalia* based on its good growth performance exhibited during the study period.

In the laboratory experiments, mass mortality at the three temperatures during the initial five days suggests immature spores and not temperature effects. During the subsequent days it is clear that the condition of 20 °C was deleterious for *Sarcothalia* germlings. However, the significant differences on survival found for 10 and 15 °C during the late phase of the experiment can not be completely explained, especially if one takes into account that 10 °C is a common low temperature in the cold waters of the southern area of distribution of the species. The minimum winter temperature of the water recorded at the Marine Station pier in July 1996 was 10.5 °C. A pos-

sible explanation is that this behavior results from the expression of an adaptive physiological response for living in warmer waters than the rest of the southern populations.

From the results of growth versus temperature and irradiance it was determined that conditions of about 15 °C, 24–120 μmol photon $m^{-2}s^{-1}$, full strength Provasoli enriched sea water, and 12 h light: 12 h dark photoperiod are suitable conditions for the early development of the germlings before outplanting to the sea.

The field growth shown by *Sarcothalia* in this study certainly agrees with the fluctuations of the wild population showed by Avila et al. (1996) for commercial beds of the same species in Chiloé Island, about 650 km south from Coliumo Bay. In Chiloé, *Sarcothalia* populations reach maximal growth from January to March and showed a biomass of ca. 1300 g m^2 (wet weight), similarly to the responses of the wild population at Concepción bay (Poblete et al., 1985). The available information permits to conclude that the biomass of about 2000 g per meter of vertical line, recorded in this study, can be considered a good yield, specially when three-fold yields could be obtained be increasing the length of seeded ropes suspended from the floating systems. These results are more promising than the yield of 400 g of *Gracilaria* per meter of horizontal rope obtained after 9.5 months reported as the highest yield by Doty and Fisher (1987: p. 35, Figure 5) in a pilot experiment done in Malaysia. On the other hand, our results are comparable with the 2.5 kg per meter of horizontal line shown by *Gracilaria chilensis* after 9 months of growth in the estuary of the Tubul River in central Chile (Alveal et al., 1997). The fast growth shown by the plants on the ropes allowed them to reach a harvest size of 34–40 cm length in about nine months, which is a reasonable term to be considered for a productive seaweed farm.

The red alga *Iridaea cordata* (Turner) Bory from the northern hemisphere shares several morphological as well as ecological and biological characteristics with *Sarcothalia*. The results of the present study agree in general with the experiments done by Mumford (1977) with *Iridaea* growing on small nets placed near the bottom at various locations in Puget Sound and in San Juan de Fuca Islands in the northwest coast of North America. In a more comparable experiment done by Waaland (1973), the maximal growth rate of *Iridaea* growing on vertical lines occurred, like *Sarcothalia*, at approximately 3–4 m depth.

The optimal season to start the cultures in the field appears to be early autumn, due to the minimal incidence of fouling during this season. In addition, efforts to develop programs on epiphyte control to apply during late spring and summer should be done. Maintaining culture ropes deeper during the warm seasons would probably decrease the fouling and epiphyte attack, as suggested by the results of this study. Other advantages of this type of culture is the possibility of using carpospores or tetraspores for seeding ropes, when a farmer needs to program the production for manufacturing λ or κ carrageenan. Presently, the raw material for the carrageenan market is dominated by Philippines and Indonesian species of *Kappaphycus* and *Eucheuma*, and by the Chilean *Gigartina skottsbergii* (Bixler, 1996), so the commercial application of these techniques for *Sarcothalia* production should be only considered when the economic balance between profit and investment is convenient.

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