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Growth rates and agar properties of three gracilarioids in suspended open-water cultivation in St. Helena Bay, South Africa

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Abstract

Relative growth rates (RGRs), yields and agar characteristics of three gracilarioid isolates (*Gracilariopsis* sp. from St. Helena Bay, and *Gracilaria gracilis* isolates from Langebaan Lagoon and Saldanha Bay) were measured to assess the suitability of a site in St. Helena Bay for suspended cultivation. The gracilarioids were grown on polypropylene ropes and 'netlon' lines, and the RGRs were 4.0–11.0% d^{-1} and 5.0–7.0% d^{-1}, respectively. The RGR of the Langebaan isolate of *G. gracilis* grown on ropes was significantly higher than the RGR of other isolates. The mean net yield for the Langebaan isolate grown on 'netlon' lines was 2.6 ± 0.9 kg wet wt m^{-2} 30 day^{-1}. The cultured gracilarioids were extracted for native and alkali treated agars. The mean native agar yield over the entire period was 39.0% dry wt. Alkali pretreatment reduced the yield by 55%, but significantly increased gel strength. High gel strengths (>750 g cm^{-2}) were measured in agars from *Gracilariopsis* sp. and Saldanha *Gracilaria gracilis* in mid-summer and winter. The dynamic gelling and melting temperatures of native and alkali treated agars varied among the gracilarioids. The mean gelling and melting temperatures of agars were about 39.0 °C and 86.0 °C, respectively. The 3,6-AG content ranged from 29% to 38% for native agars and 34–45% for alkali treated agars. While these results indicate that this site is suitable for gracilarioid cultivation, occasional low-oxygen events in St. Helena Bay lead to production of hydrogen sulphide in the sea water ('black tides'). Such events killed most inshore biota (including seaweeds) in 1994 and 1998. This frequency (on average 1–2 per decade) and duration (maximum 2 weeks) would have to be considered in planning commercial seaweed farming in St. Helena Bay.

Introduction

The high value of gracilarioids for human food, abalone feed and agar production (Critchley, 1993) has led to the development of farming systems in the ocean and in ponds (Santelices & Doty, 1989). Commercial farming of gracilarioids is currently taking place in Chile, China, Taiwan and Namibia (Critchley & Ohno, 1998). Brazil, Israel, Mexico, the Philippines, South Africa and Venezuela are assessing the possibility of farming gracilarioids (Critchley & Ohno, 1998). The increasing interest in mariculture of gracilarioids has also led to the screening for species, strains and clones with superior growth and agar qualities (Patwary & van der Meer, 1983; Santelices & Ugarte, 1990).

A commercial *Gracilaria* industry has existed in South Africa since at least 1951, based on collections of beach-cast *G. gracilis* at Saldanha Bay (Anderson et al., 1989). However, in 1974, beach casts ceased and the industry collapsed due to construction of an ore jetty (Anderson et al., 1989). Since then, collections have not fully recovered and have collapsed several times, making the resource unreliable for export and local agar production (Anderson et al., 1996). In an effort to develop a stable *Gracilaria* industry in South Africa, the mariculture of *G. gracilis* was experiment-

ally investigated in Saldanha Bay, and relatively high growth rates obtained for several years (Anderson et al., 1996). However, there were problems with poor growth in some summers, and in a subsequent commercial test, mussel settlement reduced the production to below commercially viable levels. Expanding harbour activities may also threaten the ecological health of Saldanha Bay, and increase water-space conflicts (Smit, 1998). It is therefore necessary to find alternative sites for the cultivation, particularly where there are natural populations to supply stocking material.

There are few sheltered sites along the South African coastline with populations of gracilarioid algae. The habitats on the east coast are all estuaries, and with a few exceptions (e.g. Knysna & Swartkops) are unlikely to be productive because they are too small, seasonally flooded and turbid. The few large estuaries are mainly used for recreation, and any mariculture activity on the surface of the water is unlikely to be allowed. The other sites are Langebaan Lagoon and St. Helena Bay on the west coast. Since Langebaan Lagoon is part of the West Coast National Park, and Saldanha is not ideal for *Gracilaria*, St. Helena Bay appears to offer the greatest promise for extensive gracilarioid farming. There is also a growing interest in mariculture of *G. gracilis* from the local fishing and business community in St. Helena Bay. The community interests and presence of natural stocks of *G. gracilis* in St. Helena Bay, coupled with the problems at Saldanha Bay, led to this study.

The purpose of this study was to investigate the suitability of St. Helena Bay for the suspended cultivation of gracilarioids by measuring on an experimental scale the growth rate and productivity of three local gracilarioids, *Gracilariopsis* sp. and two isolates of *Gracilaria gracilis*. The quantity and quality of the agars from the cultivated material were also analysed to assess their commercial potential. Agar quality factors measured were 3,6-anhydrogalactose content, gel strength, and dynamic gelling and melting temperatures.

Materials and methods

Study site and gracilarioid collection sites

The study was done in St. Helena Bay on the west coast of South Africa (Figure 1). The experimental raft was anchored 50 m from a disused jetty in Vioolbaai, at about 5 m depth. St. Helena Bay receives some run-off nutrients from a nearby (1.5 km) fish factory and from the Berg River. The gracilarioid isolates were collected from three locations. *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham was collected in Saldanha Bay and Langebaan Lagoon, designated here as the Saldanha and Langebaan isolates, respectively. *Gracilariopsis* sp., identified only by the absence of nutritive filaments in cystocarpic material (R.J.A., unpublished data), was collected near Laaiplek in St. Helena Bay.

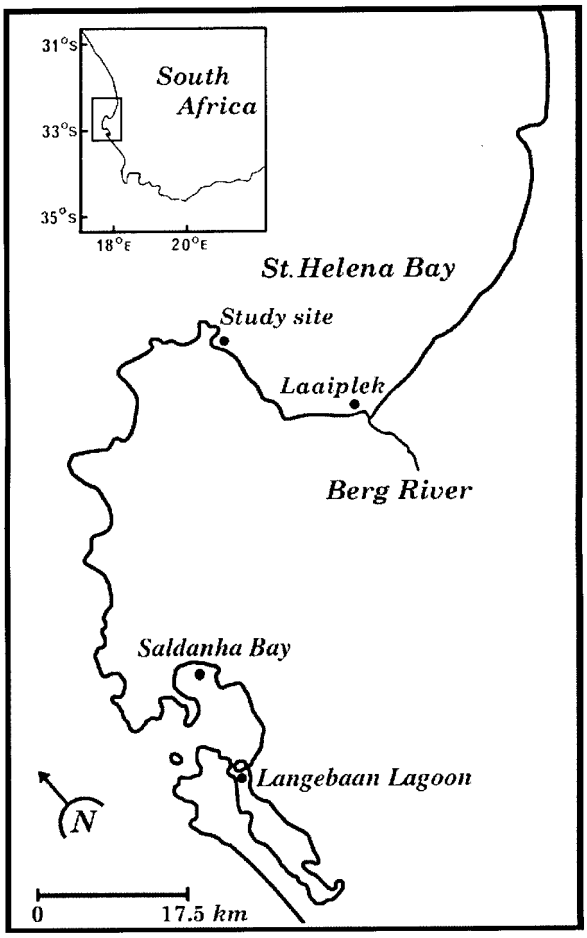

Figure 1. St. Helena Bay and sites where gracilarioid material was collected for cultivation (dots).

Environmental factors

Various environmental factors were measured. Continuous water temperature measurements were made at hourly intervals with submerged electronic recorders, calibrated to 0.5 °C, and data were averaged for each day. Recorders were placed at a depth of about

50 cm (the position of the experimental plants) on the raft. Water temperature was measured for most of the experiment, except for September 1997. Surface water samples for nutrient determinations (phosphate, nitrite, nitrate) were collected every third day near the raft and analysed on an Autoanalyser (Technicon Corp). Nitrite levels were mostly less than 0.1 μm and are not presented here. Photon flux density (PFD), salinity and pH were measured occasionally at 50 cm depth. PFD was measured with a LI-COR's LI-193SA spherical quantum sensor and LI-1000 datalogger; salinity by a hand refractometer (Atago S-20E, Japan) and pH with an Orion Triode pH electrode (model 91-57 BN).

Cultivation techniques and growth measurements

The gracilarioids were grown on a suspended rope system as described by Dawes (1995) and Anderson et al. (1996). This consisted of a rectangular rope frame suspended between anchors and floats at 50 cm below the water surface. The plant material was either attached to polypropylene rope (6 mm, 3 ply) or threaded sideways through 'netlons' or 'Superope', a plastic, tubular netting with a mesh size of approximately 20 mm made for *Gracilaria* farming (van Leer Plastics, South Africa). The plant material selected was clean, healthy and about 20–30 cm long. If the epiphyte *Ceramium diaphanum* (Lightfoot) Roth was present it was manually removed. In August 1998, gracilarioid material was highly epiphytised and was treated with fresh water for five minutes. Every second month, the gracilarioid seeding material was replaced with fresh material from the relevant populations. However, for October 1997 and August 1998, the same material was re-stocked for three consecutive months, when fresh benthic material was unavailable.

Two types of experiments were done. Every 3 months, net yield was measured using the commercial 'netlon' method, and every month relative growth rate was measured using tufts of plant material tied to polypropylene ropes. The latter method is much quicker to set up, but cannot give an estimate of commercial yields because the stocking weight is too low.

The 'netlon' lines were stocked with *G. gracilis* seedlings using the 'hook method' as described by Dawes (1995). This involved a series of wire hooks on a wooden board where thalli were pulled through the sides of the 'netlon' tube so that plants hung out from both sides. Four replicate 'netlons', each with a stocking weight density of 400 g wet wt m^{-1} (commercial stocking density in Namibia), and 5 m long, were used during each cultivation period. Only the Langebaan isolate was used for these tri-monthly yield experiments. For the growth rate experiments, tufts weighing between 20 and 24 g were affixed to polypropylene ropes by cable ties at 20 cm intervals and stocking weight density of 60–75 g wet wt m^{-1}. Two to three ropes, each with 15 replicate tufts were used every month for each isolate. The 'netlons' and ropes were installed horizontally on the suspended raft at a spacing interval of 75 cm to avoid abrasion between lines, and self-shading. In addition, the percentage of *Ceramium diaphanum* load (by weight) was determined, at the end of each growth period, from five random samples. Individual tufts of known weights were brought to the laboratory, all visible *C. diaphanum* epiphytes manually removed and the material dried in a ventilated oven for 48 h at 70.0 °C. The dried material was cooled in a desiccator over silica gel. Epiphytes and host were weighed and the extent of infestation expressed as a percentage of the gracilarioid weight. This weight of epiphyte was subtracted from the harvested material for the calculation of net yield or RGR. The growth and environmental measurements were made from July 1997 to November 1998.

The growth rate and net yield were obtained as follows: the mean monthly relative growth rate (RGR), expressed as % increase in wet weight per day was calculated according to the following formula:

$$\mathrm{RGR} = [(\mathrm{Wt}/\mathrm{Wo})^{1/t} - 1] \times 100,$$

Where Wo = wet weight at start, Wt = wet weight at time t and t = time intervals (days).

The net yield (Yn) was calculated as the difference between initial and final fresh weights, expressed on an areal basis (kg m^{-2}) and taking into account that 'netlons' were spaced 75 cm apart. Results were standardised for a 30 day period according to Anderson et al. (1996).

Agar extraction

Monthly samples were collected randomly from harvested plants, and dried and ground to a fine powder using a Wiley mill (40-mesh size). To evaluate the commercial value of the algal material, agar extraction was also done using alkaline pretreatment. Agar was extracted on alkali-treated and untreated (native) algal materials in three replicates according to

the procedures of Craigie & Leigh (1978) with some modifications. Native agar extraction was done using 10 g of acetone-extracted algal powder which was initially hydrated in 400 mL distilled water for 5 h. The suspension, in a 1-L beaker was boiled in a water bath at 97.0 ± 2.0 °C for 90 min with continuous stirring. A preliminary study had established these conditions as suitable for extracting agar from *G. gracilis*. For alkali pretreatment, 10 g of acetone-extracted algal powder was treated with 400 mL of 4% NaOH solution at 85.0 ± 2.0 °C for 1 h. The residue was rinsed thoroughly in running tap water, drained and placed in 400 mL of distilled water. The pH of the mixture was adjusted to 6.3 ± 0.2 with HCL or NaOH and left overnight. Prior to extraction, the pH of the suspension was adjusted as above and it was incubated in a water bath as described for the untreated material. To each of the hot suspensions, 15 g Celite 545 was added and stirred for 30 min, and the hot slurry pressure filtered through Whatman filter paper (8 μm, porosity) in a preheated pressure filter holder (Sartorius, SM 16274). The residue was then removed from the filter paper, placed in 200 mL boiling distilled water and re-extracted as above. The filtrate was poured into plastic trays, left to gel at 20.0 °C, frozen and then thawed. The gel matrix was redissolved in 200 mL boiling distilled water, cooled and freeze-thawed again. Finally the thawed gel was oven-dried at 45.0 °C to constant weight, cooled in a desiccator over silica gel and weighed to calculate agar yield as a percentage of the dry weight.

Agar gel analysis

The physical properties (gel strength, dynamic gelling and melting temperatures) were determined in triplicate using a 1.5% gel solution prepared from agar samples. To measure gel strength, a hot agar solution (1.5% w/v, 40 mL) was poured into plastic beakers (50 cm diameter, 65 cm height), covered with aluminium foil and allowed to stand overnight at 20.0 °C. Gel strength was measured with a laboratory made apparatus that measures the force required to break a gel surface. The apparatus consists of a 1 cm^{-2} stainless steel cylindrical probe connected to a motor drive and, a Sartorius balance attached to a PC with Winwedge® version 1.2 software. Dynamic gelling temperature was measured by pouring a hot agar solution (10 mL, w/v) into a test tube (diameter 22 mm, height 142 mm) and the test tube was covered with a double holed cork stopper. A thermometer (0.1 °C sensitivity) was inserted through a hole in the centre of cork and then the solution was allowed to cool at a rate of 1.0–2.0 °C min^{-1}. Glass beads (3 mm) were periodically introduced onto the surface of the solution through the other hole. Dynamic gelling temperature was recorded when the bead failed to sink through the gel. The gelled sample was equilibrated overnight at 20.0 °C, and melting temperature was measured by placing a glass bead on the surface of the gel in a test tube clamped in a water bath and warmed at the rate of 1.0–2.0 °C min^{-1}. Melting temperature was recorded when the glass bead sank to the bottom. The chemical analysis involved measuring triplicate samples for 3,6-anhydrogalactose content (3,6-AG) using the resorcinol-acetal reagent method as described by Yaphe & Arsenault (1965). The 3,6-AG content was expressed as a percentage of the agar extract dry weight. For purposes of comparison, agar quality measurements (gel strength, 3,6-AG, gelling and dynamic melting temperatures) were also performed with Difco Bacto-agar, lot no. 106728JC (DIFCO Laboratories, Detroit, USA) and Sigma Agarose, lot no. 38H0085 (Sigma Chemicals, St. Louis, USA).

Data analysis

Statistical analysis involved analysis of variance (ANOVA), a general linear model (GLM) and Pearson correlation procedures using the SPSS for Windows release 8.1 software package. The data were tested for normality (Lilliefors tests) and homogeneity of variances using the Levene test. Non-normal data were transformed accordingly. Tukey's Honest Significance Difference (HSD) test was applied for comparison of means. The Pearson product-moment correlation was used in the correlation of selected agar indices.

Results

Environmental factors

Spring and summer water temperatures fluctuated frequently and rapidly, between 11.0 °C and 16.0 °C. However, in February/March 1998, temperatures rose to a maximum of 19.0 °C for more than two weeks (Figure 2a). In May 1998, temperatures began dropping, and fell throughout winter, with relatively small short-term fluctuations. Although only a few temperature measurements were taken near the bottom (5 m), no major difference was observed between surface and

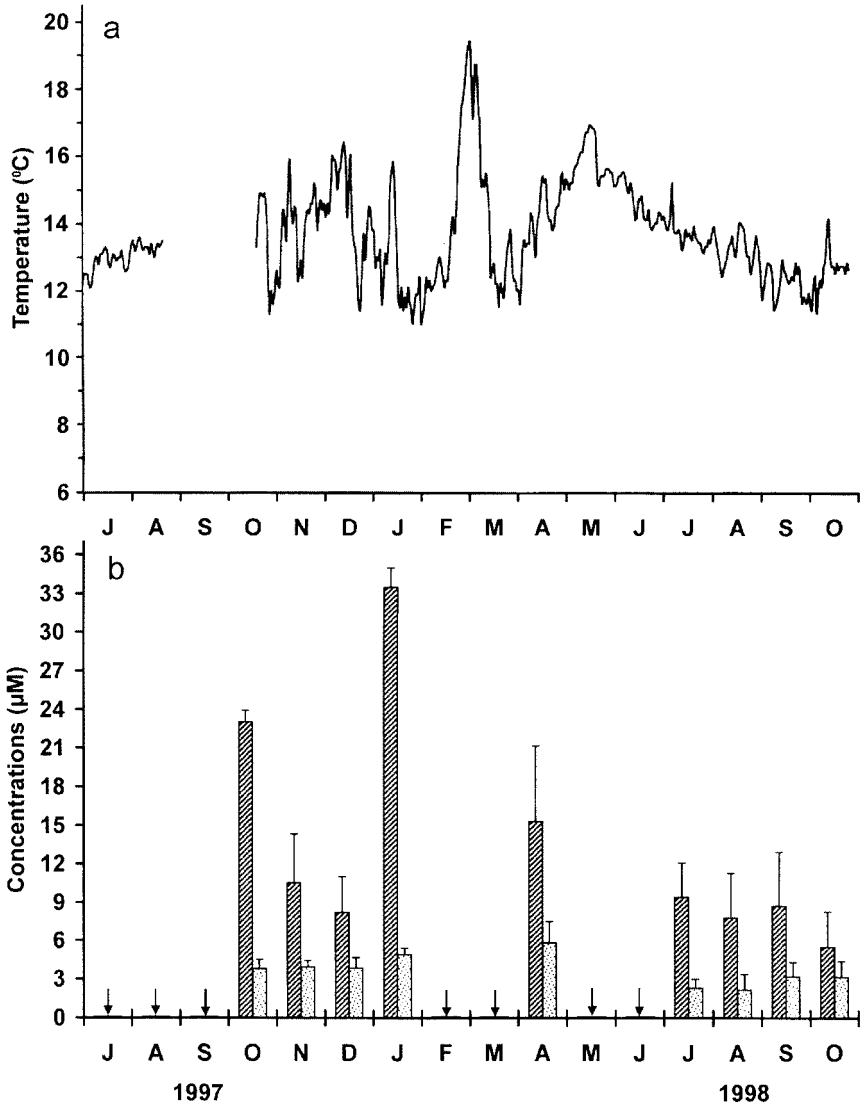

Figure 2. Water characteristics at 50 cm in St. Helena Bay, South Africa from July 1997 to October 1998: a) average daily temperature; b) average monthly nitrate (crossed bar) and phosphate (dotted bar) concentrations with standard error bars shown. Arrows indicate no data.

bottom water temperatures, an indication of a generally mixed water column at this site for most of the year.

Water samples were only collected occasionally. The highest nitrate (15.3–33.5 μm) and phosphate (5.8 μm) concentrations were recorded in summer/autumn, while their values were generally low in winter (Figure 2b). No significant difference between bottom and surface nutrient concentrations were observed. Photon flux density was generally high in summer. In November 1998 at mid-day, PFDs of 2330, 2020, 910 and 545 μmol photon m^{-2} s^{-1} were measured at the surface (0 cm), 50, 250 and 500 m depth. Salinity was 35–37‰ and pH 7.4–7.9.

Growth and production rates

In the test of relative growth rate (RGR) of the three gracilarioid grown on polypropylene ropes (Figure 3a), the Langebaan *Gracilaria gracilis* grew significantly faster (ANOVA, $p < 0.001$, Tukey HSD test). There was no significant difference in growth rates between *Gracilariopsis* sp. and the Saldanha *G. gracilis* isolate. There was no clear seasonal pattern in RGR for any of the isolates, but differences

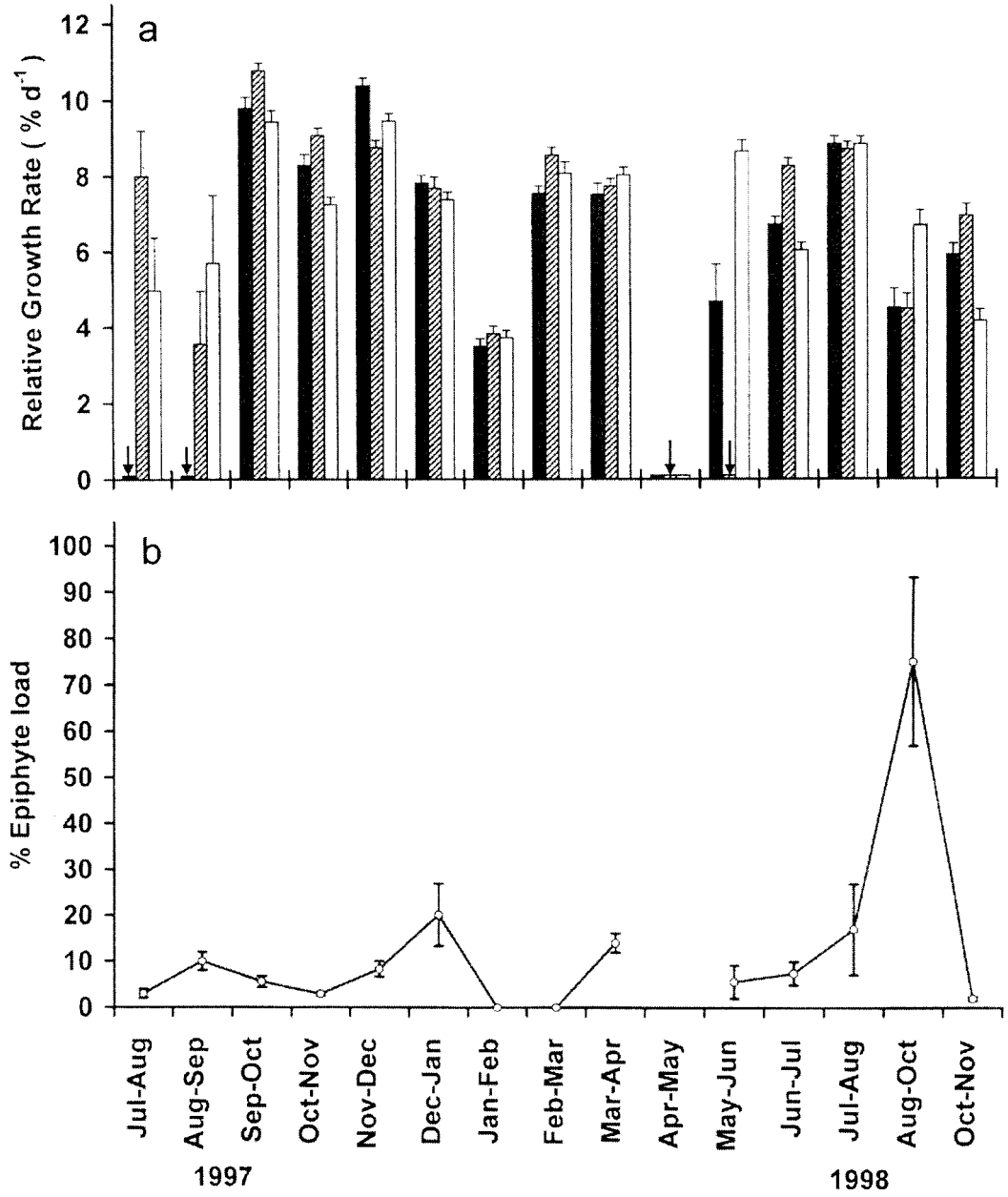

Figure 3. a) Relative growth rates of *Gracilariopsis* sp. from St. Helena Bay (black bar), *Gracilaria gracilis* from Langebaan Lagoon (crossed bar) and *Gracilaria gracilis* from Saldanha Bay (white bar) grown on polypropylene ropes on a suspended raft system in St. Helena Bay (mean ± SE, n = 20–45); b) Monthly load of the epiphyte *Ceramium diaphanum* as a percentage of the host gracilarioid weight (mean ± SE, n = 5). Arrows indicate no data.

from month to month (Figure 3a). The RGR values for October-November 1998 were lower than those in October-November 1997.

The growth of the Langebaan isolate of *G. gracilis* (in terms of RGR) was similar on ropes (Figure 3a) and 'netlons' (Figure 4) for corresponding months. The difference between the growth rates of Langebaan material grown on 'netlons' and ropes is due to the different stocking weights. The highest mean RGR and net yield of 6.7% day^{-1} and 4. 0 $\pm$ 0.1 kg wet wt m^{-2} 30 day $^{-1}$, respectively,was recorded in October-November 1997 for the Langebaan material seeded on 'netlon' lines (Figure 4). The average RGR and net yield for the Langebaan plant grown on 'netlon' lines during the study period, excluding the month of August 1997 when there was a storm, was 5.4 $\pm$ 0.7% $day^{-1}$and 2.6 $\pm$ 0.9 kg wet wt m^{-2} 30 day^{-1}, respectively.

No correlations were found between the average relative growth rates for each gracilarioid (and their combined RGR) and the measured environmental factors of surface water temperature, dry weight and epiphyte load (Figure 3b). The data for nutrient concentrations were irregular. However, we believe that the growth rate of the isolates was influenced by the quality of planting materials. For example, in August-October 1998, Langebaan and *Gracilariopsis* material, which had been used in previous months, were highly contaminated with *Ceramium* and had significantly lower RGR than freshly collected epiphyte-free Saldanha plants (ANOVA, $p < 0.001$). However, even with freshly collected material differences were also observed. For instance, fresh material was used for seeding all the isolates during the month of October-November 1998, but the growth rates of Saldanha material differed markedly from the other isolates (Figure 3a). The overall mean RGR measured from Langebaan was 67% and 17% (significantly) higher than Saldanha material and *Gracilariopsis* sp., respectively (GLM, $p < 0.001$). The Saldanha material used was thick, hollow, twisted and highly branched with small branches sprouting along the almost flat thallus forming a 'millipede like structure'.

In April-May 1998, all seaweeds on the raft were killed by an environmental event (see discussion) that also killed kelp beds, mussels and other fauna in the area. Growth was low in January-February 1998, which coincided with the highest daily temperature fluctuation (8.4 °C) measured in February 1998. Low growth observed in August-September 1997 was probably due to a strong storm (swell height: 180 cm) that dislodged kelps growing near the raft; these subsequently damaged the plants on the raft by entangling and breaking them.

The gracilarioids on the raft were occasionally contaminated by epiphytes and fouling organisms. The major epiphyte was *Ceramium diaphanum*, which occurred throughout the study period, reaching amounts of between 3% and 21% of the host gracilarioid, except in October 1998, when epiphyte load was very high (Figure 3b). Other organisms contaminating the gracilarioid thalli were low quantities of *Ulva, Cladophora* and *Enteromorpha*, diatoms and mussel spat.

Table 1. Analysis of variance on the yield and some properties of agar from three cultivated gracilarioid isolates (*Gracilariopsis* sp. from St. Helena Bay, and *Gracilaria gracilis* from Langebaan Lagoon and Saldanha Bay) extracted under two different conditions: treated with I N NaOH (alkali treated) and not treated (native). [A = isolates, B = alkaline status, A × B = isolates and alkaline status interaction]. Data on gel strength were $\log_{10}$ transformed

Sources of variation	df	F value	P value
Agar yield			
A	2	2.932	0.058 ns
B	1	538.5	0.001 **
A × B	2	0.741	0.479 ns
Gel strength			
A	2	2.507	0.087 ns
B	1	86.7	0.001 **
A × B	2	1.161	0.318 ns
Gelling temperature			
A	2	2.047	0.135 ns
B	1	37.31	0.001 **
A × B	2	1.228	0.298 ns
Melting temperature			
A	2	0.985	0.378 ns
B	1	0.722	0.398 ns
A × B	2	7.750	0.001**
3,6-Anhydrogalactose			
A	2	5.438	0.006 **
B	1	23.83	0.001 **
A × B	2	0.657	0.521 ns

** = Highly significant, ns = not significant.

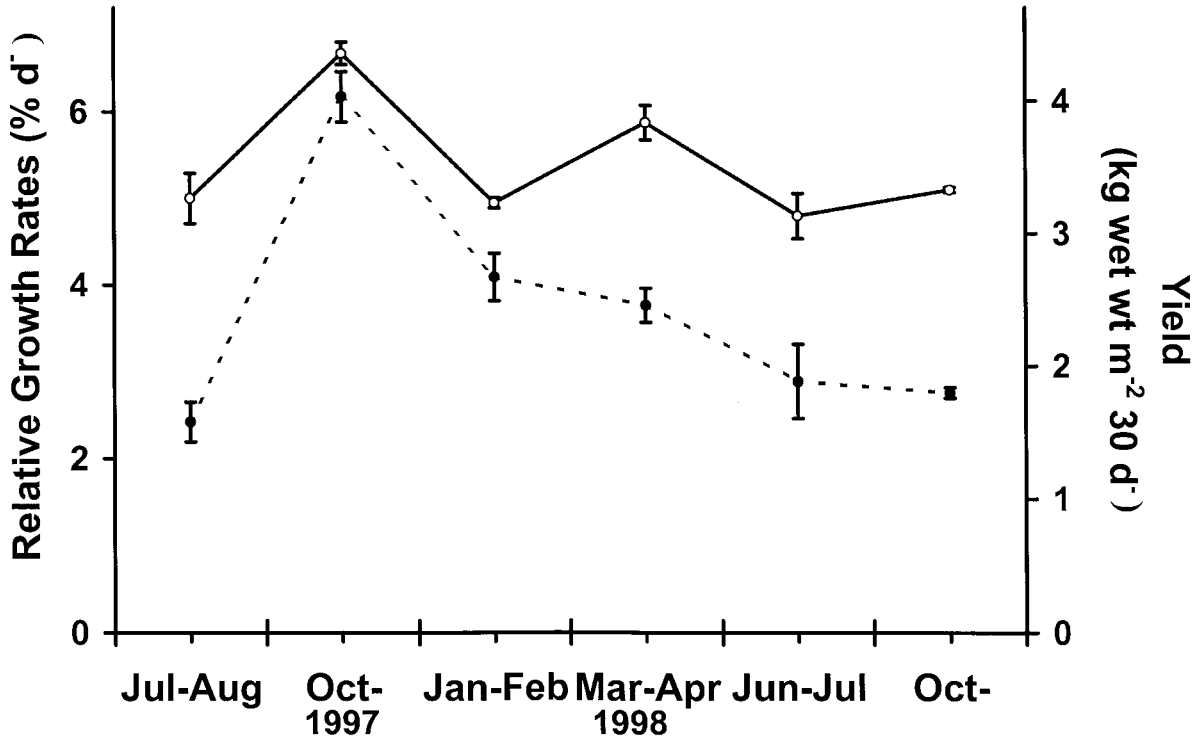

Figure 4. Mean relative growth rate (solid line) and net yield (broken line) of *Gracilaria gracilis* from Langebaan Lagoon grown on commercial-style 'netlon' lines (400 g m^{-1}) on a suspended raft system in St. Helena Bay (mean ± SE, n = 4).

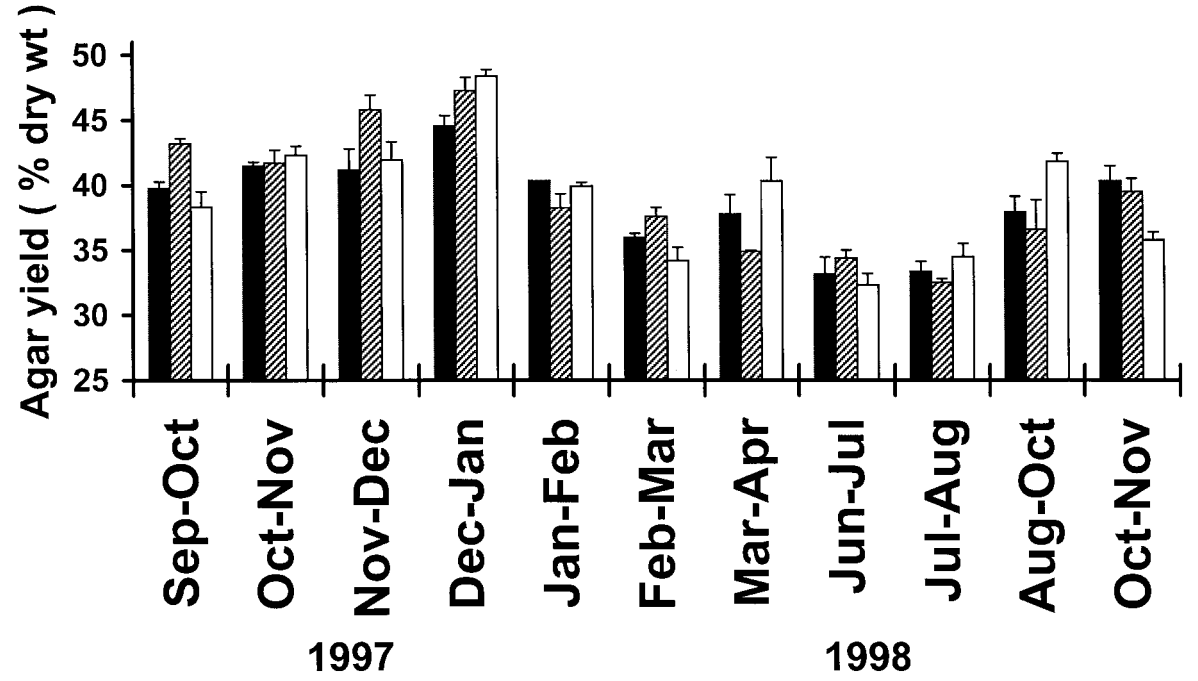

Figure 5. Native agar yields (as percentage dry wt) from *Gracilariopsis* sp. from St. Helena Bay (black bar), *Gracilaria gracilis* from Langebaan Lagoon (crossed bar) and *Gracilaria gracilis* from Saldanha Bay (white bar) grown on a suspended raft in St. Helena Bay, South Africa. Standard error bars shown (n = 3).

Agar yield

The native agar yields from the gracilarioid isolates varied during the study period, with higher yields in summer than in winter (Figure 5). Native agar yields obtained over the study period showed no statistical differences between the three isolates. The average agar yields for alkali treated material from *Gracilariopsis* sp., Saldanha and Langebaan *G. gracilis* isolates was 16.5 ± 2.7, 19.2 ± 2.8, and 20.1 ± 4.7%, in that order. The alkali pretreatment statistically reduced the agar yield by 55% (Table 1). There were no significant correlations between agar yield from the isolates, and temperature, relative growth rate, epiphyte cover or dry weight (data not shown).

Agar gel properties

The mean gel strengths of alkali treated agars for the three gracilarioid isolates are shown in Figure 6a. Gel strengths were highest in December-January and June- July for the three isolates. There was a significant increase in gel strength following alkali treatment (ANOVA, $p < 0.001$) but no significant gel strength difference among the isolates was observed (Table 1). The gel strengths of the *Gracilariopsis* sp. and *G. gracilis* Saldanha agars showed significant correlations with melting and gelling temperatures (Table 2), but none was observed in Langebaan *G. gracilis* (data not shown). The average gel strength for native agar from *Gracilariopsis* sp., Saldanha and Langebaan *G. gracilis* isolates was 284 ± 71, 313 ± 124, and 335 ± 144 g cm^{-2}, in that order.

The 3,6-anhydrogalactose content (3,6-AG) of agars varied significantly among the three isolates and with the effect of alkali treatment. Alkali pretreatment of agars significantly increased 3,6-AG in the three isolates investigated (Table 1), and the Langebaan *G. gracilis* plants exhibited a significantly higher 3,6-AG content than the other isolates. Gracilarioid isolates generally showed little variation in 3,6-AG content during the culture period (Figure 6b). Although the average 3,6-AG content of alkali treated agars was very high it did not significantly correlate with any of the agar indices (Table 2). The amount of 3,6-AG in the native agars ranged from 35 to 37%.

The dynamic gelling and melting temperatures of alkali treated agars varied among the gracilarioid isolates and were significantly higher after alkali treatment (ANOVA, $p < 0.001$). Although the melting temperatures of the three isolates showed little variation throughout the culture months, all alkali treated agars exhibited the highest melting temperature (> 87.0 °C) in December-January (mid summer) (Figure 6c). Melting temperatures of alkali treated agars of the Langebaan isolate varied between 86.0 and 90.0 °C, those of Saldanha from 83.0 to 88.0 °C and *Gracilariopsis* sp., from 82.0 to 87.0 °C. The gracilarioid isolate and alkali pretreatment of the algal materials had no significant influence on the melting temperatures but their interaction had a significant effect (ANOVA, $p < 0.001$) (Table 1). In Saldanha *G. gracilis* agar, the gelling temperature was positively correlated with melting temperature, but not in the other isolates (Table 2).

Discussion

Growth and production rates

The mean relative growth rate of the gracilarioids in this study was 7.5 ± 0.4% d^{-1}, which is within the range of 5.0–10.0% d^{-1} reported for most *Gracilaria* species (McLachlan & Bird, 1986; Rueness et al., 1987; Molloy & Bolton, 1996). *G. gracilis* from Langebaan Lagoon grew significantly faster than the other gracilarioids, but it is important to note that phenotypic plasticity in gracilarioids is a very common phenomenon (Critchley, 1993) and may contribute to differences within a species.

Although there was no strictly seasonal pattern of growth, the gracilarioid isolates exhibited the highest (9.5–10.8% day^{-1}) growth rates in September-October, probably as a result of increasing temperatures, irradiance, and nutrient levels. During the same period the highest net yield of 4.0 kg m^{-2} 30 day^{-1} was also recorded on the 'netlons'. Average nitrate (10.5–23.0 μm) and phosphate (3.8–4.0 μm) levels were highest in summer. These levels are consistent with those of freshly upwelled Benguela water (6.0–8.0 μm phosphate and 15.0–30.0 μm nitrate), as reported by Brown & Hutchings (1987). It is also likely that some nitrate and phosphate reaches the site from fish-factory waste, but the area shows no signs of pollution. Photon flux density levels are unlikely to be limiting in suspended cultivation, particularly in spring or summer. Engledow & Bolton (1992) reported a light saturation level 80 μmol photon m^{-1} s^{-1} for growth of *G. gracilis*, well below the midday PFD of 2020 μmol photon $^{-1}$ s^{-1} measured in St. Helena Bay in October (spring).

Low growth rates and net yields in January-February 1998 coincided with a wide temperature

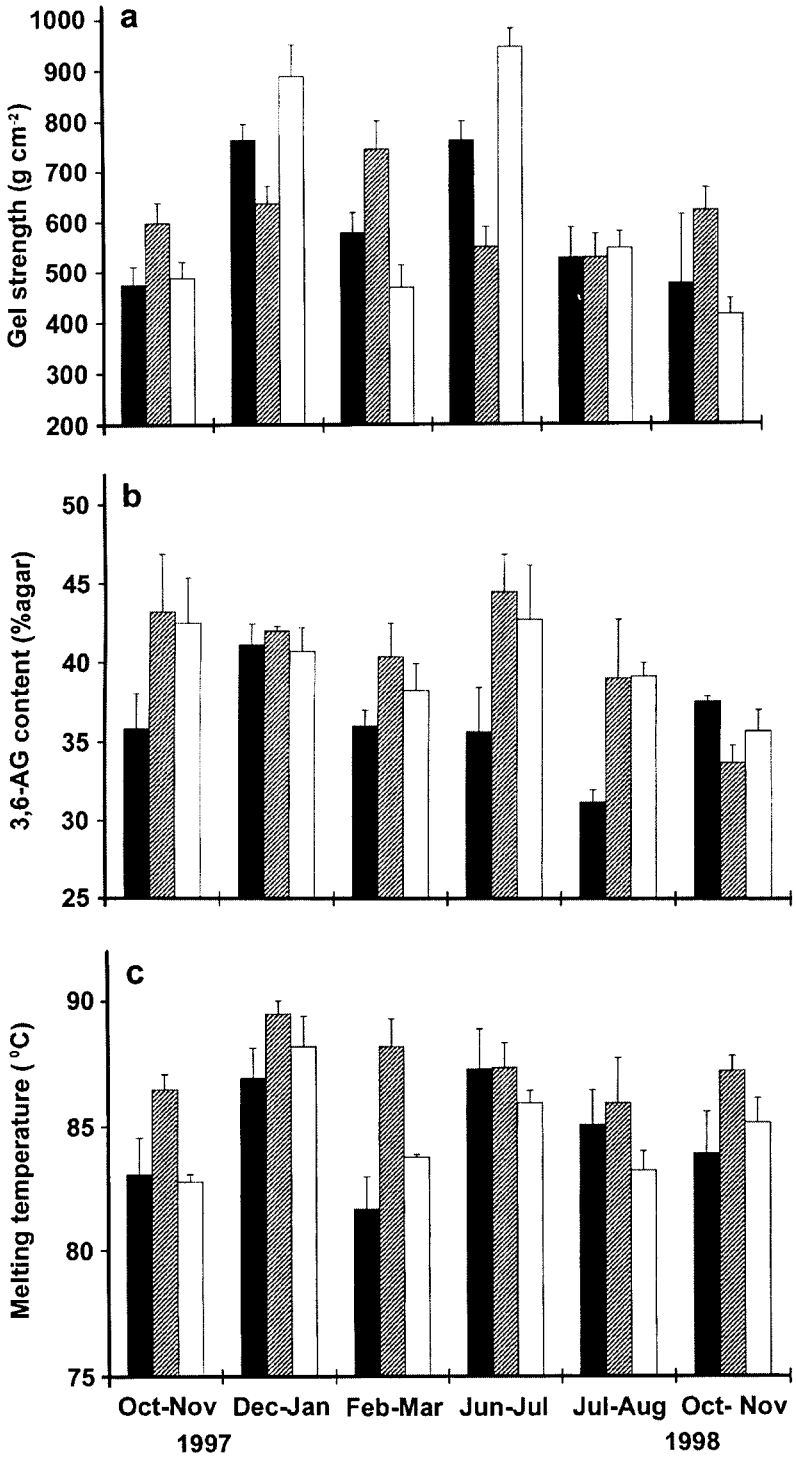

Figure 6. Physical and chemical properties of alkali treated agars from *Gracilariopsis* sp. from St. Helena Bay (black bar), *Gracilaria gracilis* isolate from Langebaan Lagoon (crossed bar) and *Gracilaria gracilis* isolate from Saldanha Bay (white bar) grown on a suspended raft in St. Helena Bay, South Africa. Standard error bars shown (n = 3).

Table 2. Correlation coefficient matrix of some physical and chemical properties of alkali treated agars: gel strength, melting temperature, gelling temperature and percent 3,6-anhydrogalactose (bottom matrix, *Gracilariopsis* sp. from St. Helena Bay; top matrix, *Gracilaria gracilis* from Saldanha Bay). No significant correlation coefficient for *G. gracilis* from Langebaan Lagoon in any of the four properties was observed (data not shown)

	Gel strength ($g\ cm^{-2}$)	Gelling temperature (°C)	Melting temperature (°C)	3,6-anhydrogalactose (%)
Gel strength	–	0.662*	0.716**	0.266
Melting T.	0.549*	–	0.878**	0.099
Gelling T.	0.587*	0.026	–	0.106
3,6-AG	0.368	0.421	0.164	–

* Significant at $p = 0.05$, ** Significant at $p = 0.01$.

fluctuation (11.0–19.4 °C) and a high monthly mean of 15.6 °C in February (the highest recorded in this study). However, it is not likely that high temperature reduced growth because Engledow & Bolton (1992) showed that the optimum temperature for *G. gracilis* growth is 25.0°C. Rather, low growth was likely to be a result of low nutrients, because in the Benguela system, there is an inverse relationship between temperature and nutrient levels, and water warmer than about 16.0 °C is likely to be nutrient poor (Bailey & Chapman, 1985). Anderson et al. (1996) showed that in summer in Saldanha Bay, sustained periods of high water temperature (over 16 °C) are correlated with periods when the *G. gracilis* grew very slowly, and exhibited symptoms of nitrogen starvation, such as loss of pigments, bleaching and death. The low growth in August-October for *Gracilariopsis* sp. and Langebaan isolates; and June 1998 for *Gracilariopsis* sp. was partly due to high epiphyte contamination by *Ceramium diaphanum*. The quality (apparent health) of planting material has also been shown to affect growth of gracilarioids. Smit (1998) demonstrated that the growth rate of healthy-looking, dark *G. gracilis* was significantly higher than apparently unhealthy, slightly bleached plants that had been in surface cultivation for several months. He attributed the difference to physiological changes in nutrient kinetics and photosynthesis, as well as possible changes with the increased thickening of plants grown near the surface for several months. The results of this study support these observations.

In April-May 1998 all the plants on the raft died due to a 'black tide'. This term is used to describe low oxygen events that follow massive plankton blooms, which result in the production of hydrogen sulphides in sea water (Matthews & Pitcher, 1996). Although the hydrogen sulphide concentration was not measured in 1998, high toxic concentrations (128 μmol L^{-1}) of hydrogen sulphide were recorded in 1994 during a similar black tide in St. Helena Bay (Matthews & Pitcher, 1996). The effect of hydrogen sulphide on South African gracilarioids is not known, but Kim (1970) reported that gracilarioids cannot survive high concentrations. Black tides were observed in 1994, 1997, 1998 (this study) and 1999 (R.J.A., unpublished) but not all were fatal to seaweeds. These black tides all occur in late summer, last for periods of several days to 2 weeks. They do not occur every year, and are only severe enough to affect seaweeds about once or twice a decade (R.J. A. unpublished). The production of toxic levels of hydrogen sulphide is the last series of events that take 1–2 weeks to develop, and so some preparations can be made to reduce the effects on a farm. For example, the bulk of the crop could be quickly harvested, and seed stock stored on onshore tanks if necessary.

The net productivity of *Gracilaria* varies with the species and culture techniques. Net yields of *Gracilaria* ranging from 18 to 127 t dry wt ha^{-1} y^{-1} have been reported. In this study, the average net yield from the commercial stocking density of 400 g wet wt m^{-1} would be extrapolated to 47 t dry wt ha^{-1} y^{-1}. This net yield is based on an average dry weight of 14.5% dry weight, which is lower than the commercial 20% dry weight ratio used (Chiang, 1981). This net yield is higher than the reported 18–29 t dry wt ha^{-1} y^{-1} for pond cultivation of *G. tikvahiae* in Florida (Hanisak & Ryther, 1984). A higher yield was recorded in this study than in other suspended raft systems in the southern Africa region. The Namibian *G.*

gracilis commercial farm yields 27–37 t dry wt ha^{-1} y^{-1} (K. Rotmann, pers. comm.) while Anderson et al. (1996) estimated 40 t dry wt ha^{-1} y^{-1} for Saldanha *G. gracilis*, based on a small scale experimental raft. Lapointe and Ryther (1978), who reported a very high production of 127 t dry wt ha^{-1} y^{-1} for *G. tikvahiae,* emphase that small-scale experimental data show only the potential productivity of the seaweed but that these results cannot be extrapolated to a large-scale commercial operation. The high net yield in our study may be attributed to high nutrient levels in St. Helena Bay, considered to be one of the most productive areas in the Benguela systems (Bailey & Chapman, 1985).

Agar yield and quality

The agar yields from the gracilarioid isolates in this study ranged from 34.0 to 47.0% and 15.0 to 27.0% for native and alkali treated agars, respectively. These are comparable to the 41.6–49.1% agar (native) yields reported for Namibian *G. gracilis* (Wilson & Critchley, 1998) and the 43.3% obtained for *G. chilensis* in Chile (Matsuhiro & Urzua, 1990). The native agar yields from the gracilarioids in the present study are much higher than previous report from the same species (Engledow & Bolton, 1992), probably because of different extraction methods (McLachlan & Bird, 1986). However, agar yields after alkali treatment were similar to those reported by Anderson et al. (1996) for Saldanha *G. gracilis.* Here, high agar yields were obtained in December-January (mid summer) and low yields in winter, similar to the results of Hoyle (1978), for Hawaiian gracilarioids. A high agar yield during or immediately after the growing season seems to coincide with the peak of assimilatory activity in the plants. Although agar yield was not significantly correlated to dry wt, the highest dry wt was observed during this period of high growth (unpublished data). However, the absolute agar yields measured in this study should be interpreted with caution because the extraction method may have resulted in floridean starch inclusions with extracted agars. Chiles et al. (1989) observed a substantial amount of floridean starch in such agar preparations.

High gel strength in gracilarioid agars has generally been observed during periods of high growth rates, in summer months or at peak plant abundance (Lahaye & Yaphe, 1988; Pickering et al., 1990). Hoyle (1978) observed that agars from both *G. coronopifolia* and *G. bursa-pastoris* in Hawaii showed seasonal changes in gel strength. However, these changes did not occur at the same time in both species and the gel strength of *G. coronopifolia* agar declined in late summer. Other studies have reported agars with very little or no variation in gel strength through out the year (Durairatnam, 1987; Anderson et al., 1996). In this study, marked seasonal variability was observed in gel strength in agars, especially from *Gracilariopsis* sp. and Saldanha *G. gracilis* isolates. Both isolates exhibited peaks of high gel strength (>750 g cm^{-2}) in December-January and June-July but there was no clear seasonal pattern. Hoyle (1978) also observed bimodal patterns of high gel strengths during both summer and winter months for *G. bursa-pastoris* in Hawaii.

The present results show that alkali treated gracilarioid isolates produced agars of relatively high mean gel strength (612 g cm^{-2}), high dynamic melting temperature (85.7 °C) and high gelling temperature (38.8 °C). The gel strength values were higher than Difco Bacto agar (444 g cm^{-2}) but lower than Sigma agarose (1345 g cm^{-2}). All gelling and melting temperatures obtained in this study meet the specifications of the United States Pharmacopoeia standards (Armisen, 1995). From the data presented here, St Helena Bay may be a suitable site for gracilarioid cultivation, to produce reasonably good agars. The next stage would appear to be a pilot-scale commercial farm, where the economics of gracilarioid cultivation could be quantified. Possibly the main threats to seaweed farming here are high hydrogen sulphide events associated with black tides; the frequency and duration of such events would have to be taken into account in planning such farming.

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