

Regenerative capacity of *Gracilaria* fragments: effects of size, reproductive state and position along the axis*

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Abstract

Field farming of *Gracilaria chilensis* in Chile is mainly based on the regenerative capacity of thallus fragments. This study evaluates experimentally the effects on growth of the few organismic determinants that the farmer is capable of effectively handling in the field. No differences were found in the length increments of ramets with and without apices, a surprising result in view of the generally accepted idea that the most important growth meristem in this group of algae is located in the apex of the thallus. Total elongation and increase in weight of fragments with an initial length of up to 20 cm are a function of the initial length of the ramet, suggesting that intercalary growth makes a significant contribution to growth. Vegetative female axes grow faster, (in terms of length and weight) and produce more laterals than fertile female axes, and these faster than fertile tetrasporic branches. No differences in the growth of ramets originating from proximal or distal parts of axes were found.

Introduction

Field farming of *Gracilaria chilensis* is mainly based on the regenerative capacity of thallus fragments (ramets). The literature contains information on the growth effects of key environmental factors on *Gracilaria* (see Hoyle, 1975; McLachlan & Bird, 1986; Hanisak, 1987; Santelices & Doty, 1989 for reviews) and more recent studies have evaluated the effects of planting and harvesting techniques on the regeneration and production capacity of *G. chilensis* (Westermeyer *et al.*, 1991; Pizarro & Santelices, 1993; Buschmann *et al.*, 1995). In contrast to this, seemingly no studies have addressed the problem of how the organismic components, other than genetic, could affect the capacity for growth and regeneration. The initial fragment size, the reproductive state of the fragment and the original position of a ramet along an axis are among the few organismic determinants that the farmer can effectively handle in the field, improving production if dealt with properly. The aim of the present study was to evaluate the effects

of these determinants on the regrowth of fragments of *Gracilaria chilensis*.

Materials and methods

General experimental conditions

Specimens used in all the experiments were collected at Maullín, near Puerto Montt, southern Chile (41°36'S; 73°36'W). Prior to each experiment, the plants were washed with micro-filtered seawater (0.45 µm) and acclimated for 30 days by keeping them under controlled conditions of temperature (14–16 °C), photoperiod (12:12 h L:D) and photon flux density (40–90 µmol m⁻² s⁻¹). The culture medium used was SWM-3 (McLachlan, 1973), with a salinity of 32–34‰ which was changed every 6 days.

During the experiments, the base of individual ramets were clamped between microscope slides in order to keep them at the bottom of 1000 ml beakers filled with 900 ml culture medium. Air bubbling was permanent in all cultures. Length, blotted weight and num-

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ber of branchlets were recorded at the beginning and at the end of each experiment. Specific (daily) growth rates were calculated as described by Brinkhuis (1985). All experiments lasted 30 days. Statistical analyses of results included t-test, ANOVA and Spearman correlation coefficient (Sokal & Rohlf, 1969).

Specific experimental conditions

Effect of initial length

Three experiments were done to evaluate the effects of initial length of ramets on their elongation and growth increments. In the first experiment, two morphotypes collected at Maullín were used. One was thin and profusely branched while the other was thick and very sparsely branched. Eight plants (genets) from each morphotype were used. From each plant a 9-cm long axis was randomly selected. The apical 1 cm was cut in 4 of the axes, while the remaining 4 were cut at the bottom and used as controls (with apical tips). All thalli were incubated under the above mentioned controlled conditions and the length increments measured after 30 days.

In the second experiment, five, 60 cm-long axes, each from 5 genets from the thick, sparsely-branched morpho from Maullín were used. From each axis, 36 ramets were selected and separated into 6 groups of 6 ramets. The ramets in each group were cut to 3, 4, 5, 6, 7 and 8 cm (total length). All ramets were incubated under the same culture conditions previously described. The length and wet weight of each ramet was measured at the beginning and at end of the experiment.

The third experiment of this series was similar to the second experiment, but the initial lengths of the experimental ramets were 8, 10, 12, 14, 16 and 18 cm and the container used were 2000-ml beakers filled with 1800-ml of culture medium.

Effect of the presence of reproductive structures

Four fertile cystocarpic and four fertile tetrasporic genets from Maullín were used. Ten ramets with and 10 without cystocarps were selected from each fertile cystocarpic genet. Vegetative ramets of tetrasporic genets became fertile under the incubation conditions used, therefore fertile ramets was the only kind of tetrasporangial thallus used. At the beginning of the experiment, all ramets were cut to 5 cm (total length). The number of cystocarps, the length, wet weight and number of secondary branches were registered at the

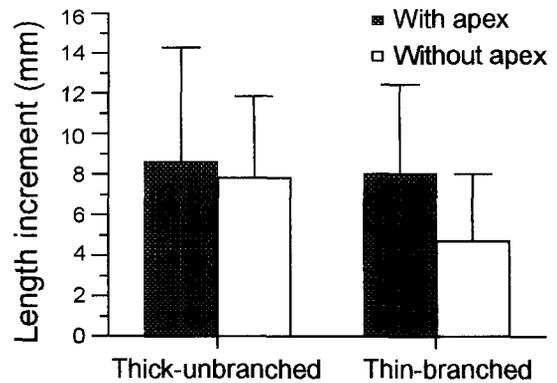


Fig. 1. Length increments of ramets with and without apical tips in two morphotypes of *Gracilaria chilensis* from Maullín.

beginning and at the end of the experiment. All ramets were incubated for 30 days under the above controlled conditions.

Effect of position of the ramet

Five, long (1.3 m) genets from Maullín were used in this experiment. Six ramets from the most basal portion and another six from the most apical portion of each of these genets were removed. At the beginning of the experiment, the length of both types of ramets (basal and apical) was adjusted to 8 cm by cutting the bottom of the ramet. The initial and final wet weight and length were used to evaluate position-related differences.

Results

Effects of initial length

No differences were found in the length increments of ramets with and without apices using both of the morphotypes of *G. chilensis* which were studied from Maullín (Fig. 1). The corresponding t test values are = 0.747; $p = 0.462$ for the thick-unbranched form and 1.861 and $p = 0.059$ for the thin-branched form.

Results of the second (Fig. 2) experiment indicate that specific growth rate, total weight and total length increments all are a function of the initial length of ramets. The specific growth rate decreased as initial length increased while the opposite occurred with the total length and total weight increments. The significant linear relation found between length increments and initial length ($y = 0.38 + 2.25x$; $r^2 = 0.61$; $p = 0.0001$) suggests that intercalary growth occurs in the first 8 cm. Weight also increases as a function of

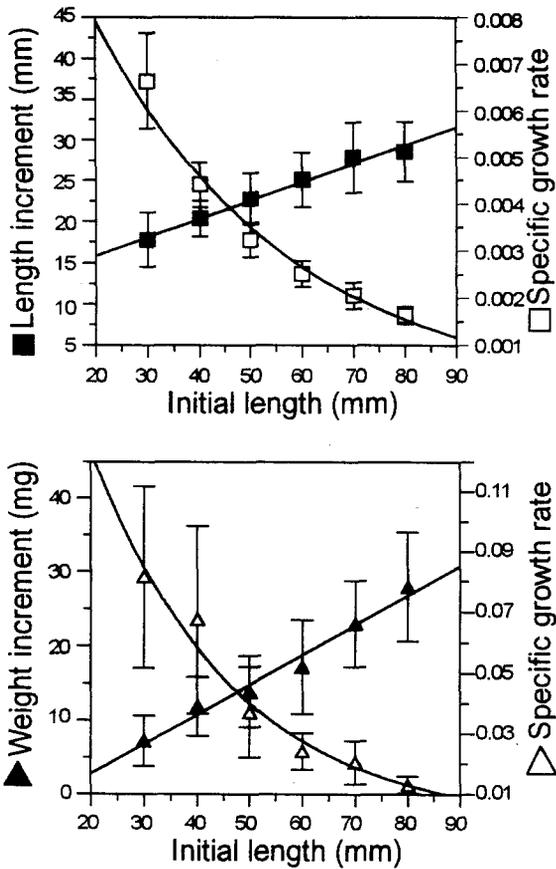


Fig. 2. Specific growth rates, total weight and total length increments as a function of the initial length in thallus fragments to 8 cm long.

initial length ($y = -5.21 + 4.02x$; $r^2 = 0.66$; $p = 0.0001$) due to increased elongation and increased production of lateral branches. Specific growth rates based on length ($y = 0.01.10^{(-0.12x)}$; $r^2 = 0.93$; $p = 0.0001$) and on weight increments ($y = 0.26.10^{(-0.16x)}$; $r^2 = 0.79$; $p = 0.0001$) maintain negative relations with initial length, indicating that the contribution made by intercalary growth decreases with increasing distance from the apex.

Examination of the data gathered for each individual ramet (Fig. 3) highlights inter- as well as intra-genet variation. Genet 3 generally grew slightly more and genet 1 slightly less than the other genets. On the other hand, ramets of the same genet (e.g., genet 1) show a range of inter-ramet variation that is about twice that shown by other genets (e.g., genet 5). Although the data in Fig. 3 are based on weight increments, the pattern emerging from length increments is essentially the same.

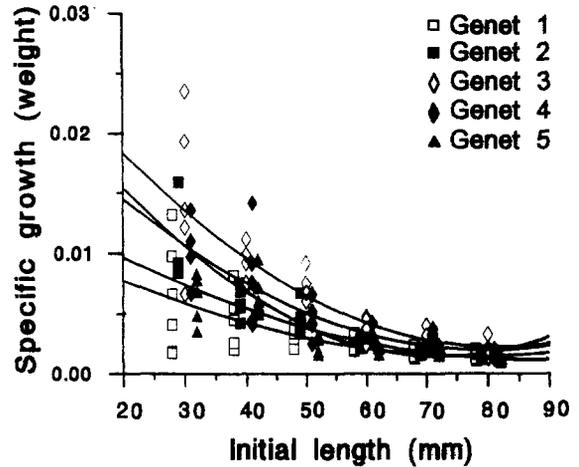


Fig. 3. Inter and intraclonal variation in specific growth rates. Lines are the exponential fit for each genet ($p < 0.05$).

The pattern of growth as a function of initial length described for tips which were initially 3 to 8 cm-long persists in longer ramets (Fig. 4). Specific growth rate decreases significantly with initial length ($y = 0.009.10^{(0.005x)}$; $r^2 = 0.37$; $p = < 0.0001$ for length increments; $y = 0.06.10^{(-0.01x)}$; $r^2 = 0.73$; $p = < 0.001$ for weight increments), while total length and weight increments are a positive function of initial length ($y = 9.41 + 0.53x$; $r^2 = 0.84$; $p = 0.024$ for length and $y = -55 + 1.11x$; $r^2 = 0.95$; $p < 0.01$ for weight values). In comparison to shorter tips (Fig. 2), the total elongation rates of these longer tips are higher and support the interpretation of significant ramet elongation by intercalary growth. On the other hand, the actual weight increments per unit length of larger ramets are much greater (Fig. 4) than in shorter (Fig. 2) ramets, probably due to the activity of increasingly abundant branchlets in the experimental ramets.

Effect of reproductive state

Weight and length increments shown by the vegetative ramets of the Maullín strain were significantly greater (Fisher PLSD test; $F = 10.91$; $p < 0.05$ for length and 41.805 ; $p < 0.05$ for weight increments) than the values shown by fertile cystocarpic branches (Fig. 5). In turn, this last type of branchlet grew significantly more ($p < 0.05$ for both characters, Fisher PLSD test) than the fertile tetrasporangial thalli.

The negative correlation between reproductive state and growth exhibited by the fertile cystocarpic branchlets of *Gracilaria chilensis* is also found when

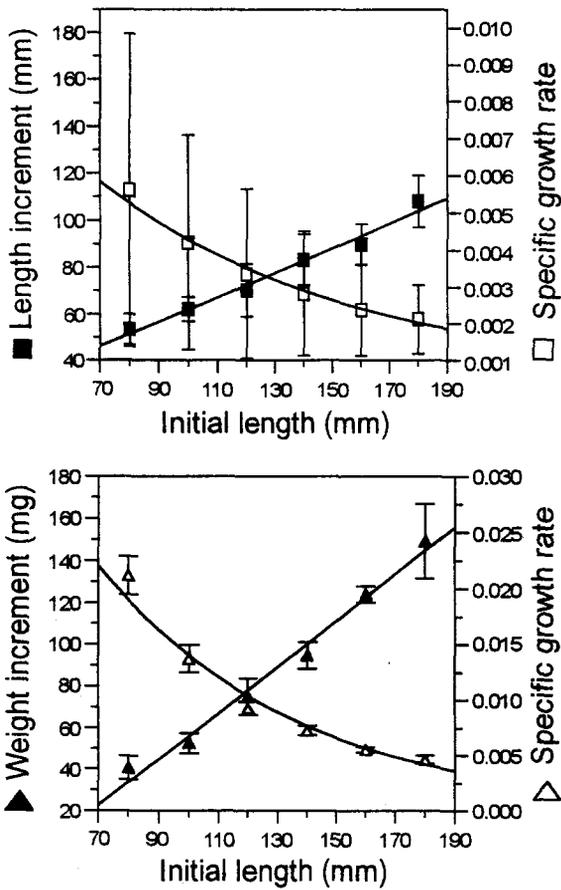


Fig. 4. Specific growth rates, total weight and total length increments as a function of the initial length in thallus fragments up to 18 cm long.

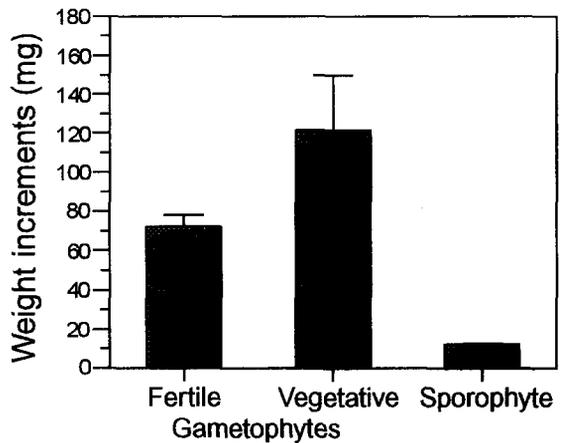
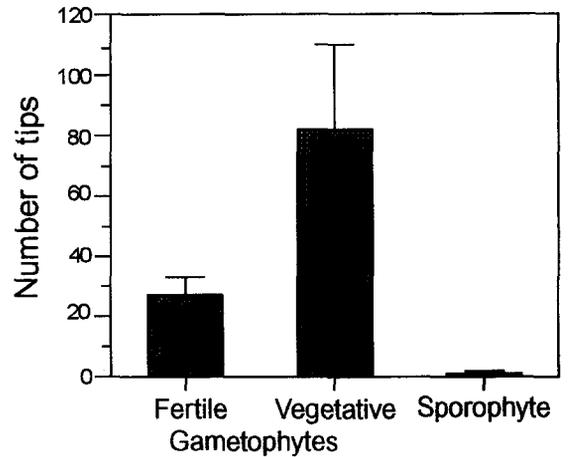
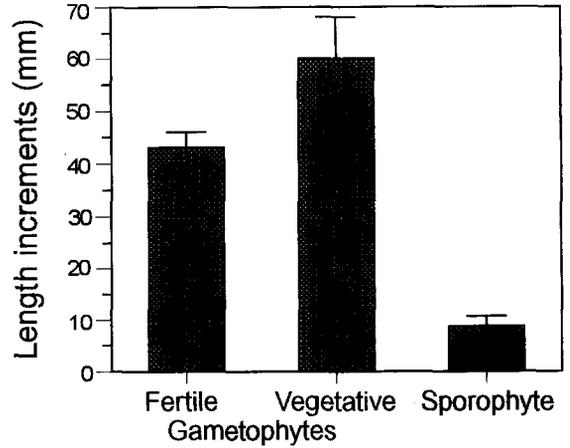


Fig. 5. Length, branch number and weight increments in fertile cystocarpic, vegetative cystocarpic and fertile tetrasporic thalli of *Gracilaria chilensis*.

the number of cystocarps is considered (Fig. 6). There was a significant negative correlation between both the increment in length and number of branchlets produced and the number of cystocarps present on a ramet ($y = 49.341 - 1.0797x$; $r^2 = 0.28$; $p = 0.005$ for length increments and $y = 46.11 - 2.8461x$; $r^2 = 0.35$; $p = 0.0001$ for increment in number of branchlets). However, a positive but non-significant correlation was found between weight increments and number of cystocarps ($y = 68.484 + 2.0192x$; $r^2 = 0.041$; $p = 0.21$).

Effects of position

No significant growth differences were found in terms of weight ($F = 2.926$; $p = 0.1012$) or length increments ($F = 0.21$; $p = 0.651$) between the basal and apical ramets of the experimental genets (Fig. 7).

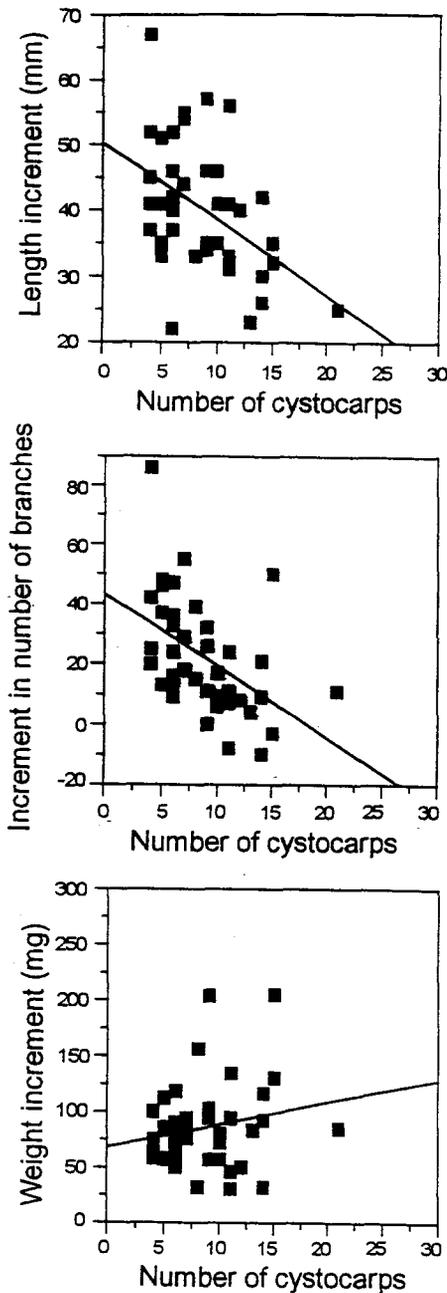


Fig. 6. Length, weight and branch number increments of fertile cystocarpic branchlets, as a function of number of cystocarps.

Discussion

Results gathered in this study indicate that the field manipulation of two organismic determinants, initial size and reproductive state of the *G. chilensis* fragments to be regenerated, could improve production.

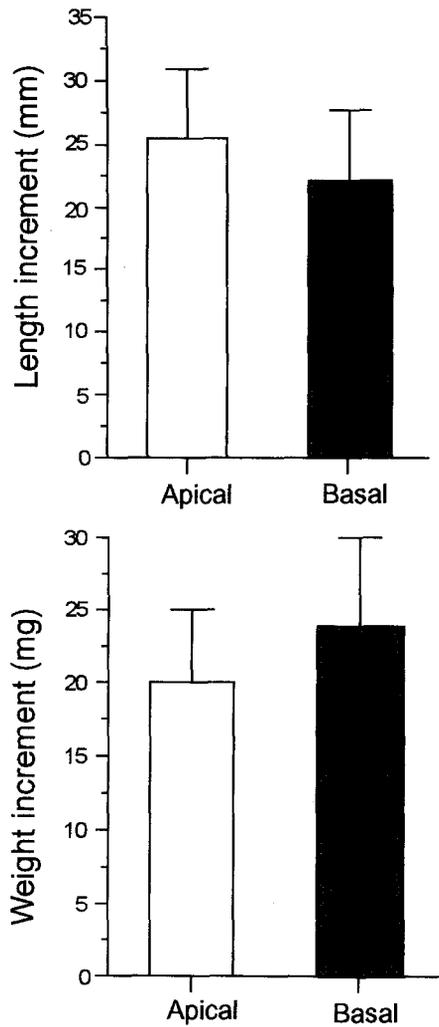


Fig. 7. Comparison of length and weight increments between apical and basal ramets.

The lack of elongation differences between ramets with and without apical tips is surprising in view of the generally accepted idea that the most important growth meristem in this group of algae is located in the apex of the thallus (e.g. Hoyle, 1975; McLachlan & Bird, 1986). Although some growth activity is occurring in that part of the axis, our results indicate that intercalary activity is more important for increasing length of the thallus in this species. The significant correlation between length increments and initial size is found in both short and long (up to 18 cm long) ramets.

Initial length also determines weight increments. In the shorter ramets this is probably mainly due to intercalary growth. In longer ramets, the relative contribution of lateral branchlets to growth increases with increasing length, reinforcing the dependence of weight increments on initial length.

Extrapolation of these results to field farming activities requires a few empirical determinations. All cultivation sites have a carrying capacity determined by the prevailing fertility conditions (e.g., nutrients conditions, light and temperature regimes, water movement, grazers abundance) present there. On the other hand, progressively longer and bushier thalli of *Gracilaria chilensis* become increasingly more prone to being dislodged by water movement (Santelices & Doty, 1989). Therefore, the optimum fragment length to be used for field cultivation would be the longest possible one that the fertility of the farming site could support and that the average water movement intensity would allow prior to the time of harvest.

Our results indicate that the presence of reproductive structures also affects growth. These results are consistent with resource allocation theory which predicts that reproduction imposes a cost on an organism (for reviews see DeWreede & Klinger, 1988; Santelices, 1990; Mathieson & Guo, 1992). Therefore, it is expected that fertile cystocarpic branchlets would grow less than vegetative branchlets. However, our results indicate that the growth suppression exerted by the growing cystocarp is highly localized, as the vegetative and cystocarpic ramet of the same cystocarpic genet grow at significantly different rates.

Since the tetraspores are microscopic, borne superficially and dispersed over the entire thallus surface, it is very difficult to measure, in terms of biomass or energy, the cost imposed by reproduction. However, the magnitude of growth differences between fertile cystocarpic and fertile tetrasporic branchlets is extremely large. It should be noted that differences in productivity have been reported from karyologically different phases of some species of *Gracilaria* (Edelstein, 1977; Hoyle, 1978; Kim & Henriquez, 1979; Ren & Chen, 1986). McLachlan and Bird (1986) have commented that these differences remain unclear and are assumed to be insignificant. However, Santelices and Doty (1989) have argued that since the two phases may have different modes of living, it would not come as a surprise that such differences in productivity may exist. The data gathered in this study support the prediction of such differences among fertile thalli. We are as yet unaware if vegetative thalli also exhibit such a difference in production.

The overall conclusion emerging from the growth comparison among karyologically different phases is that farmers should prefer cystocarpic over tetrasporic thalli. The growth rate of the former, even when fertile, is greater. The presence of cystocarps on these

thalli would allow the farmer to identify and remove the branchlets which are fertile.

Acknowledgments

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