



Organismic determinants and their effect on growth and regeneration in *Gracilaria gracilis*

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Abstract

The growth of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham was examined by studying the effect of organismic determinants such as thallus length, position along the thallus and branching. Knowledge of these factors is essential in order to increase production from suspended seaweed rafts seeded with vegetative *G. gracilis* fragments. Seeding netlons with seaweed material freshly collected from subtidal populations provided up to 30% higher relative growth rates than seaweed maintained on the netlons for successive months. Initial seedstock length greatly affected growth rates and yields such that 30-cm thalli fragments resulted in growth rates 14% higher than for 10-cm fragments. This difference is suggested to be due to the higher contribution to overall biomass by growth of lateral branches. Comparisons of the growth of apical and basal fragments suggest that growth takes place over the entire length of the thallus, but that the apex contributes more to overall elongation than does the proximal part. The removal of apical meristems resulted in an enhanced branching frequency with production of four times as many branches as intact fragments. Evidence is also provided for extensive morphological differentiation following long periods of rapid growth. These thalli have very high frequency of branching, are hollow due to the disintegration of medullary cells and are considered to be completely senescent. These factors have implications for the successful cultivation of *G. gracilis* on commercial mariculture systems.

Introduction

Gracilarioid seaweeds contribute to more than half the world's agarophyte production (Fletcher, 1995). They are cultivated in countries such as Chile, China, Taiwan, Namibia and South Africa (Dawes, 1995; Friedlander & Levy, 1995; Smit et al., 1997). Characteristics of *Gracilaria* spp. that make them desirable for cultivation are fast growth rate, good agar yield and quality and relative ease of growth (Buschmann et al., 1995). The most important attribute is that almost all cultivated species reproduce solely through fragmentation, leading to a high regenerative capacity (Hurtado-Ponce, 1990; Santelices & Varela, 1995). This, along with the fact that *Gracilaria* species are morphologically plastic (Dawes, 1993; Meneses, 1996), has initiated many studies focusing on selection

of strains with good gel characteristics and fast growth rates (Levy & Friedlander, 1990).

Several studies on *Gracilaria* have been done in southern Africa since World War II. Most of these have dealt with the ecology of *Gracilaria* (Isaac, 1956; Simons, 1977; Anderson et al., 1993; Molloy & Bolton, 1995; Anderson et al., 1996b). Several other studies have looked at ecophysiological aspects of *Gracilaria* cultivation (Engledow & Bolton, 1992; Anderson et al., 1996a; Rebello et al., 1996; Smit et al., 1997; Anderson et al., 1998; Smit, 1998) or dealt with commercial aspects of its utilisation (Rotmann, 1990). The recent interest in seaweed mariculture in southern Africa however, has necessitated research into factors inherent in *Gracilaria* itself, which affect growth and regrowth performance. Santelices and Varela (1995) termed these factors 'organismic determinants'. Since

productivity of *Gracilaria* is tightly coupled to vegetative regeneration, it is important to understand the processes leading to the development of new tissue and to apply this knowledge to seaweed mariculture operations in order to enhance production. Despite the wealth of literature available on the ecophysiological aspects of seaweed growth, little has been done in order to understand growth in the organism itself (Santelices & Varela, 1995). However, the evaluation of organismic determinants such as reproductive state, size of thallus fragments and the position of fragments along the thallus axis are known to be important in affecting growth (Santelices & Varela, 1995). The aim of this study was therefore to evaluate the effect of such factors on the mode of growth and regrowth in *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham from Saldanha Bay with the view of applying this knowledge to *Gracilaria* mariculture operations in the region.

Materials and methods

In situ determination of effects of seed stock quality on growth

The hypothesis that the quality of seed stock affects growth and production of *Gracilaria gracilis* on a suspended raft was tested by comparing the growth rate of naturally occurring *Gracilaria* from Saldanha Bay with that of seaweed previously grown on a suspended raft. The raft used in these experiments in Saldanha Bay was similar to that described by Dawes (1995) and Anderson et al. (1996a). The experiment was conducted during winter between May and August 1996. Thallus fragments of cultivated seaweed were frequently broken due to harvesting techniques and measured between 15 and 20 cm in length. They were often infested with *Ceramium* sp. when grown on the raft (see Anderson et al., 1998). Before seeding *Gracilaria* onto the lines, most *Ceramium* was manually removed although some remained which could later regenerate. Apart from epiphyte contamination and broken thalli the harvested *Gracilaria* was in good condition. *Gracilaria* collected from the natural populations was very healthy and up to 1.7 m in length and showed very little epiphyte infestation.

Netlons were seeded with cultivated or naturally occurring *Gracilaria* (four replicate lines) to an initial stocking density of 1.1 to 1.2 kg (wet) per 2.5 m. Seaweed tufts used for seeding were approximately 20 cm in length, spaced at 20 cm intervals along

the netlon line. Netlons ('superope') are plastic tubular netting with a mesh size of approximately 20 mm supplied by Van Leer Plastics, Johannesburg, South Africa (Dawes, 1995; Anderson et al., 1996a). Netlons were spaced 0.75 m apart on the raft to avoid abrasion against adjacent lines and to minimise self-shading. They were removed, weighed and restocked once a month (May to July) with harvested and newly collected material from natural populations and relative growth rates (RGR) calculated and expressed as percent wet mass increase day⁻¹. The equation used for the calculation of RGR is

$$RGR = \frac{\ln(W_2/W_1)}{n} \cdot 100$$

where W_2 and W_1 are final and initial mass respectively and n is the number of days. Results were analysed using a two-way ANOVA (Statistica for Windows Release 5.1) where appropriate.

In situ determination of the growth rate of different lengths of thalli

During August 1996 the RGR and yield of *Gracilaria* were compared among three different thallus length classes to test the hypothesis that longer thalli produce more biomass than an equal mass of shorter thalli. Plants were collected from benthic populations and 45 individuals selected and cut to lengths of 10, 20 and 30 cm. Each tuft was weighed to between 15 and 24 g (wet). Individual tufts (15 individual tufts per rope) were numbered and attached at 20 cm intervals with plastic cable ties on each of three 6 mm polypropylene ropes. No attempt was made to randomise thalli of different lengths between ropes and consequently, each rope contained tufts of the same lengths. Previous experiments indicated that there was no significant difference in growth of *G. gracilis* on different netlons when they were placed adjacent to each other (RJ Anderson and AJ Smit, unpublished). Seeded ropes were attached to the raft and growth determined a month later by recording the fresh mass and length of each tuft. Negligible epiphyte material was present eliminating the need for epiphyte correction. Growth was expressed as RGR using calculations for both length and mass data. Results were analysed using an one-way ANOVA and Pearson product-moment correlations (Statistica for Windows Release 5.1).

Standard conditions for laboratory growth experiments

Healthy plants collected from Saldanha Bay were placed in a laboratory holding tank until required, usually within a week of collection. Before each experiment, thallus fragments were rinsed in 0.45 μm filtered seawater, most of the visible epiphytes removed manually and treated with freshwater and povidone-iodine to kill any remaining macroalgal epiphytes and diatoms. The povidone-iodine treatment involved soaking fragments for 1 minute in a solution containing 0.5% (w:v) povidone-iodine in distilled water containing a few drops of wetting agent, followed by a thorough rinse in distilled water. Plants were acclimatised for five days to experimental conditions of 18 °C, a 16 : 8 (light : dark) photoperiod and a light intensity of 80 to 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Osram cool-white fluorescent tubes). One-third strength standard Provasoli's Enriched Seawater (McLachlan, 1973) was used as culture medium in acclimation and experimental phases.

Standard growth conditions involved individual seaweed fragments (five replicates) being placed separately into 100-mL Erlenmeyer flasks and receiving aeration through disposable pipette tips attached to plastic air-tubing. The fresh mass of each fragment was determined every three days while replacing the culture medium. Fragment lengths were measured to the nearest millimetre and total number of lateral branches counted. RGR was calculated from the final slope of the resulting exponential growth curve when cumulative biomass was plotted against time.

Growth comparison between thallus base and tip

To determine the contribution of intercalary growth to overall growth, the change in wet biomass and length of five 10 mm tips cut from lateral branches were compared to that of the same length fragments removed from the base of the branch. All thallus fragments used in the experiment were taken from the same plant.

Effect of removal of apical tip on growth

The growth of five 12 mm *Gracilaria* thallus tips, of which 2 mm of the apex was removed (i.e. starting length of 10 mm), was compared to that of five 10 mm fragments with intact apical meristems.

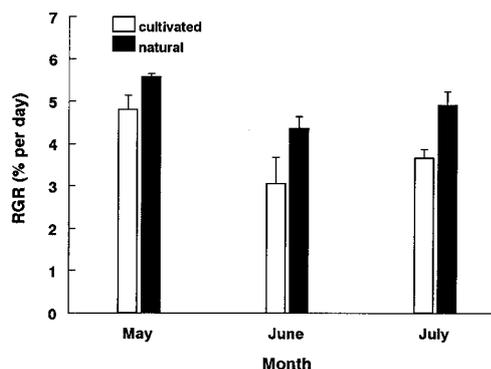


Figure 1. RGRs of freshly collected and reseeded *Gracilaria gracilis* on a suspended seaweed cultivation raft (standard error bars shown, $n = 4$).

Results

The effect of seedstock quality

The RGR of *Gracilaria* collected from natural populations differs markedly from that of cultivated seaweed when grown on the raft (Figure 1). RGRs obtained from netlons seeded with naturally occurring *Gracilaria* were 19.4, 29.8 and 21.5% higher than those of netlons seeded with cultivated seaweed for May, June and July respectively (as determined from the mean monthly RGR). A two-way ANOVA indicated these differences to be significant at $p < 0.05$ between seeding treatments (d.f. = 1, $F = 14.14$) and over the three month experimental treatment (d.f. = 2, $F = 7.88$). A Tukey HSD test showed that the between-month difference in RGR for netlons seeded with cultivated *Gracilaria* was insignificant at $p > 0.05$, while for May, netlons containing naturally occurring *Gracilaria* had a significantly higher RGR compared to the other two months. No significant interaction between seeding treatment and time was found (d.f. = 2, $F = 0.27$). At the end of the experiment the reseeded seaweed that had been maintained on the raft for the full three month period appeared morphologically and anatomically different from *Gracilaria* obtained from natural populations. The main axes of these plants were thick and twisted with a high branching frequency. The morphologically differentiated specimen had 14.9 ± 8.3 (mean \pm SD) branches cm^{-1} with the main axis being 3.2 ± 0.3 mm in diameter. The normal form had 1.0 ± 0.3 branches cm^{-1} and a thickness of 1.7 ± 0.1 . In the differentiated form the medullary cells disintegrated resulting in a hollow interior. In some laboratory culture studies *Gracilaria*

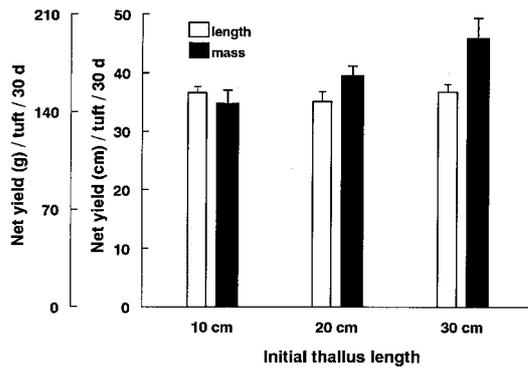


Figure 2. Net yield of *Gracilaria gracilis* initially seeded as 10-, 20- and 30-cm lengths. Yield is calculated from length and mass data and normalised to a month (standard error bars shown, $n = 15$).

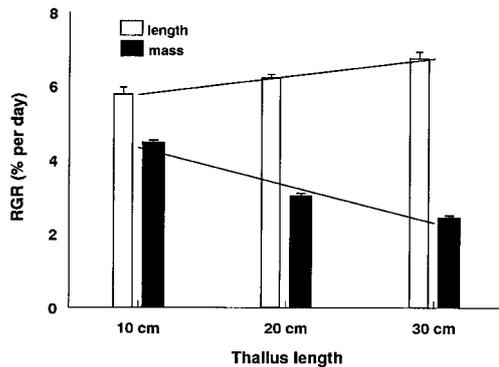


Figure 3. The effect of initial thallus length on RGR of *Gracilaria gracilis* expressed as % length and wet mass day^{-1} . Vertical bars indicate treatment means and whiskers are standard errors ($n = 15$); sloping lines are linear regressions showing growth rate as a function of initial seeding length.

gracilis with such a thick, hollow main axis floated to the surface as the interior filled with gas.

In situ determination of the growth rate of different lengths of thalli

Figure 2 shows the net yield of *Gracilaria* initially seeded as three different thallus lengths as calculated from change in biomass or length over 30 days. Initial thallus length had a significant effect on net yield per tuft expressed in terms of mass (one-way ANOVA; $p < 0.05$, d.f. = 2, $F = 4.66$) so that the 30 cm tufts produced $21.8 \pm 5.1\%$ more biomass than the 10 cm tufts. Difference in yield expressed as length produced over 30 days were not significant between the three treatments (one-way ANOVA; $p > 0.05$, d.f. = 2, $F = 0.44$), so that the same increase in length was produced from 10, 20 and 30 cm thalli segments. RGR was significantly different between the three length

groups for both the mass and length calculations (one-way ANOVA; $p < 0.001$, length: d.f. = 2, $F = 9.97$, mass: d.f. = 2, $F = 236.06$). RGR expressed in terms of length showed a positive dependency ($r = 0.567$ at $p < 0.001$) on initial seeding length so that the RGR for the 30 cm treatment was $14.2 \pm 2.8\%$ higher than for the 10 cm treatment. The slope of the regression line was negative when RGR is expressed in terms of mass ($r = -0.932$; significant at $p < 0.001$; Figure 3).

Effect of removal of the apical meristem on growth

The final mass of the thallus fragments with and without apical meristems were not different at the end of the experimental period of 14 days ($p > 0.05$, Student's *t*-test). There was some evidence of a greater increase in length for the treatment with the intact apical meristem (71.4 ± 3.4 and 62.0 ± 3.7 mm for fragments with and without meristems respectively). Although this difference was not significant ($p > 0.05$) it resulted in a significantly different RGR between the treatments when calculated in terms of change in length day^{-1} (Table 1). In contrast, RGR's expressed in terms of mass were not significantly different. An examination of the branching frequency of the treatments suggests that lateral branch development is under control of the apex so that the growth and development of lateral branches are promoted when the tips are removed. *Gracilaria* fragments with the apical cells removed had 5.9 ± 0.5 lateral branches cm^{-1} of thallus compared to 1.4 ± 0.3 branches cm^{-1} for the intact thallus pieces ($p < 0.001$, Student's *t*-test).

Growth comparison between thallus base and tip

Table 2 gives the RGR's for 10 mm *Gracilaria* thalli cut from either the tip or base of a thallus. RGR of the alga when expressed as percent mass and length day^{-1} was significantly higher for the apical 10 mm of the thallus compared to 10 mm fragments cut from the base of the thallus. The basal areas however, produced 42% more lateral branches per 10 mm than did the apical region and new branch development was evident from the cut surface of proximal end of the basal fragments. Neither basal nor apical fragments had any developed or developing branches at the start of the experiment.

Table 1. RGRs and final lengths and weights of *Gracilaria gracilis* fragments with the apical meristem removed and with meristem intact. Results of t-tests are also shown (mean \pm SD)

	RGR (% length day ⁻¹)	RGR (% mass day ⁻¹)	Final length (mm)	Final mass (g)
with tip	14.3 \pm 0.4	20.7 \pm 0.8	71.4 \pm 3.4	0.038 \pm 0.003
no tip	11.5 \pm 0.5	19.2 \pm 0.7	62.0 \pm 3.7	0.042 \pm 0.003
<i>p</i>	0.001	0.191	0.098	0.356
<i>n</i>	5	5	5	5

Table 2. RGRs and number of lateral branches per 10 mm for tips and bases of *Gracilaria gracilis* thalli. Probability values indicate the significance of the Student's t-test between tips and bases (mean \pm SD)

	RGR (% length day ⁻¹)	RGR (% mass day ⁻¹)	Number of lateral branches cm ⁻¹
Tip	20.4 \pm 1.4	41.8 \pm 2.7	3.8 \pm 0.5
Base	5.6 \pm 1.9	25.0 \pm 6.1	6.6 \pm 0.9
<i>p</i>	0.035	0.000	0.021
<i>n</i>	5	5	5

Discussion

Results indicate that an understanding of the mode of growth in *Gracilaria* can be used to significantly enhance production from suspended rafts. Seeding with newly collected *Gracilaria* results in a higher RGR than seaweed maintained on the netlons for three successive months. Similarly, Buschmann et al. (1995) found the productivity of *Gracilaria* cultivated on the sea-bottom in southern Chile to decrease over a period of two to three years. Two hypotheses were provided to explain this decrease. Firstly, repeated harvesting resulted in the removal of apical meristems leaving older, slower growing tissue behind. Secondly, harvesting resulted in the loss of stocking algae. For the present study, the decrease in productivity can be explained by the difference in quality of seed stock. Although seaweed harvested from rafts and natural benthic populations were both healthy, the harvested material was generally broken due to handling, resulting in shorter fronds. The effect of thallus length on growth is evident such that longer fronds yield more biomass per tuft than shorter thalli of the same mass. Despite a decreased RGR (as length day⁻¹) for shorter thalli, initial thallus length did not affect the net length produced by the seaweed. Thus, for longer fronds to

attain a larger biomass they have to undergo a change in growth habit in order to accommodate the extra biomass (i.e. in longer thalli the contribution of lateral branches to biomass production increased). This change most likely results from the increased number of branches or more rapid growth from existing lateral branches, providing greater potential for biomass production.

Reseeding with harvested material is associated with morphological and anatomical changes, namely increased branching frequency and the development of a thick, twisted main axis with a schizogenic cavity. Meneses (1996) also found natural and artificial populations of *Gracilaria chilensis* Bird, McLachlan et Oliveira to show distinct morphological and anatomical characteristics. Gutierrez and Fernández (1992) found that water motion accounted for differences in thallus morphology observed in *Chondrus crispus* Stackhouse, and suggested polymorphism to be a mechanical adaptation to different hydrodynamic conditions. In the current study, the higher branching frequency is probably the result of cortical damage incurred during seeding (M Steentoft, pers. comm.). Alternatively, an 'apical dominance effect' due to thallus breakage during successive harvests could result in axes without apical meristems (see discussion later)

followed by a proliferation in the number of lateral branches.

It is unclear as to what the ecological significance of the formation of a schizogenic cavity in the main axis may be. Flotation with the aid of gas-filled organs is often found in large brown seaweeds (kelps and fucoids) and has traditionally been associated with an adaptation to living in a low-light environment as it allows the fronds to float to the surface (Russell, 1978). It is unlikely that flotation resulting from trapping oxygen inside the axis is a response to the low light intensities experienced on the raft since this phenomenon does not occur in natural benthic populations. The development of a hollow main axis in *G. gracilis* has also been noted in intensive unialgal laboratory cultures where the optimal growth conditions result in RGR's as high as 48% day⁻¹ (A.J. Smit, unpublished data). According to M. Steentoft (pers. comm.), hollow thalli have only been observed once before and are considered senescent. They are most likely the result of rapid growth never experienced in the wild. Seeding *Gracilaria gracilis* onto netlons results in higher growth rates than are normally found in benthic populations suggesting that morphological differentiation could be the consequence of enhanced growth under artificial conditions.

In their study on *G. chilensis*, Santelices and Varela (1995) suggested that intercalary growth is more important than apical growth in contributing to elongation since thallus length increment was found to be positively related to thallus length up to 20 cm, while RGR was inversely related to length. This is contrary to studies on *Gracilaria confervoides* (L.) Grev. (= *G. gracilis*) and *G. debilis* (Forsskål) Børgesen in which it was suggested that elongation due to growth of the intercalary tissue does not occur (Isaac, 1956; Goldstein, 1973). According to M. Steentoft (pers. comm.), *G. gracilis* growth occurs throughout the thallus and not particularly near the apex. Our data seem to support the observation that growth (as thallus elongation) is significant over the entire thallus, however, it was found that the apex contributes more to overall elongation than does the proximal part of the thallus. During cultivation, additional increase in mass is attained through lateral branch proliferation of the basal parts of the thalli. Although increments in length of fragments with and without apical meristems were not found to be significantly different, such a difference might be obtained with greater replication. This is supported by the greater RGR measured in apical fragments or fragments with an intact apex, compared

to that of basal fragments or fragments with the apex removed. Net length increment is not affected by the initial thallus length suggesting that most of the new length produced originates in the apical regions. In a situation where intercalary growth is more important to overall elongation (e.g. *G. chilensis*) one would expect to find a direct linear correlation between initial thallus length and length increment.

Gracilaria gracilis exhibits an apparent apical dominance effect as there is an increase in the degree of branching in fragments from which the apices have been excised. Apical dominance has been shown to exist in several brown algae with well-developed apical cells or meristems (Moss, 1965, 1970; Chamberlain et al., 1978). Branching also appears to be a function of distance from the tip of the thallus as basal fragments had a higher branching frequency than fragments from the apical region. This may be due to the control of the apex on branch development (M. Steentoft, pers. comm.).

Our study shows thallus length and branching frequency to be important factors affecting the growth of *Gracilaria gracilis*. This may be relevant to *Gracilaria* cultivation since growth and production is linked to morphological differentiation, particularly the high branching frequency of seaweed cultivated for an extended period of time. There is also evidence that the morphological differentiation, in particular thallus thickness, is accompanied by physiological change in *G. gracilis* (Smit, 1998) which could contribute to reduced growth performance. Similar responses have been shown for a variety of other macroalgae (Markager and Sand-Jensen, 1996). *Gracilaria* farming have been shown to be technically feasible in southern Africa (Dawes, 1995, Anderson et al., 1996a; Smit et al., 1997). To enhance production from these systems, an understanding of the organism itself is essential in order for the farmer to make wise decisions regarding the seedstock selection. For example in Namibia seaweed growers now routinely use seaweed from natural populations as seed stock since they experienced reduced production when cultivated material was used (J. Fliedl, pers. comm.). Before the full potential for seaweed mariculture can be realised, continued research into intrinsic growth-determining factors is needed, in addition to 'normal' ecophysiological research.

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References

- Anderson BC, Smit AJ, Bolton JJ (1998) Differential grazing effects by isopods on *Gracilaria gracilis* and epiphytic *Ceramium diaphanum* in suspended raft culture. *Aquaculture* 169: 99–109.
- Anderson RJ, Levitt GJ, Keats DW, Simons RH (1993) The role of herbivores in the collapse of the *Gracilaria* resource at Saldanha Bay, South Africa. *Hydrobiologia* 260/261: 285–290.
- Anderson RJ, Levitt GJ, Share A (1996a) Experimental investigations for the mariculture of *Gracilaria* in Saldanha Bay, South Africa. *J. appl. Phycol.* 8: 421–430.
- Anderson RJ, Monteiro PMS, Levitt GJ (1996b) The effect of localised eutrophication on competition between *Ulva lactuca* (Ulvaaceae, Chlorophyta) and a commercial resource of *Gracilaria verrucosa* (Gracilariaceae, Rhodophyta). *Hydrobiologia* 326/327: 291–296.
- Buschmann AH, Westermeyer R, Retamales C (1995) Cultivation of *Gracilaria* on the sea bottom in southern Chile: A review. *J. appl. Phycol.* 7: 291–301.
- Chamberlain AHL, Gorham J, Kane DF, Lewey SA (1978) Laboratory growth studies on *Sargassum muticum* (Yendo) Fensholt. II. Apical dominance. *Bot. mar.* 22: 11–19.
- Dawes CJ (1993) Phenotypic plasticity in laboratory cultivars and field plants of *Gracilaria tikvahiae* (Gigartinales, Rhodophyta). *Cryptogam. Bot.* 3: 345–352.
- Dawes CP (1995) Suspended cultivation of *Gracilaria* in the sea. *J. appl. Phycol.* 7: 303–313.
- Engledow HR, Bolton JJ (1992) Environmental tolerances in culture and agar content of *Gracilaria verrucosa* (Hudson) Papenfuss (Rhodophyta, Gigartinales) from Saldanha Bay. *S. Afr. J. Bot.* 58: 263–267.
- Fletcher RL (1995) Epiphytism and fouling in *Gracilaria* cultivation: An overview. *J. appl. Phycol.* 7: 325–333.
- Friedlander M, Levy I (1995) Cultivation of *Gracilaria* in outdoor tanks and ponds. *J. appl. Phycol.* 7: 315–324.
- Goldstein ME (1973) Regeneration and vegetative propagation of the agarophyte *Gracilaria debilis* (Forsskål) Børgesen (Rhodophyceae). *Bot. mar.* 16: 226–228.
- Gutierrez LM, Fernández C (1992) Water motion and morphology in *Chondrus crispus* (Rhodophyta). *J. Phycol.* 28: 156–162.
- Hurtado-Ponce AQ (1990) Vertical rope cultivation of *Gracilaria* (Rhodophyta) using vegetative fragments. *Bot. mar.* 33: 477–481.
- Isaac WE (1956) The ecology of *Gracilaria confervoides* (L.) Grev. in South Africa with special reference to its ecology in the Saldanha – Langebaan lagoon. In Braarud T, Sørensen NA (eds), Second International Seaweed Symposium. Pergamon Press, London: 173–185.
- Levy I, Friedlander M (1990) Strain selection in *Gracilaria* spp. I. Growth pigment and carbohydrates characterization of strains of *Gracilaria conferta* and *Gracilaria verrucosa* (Rhodophyta, Gigartinales). *Bot. mar.* 33: 339–345.
- Markager S, Sand-Jensen K (1996) Implication of thallus thickness for growth-irradiance relationships of marine macroalgae. *Eur. J. Phycol.* 31: 79–87.
- McLachlan J (1973) Growth media – marine. In Stein JR (ed.), Handbook of Phycological Methods. Cambridge University Press, London: 25–51.
- Meneses I (1996) Assessment of populations of *Gracilaria chilensis* (Gracilariales, Rhodophyta) utilizing RAPDs. *J. appl. Phycol.* 8: 185–192.
- Molloy FJ, Bolton JJ (1995) Distribution, biomass and production of *Gracilaria* in Lüderitz Bay, Namibia. *J. appl. Phycol.* 7: 381–392.
- Moss B (1965) Apical dominance in *Fucus vesiculosus*. *New Phytol.* 64: 387–392.
- Moss B (1970) Meristems and growth in *Ascophyllum nodosum* (L.) Le Jol. *New Phytol.* 69: 253–260.
- Rebello J, Ohno M, Critchley AT, Sawamura M (1996) Growth rates and agar quality of *Gracilaria gracilis* (Stackhouse) Steentoft from Namibia, Southern Africa. *Bot. mar.* 39: 273–279.
- Rotmann KWG (1990) Saldanha Bay, South Africa: Recovery of *Gracilaria verrucosa* (Gracilariales, Rhodophyta). *Hydrobiologia* 204/205: 325–330.
- Russell G (1978) Environment and form in the discrimination of taxa in brown algae. In Irvine DEG, Price JH (eds), Modern Approaches to the Taxonomy of Red and Brown Algae. Academic Press, New York: 339–369.
- Santelices B, Varela D (1995) Regenerative capacity of *Gracilaria* fragments: Effects of size, reproductive state and position along the axis. *J. appl. Phycol.* 7: 501–506.
- Simons RH (1977) The algal flora of Saldanha Bay. *Transactions of the Royal Society of South Africa* 42: 461–482.
- Smit AJ (1998) Nitrogen environment, ecophysiology and growth of *Gracilaria gracilis* in Saldanha Bay, South Africa. Ph.D. thesis, University of Cape Town, 158 pp.
- Smit AJ, Robertson BL, du Preez DR (1997) Influence on ammonium-N pulse concentrations and frequency, tank condition and nitrogen starvation on growth rate and biochemical composition of *Gracilaria gracilis*. *J. appl. Phycol.* 8: 473–481.

