# The potential of *Grateloupia filicina* (Lamouroux) J. Agardh. for mariculture: Culture experiments and observations on shore phenology

**Honours Project by Susanne Vetter**
**Supervisors: Prof. John Bolton and Dr. Rob Anderson**
**University of Cape Town**
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## Abstract

*Grateloupia filicina* is a carrageenophytic red alga which is in demand as a carrageenan raw material and for use in food. There is therefore interest in developing a technique for mariculturing this species. Since vegetative propagation from thallus fragments has not been successful, the present study was initiated to determine suitable conditions for spore liberation and growth of *G. filicina* from spores. It was also attempted to propagate *G. filicina* sporelings from crust and thallus fragments. In addition, the proportions of plants in different life history phases were determined in August and September to test for seasonal differences in shore phenology, and whether *G. filicina* is monoecious or dioecious, since there is disagreement on this in the literature. Spore release was easy to achieve, even without stressing the parent plant. Growth of crusts was found to be fastest at 50 $\mu M.m^{-2}.s^{-1}$ and at 20°C. Carposporelings (i.e. young tetrasporophytes) grew slightly but significantly faster than tetrasporelings (i.e. young carposporophytes). Crust fragments were able re-attach to the substrate and gave rise to new upright thalli within 1-2 weeks, compared to 4-5 weeks between spore release and thallus initiation.. Attachment was weak, however, and only a small proportion of the crust fragments placed into culture regenerated. Thallus regeneration was not successful. There were seasonal differences in shore phenology during the study period: while two thirds of the plants collected in August were carposporophytes, only tetrasporophytes were found in September. No male gametophytes or spermatangia were found. It did not emerge from this study whether *G. filicina* in an annual, or what phenotypic differences are found throughout the year. Information of this kind is important for mariculture as it affects the supply of parent plants (and therefore spores) and the growth of young plants on ropes if these are released into the sea. The culturing experiments look promising; techniques (seeding ropes directly with spores or using regenerated crust fragments) still need to be refined.

## Introduction

*Grateloupia filicina* (Lamouroux) C. Agardh. is a red alga of the family Halymeniaceae (formerly Grateloupiaceae or Cryptonemiaceae) in the order Cryptonemiales. It is found world-wide in warm temperate waters and grows along the entire west coast of South Africa and eastwards as far as the Kowie area near Port Alfred (Stegenga *et al.*, in press). In South Africa, it is commonly found in intertidal rock pools, particularly in the upper intertidal zone. In Australia, on the other hand, it is largely confined to the vicinity of harbours and sheltered coasts, where it often occurs in abundance in shallow water or just above low tide level (Womersley 1994). Around Sydney, it is reported to be completely subtidal in sheltered bays (Millar, *pers. comm.*). This contrasts with the situation in South Africa, where the pools inhabited by *G. filicina* are often found on wave exposed shores.

Two varieties of this species have been described: *G. filicina* var. *filicina* and the larger *G. filicina* var. *luxurians* (Fig. 1). In culture, plants of the two varieties each retained their own morphologies, suggesting that there is some genetic difference between them; however, plants of intermediate phenotype are found in the Channel islands and the Mediterranean (Irvine 1983). In southern Australia, all specimens fall under the latter variety (Womersley 1994). In the British Isles, *G. filicina* var. *filicina* occurs in littoral pools and in the sublittoral zone down to a depth of 10 m, while var. *luxurians* occurs in lower littoral lagoons, harbours, estuaries in sheltered running water and in the sublittoral zone down to 6 m (Irvine 1983).

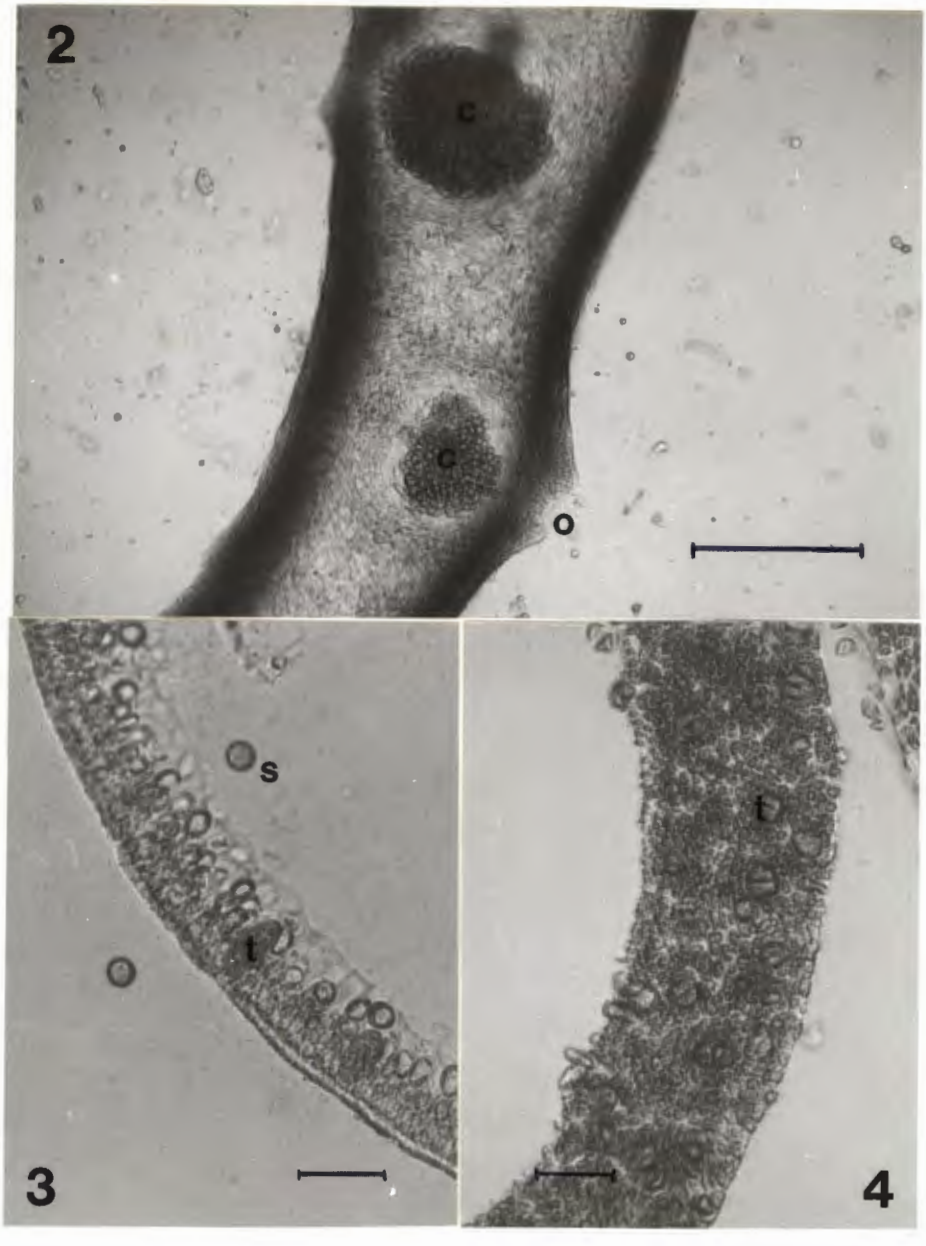
*Grateloupia* has an isomorphic life history, i.e. the tetrasporophyte and gametophyte phases look alike. Carpospores are found in scattered cystocarps which are embedded in the thallus; the cruciform tetrasporangia are found scattered in the cortex. Some details of this life history remain unresolved. Irvine (1983) describes this species as monoecious, with separate male and female plants in the gametophyte phase. In *G. filicina* var.


**Figure 1:** Habit of *Grateloupia filicina*.
A. *G. filicina* var. *filicina* (x 1).
B. *G. filicina* var. *luxurians* (x 1).
C. Part of branch with cystocarps.
(From Irvine 1983).

*filicina*, spermatangial plants are not found in the field in the British isles; elsewhere and in culture, spermatangia are scattered on the thallus and are 4-5 µm in diameter. In *G. filicina* var. *luxurians*, spermatangia are found in superficial sori 50-60 µm in diameter (Irvine 1983). Womersley (1994), on the other hand, describes *G. filicina* as dioecious in the sexual phase, with spermatangia being cut off from the outer cortical cells of the gametophyte. Figures 2-4 show sections of carposporophytes and tetrasporophytes collected in the present study.

Little is known about the seasonality of *G. filicina*; it is often described as an annual. In Australia, it is a winter annual like other members of the Halymeniaceae (Millar, pers. comm.). In the British Isles, *G. filicina* var. filicina is described as being usually annual, with greatest sizes and abundances in summer while plants overwinter until February or March. The fronds of *G. filicina* var. *luxurians* overwinter and are reported to regrow from the perennial attachment disc in spring (Irvine 1983). Bula-Meyer (1989) on the other hand reports that *G. filicina* is abundant throughout the year along the Caribbean coast of Colombia. Whether *G. filicina* is annual in the sense that it is completely absent from the shore and regrows from spores every season, or whether there are simply seasonal differences in abundance in certain environments, is not clear from the literature. In South Africa, it seems that *G. filicina* is present all year in varying abundances (Bolton and Anderson, pers. comm.).

From a commercial point of view, *G. filicina* is of dual interest. This species, among other red algae, contains carrageenan, a substance which is used as a thickener, gelling agent, stabiliser and emulsifier, and which is in world-wide demand. Its useful properties were first discovered by the dairy in the 1940's, when carrageenan was found to be an ideal stabiliser for the suspension of cocoa in chocolate milk (Chapman and Chapman 1980). Many more uses for




**Figs. 2-4:** Sections of mature carposporophytes and tetrasporophytes of *Grateloupia filicina*.
2. Carposporophyte with cystocarps (c); note the conspicuous ostiole (o).
3. Tetrasporophyte with terasorangia (t); note free tetrasopres (s).
4. Surface view of tetrasporophyte cortex with enbedded tetrasporangia (t).
Scale bars: Fig. 2: 500 μm; Figs. 3 and 4: 100 μm.

carrageenan have been discovered since then, including its addition to toothpaste, ice cream and tinned meat. More recently, a Japanese company has expressed interest in importing South African *G. filicina* for use in food.

Carrageenan is a primary constituent of the cell walls of certain red algae. Like agars and alginates, carrageenan serves a structural function analogous to that of cellulose in land plants. Whereas land plants require a rigid structure to support them against the constant pull of gravity, marine plants need a more flexible structure capable of withstanding the varying stresses of currents and wave action. For this reason, they have developed hydrophilic, gelatinous structural materials with the necessary flexibility which are collectively known as phycocolloids.

Carrageenans are sulphated polysaccharides which are grouped according to their sulphation patterns into *kappa*, *beta*, and *lambda* families. The different sulphation patterns and degrees are a result of the specificity of the sulphotransferase enzymes which provide a variety of substitution patterns for the sulphate hemiesters (Craigie 1990). Different algal species contain different carrageenans, and most algae contain a combination of different types. Few members of the Cryptonemiales have been examined in detail for their wall structure (Craigie 1990), as research has focused on members of the Gigartinales (see Nunn and Parolis 1968, 1969 and 1970; Allsbrook *et al.* 1971 and Farrant *et al.* 1971 for some detailed studies on some of the cell wall constituents of members of the Halymeniaceae; none of these include a *Grateloupia* species). The water soluble polysaccharides extracted from several members of the Halymeniaceae are highly sulphated galactans containing low levels of 3,6-anhydrogalactose. They exhibit a positive specific optical rotation and do not gel in the presence of $K^+$.

The three main types of carrageenan, *kappa*, *lambda* and *iota* each exhibit specific gel characteristics. In the industry, these are carefully chosen and blended precisely to fulfil the required functions. Different red algal species

contain one or more of the different kinds of carrageenan. At present, the carrageenan industry is dominated by *Eucheuma*, which accounts for almost 80% of the carrageenophytes harvested globally (McHugh 1991). *Eucheuma* is easily propagated vegetatively from fragmented thalli and is widely cultivated in the Philippines, Indonesia and, more recently, Tanzania. However, while *Eucheuma cottonii* and *E. spinosum* (the most widely cultured species) are good sources of *kappa*- and *iota*-carrageenan respectively, other types of carrageenan, such as *lambda*, are not produced in this genus. There is a world-wide demand for all types of carrageenans, and there is a growing interest in exploiting a larger variety of carrageenophytes, particularly cold water species. *Chondrus crispus*, for example, is harvested in Canada and France for its *lambda* carrageenan in the tetrasporophyte generation (Critchley 1993). In Chile, natural populations of *Iridaea* are harvested from the shore on a large scale; an annual harvest of 5 - 6 000 tonnes are removed (Santelices 1989). No commercial harvesting of carrageenophytes takes place in South Africa at the moment, although up to 54 tonnes per annum of *Gigartina polycarpa* (probably including *G. stiriata*) were harvested from 1956 to 1978 (Anderson et al. 1989). One of the main reasons for this is that South African carrageenophyte populations are either too sparse, too scattered or inaccessible to make exploitation economically viable (Levitt *et al.* 1995).

A number of carrageenophyes are found on South African shores, including *Gigartina* and *Iridaea* species. Little attention has been paid to *Grateloupia filicina* in the past, although it is common in South Africa and many other areas in the world. *Pachymenia hymantophora* (Halymeniaceae) was found to contain *kappa* and *lambda* carrageenan in moderate quantities (Penman and Rees 1973, cited in Craigie 1990). The type of carrageenan in *Grateloupia* has not been determined, but may be similar to other members of its family. Whether the tetrasporophyte and carposporophyte phases contain the same type of carrageenan is not known. Laboratory extractions revealed that *G. doryphora* has a carrageenan content of 38 % in the female gametophyte

phase and 33 % in the tetrasporophyte phase (Levitt, unpubl. data). As a comparison, *Hypnea* was found to contain 20 %, *Gigartina* species 35-45 %, *Aeodes* 50 % and *Iridaea* 53 %. In other words, the carrageenan content is within the range of other commercially exploited species. *G. filicina* var. *luxurians* is reported to be used in the Pacific as food and for the extraction of carrageenan (Levring et al. 1969, cited in Irvine 1983).

So far, no carrageenophytes are successfully maricultured in South Africa. Cold water carrageenophytes anywhere in the world have not been cultured as successfully as the tropical *Eucheuma* species as no cold water species have been identified which reproduce from thallus fragments, which is the fastest way of producing biomass. The fact that no viable technique for the mariculture of cold water carrageenophytes has so far been developed has led to a relative shortage of this resource in comparison to the easily maricultured *Eucheuma* species. This fact is evident from recent FOB prices for carrageenophytes: US$ 1000 for a dry ton of *Gigartina* or *Iridaea*, in comparison to US$ 700 for *Eucheuma* species (C. Dawes, *pers. comm.* in Molteno, unpubl. Honours thesis)

Still higher prices are paid for seaweeds that are used in food. For example, brown algae used as food in the Far East were reported to sell for US$ 7 000 to 10 000 per dry ton, compared to US$ 150 - 500 for brown algae sold for the extraction of algin (McHugh 1984). If the interest in South African *G. filicina* for use in food is sustained, successful mariculture of this species could be an important source of income. The high prices paid for algae which are used in food would also warrant the development of more intensive mariculture techniques, which are often too costly for the production of carrageenan.

It becomes apparent that there is a need to investigate the possibility of mariculturing *G. filicina*. The advantages of mariculture over harvesting are several: a reliable supply of the desired species is ensured without the risk of

depleting natural resources. Even where a commercially important species is abundant, there is still a need to invest considerable resources into assessing what quantities can safely be harvested before populations of the target and other species are threatened, and into monitoring the natural resource and its use. In mariculture, the quality of the algae produced can be more closely controlled and optimised, whether one is interested in carrageenan content or appearance and texture in the case of algae used as food.

lima et al. (1995) report successfully growing *G. acuminata* from fragmented crusts and upright thalli, both of which were grown from spores in culture. Migita (1988, cited in lima et al 1995) was reported to have done the same (successfully) with *G. filicina*. Vegetative propagation of *G. filicina* from thallus fragments inserted into rope were unsuccessful on the Caribbean coast of Colombia (Bula-Meyer 1989) and in Saldanha Bay, South Africa (Anderson, *pers. comm.*; see map in Fig. 5 for the location). Given the demand for *G. filicina*, the potentially high income that can be generated and the success of previous attempts to culture it, this project was initiated with the following aims:

**1.** To establish ways of inducing spore release, to determine the suitable light and temperature conditions for growing *G. filicina* in culture, and to investigate the possibility of using regenerating crust and thallus fragments for propagation. For this purpose, culture experiments were performed.

**2.** To gather ecological and phenological information relevant to mariculture and harvesting in the field and literature.

## Materials and Methods

### Shore phenology

Plants were collected from intertidal rock pools at Kommetjie beach (Fig. 2) in mid-July, mid-August and late September 1996. Plants were picked randomly from a number of different pools, and the proportions of numbers of tetrasporophytes, carposporophytes and male gametophytes of a random subsample (picked out of the bucket without looking) of this were determined. Since the life history of *Grateloupia* is isomorphic, it was necessary to section the blades and to inspect them under the light microscope at low magnification. Mature carposporangia are visible when the whole thallus is held against the light, but plants were sectioned to confirm the reproductive status, especially if carposporangia were not detected. In July, only a very few plants were sectioned, as they were collected primarily to investigate spore release and to test-run the experimental technique. In August and September, 82 and 61 plants were sectioned respectively.

### Spore release

A number of different methods have been used to stimulate spore release in red algae: temperature shock, desiccation and exposure to freshwater among others (Bolton pers. comm.). After rinsing fertile, healthy and generally epiphyte-free plants in filtered seawater, seven batches of six replicates were subjected to one of four different stress treatments.

Treatment 1: Plants were patted dry with a paper towel and laid out in the shade to desiccate them slightly. This treatment lasted 40 min, compared to 30 min in the sun or 1 h 30 min in the shade which is applied by G. Levitt (*pers. comm.*) to induce spore release in Gigartina. The desiccation period was kept shorter because unlike Gigartina, Grateloupia filicina is naturally found in pools or subtidally and presumably less tolerant of drying out.

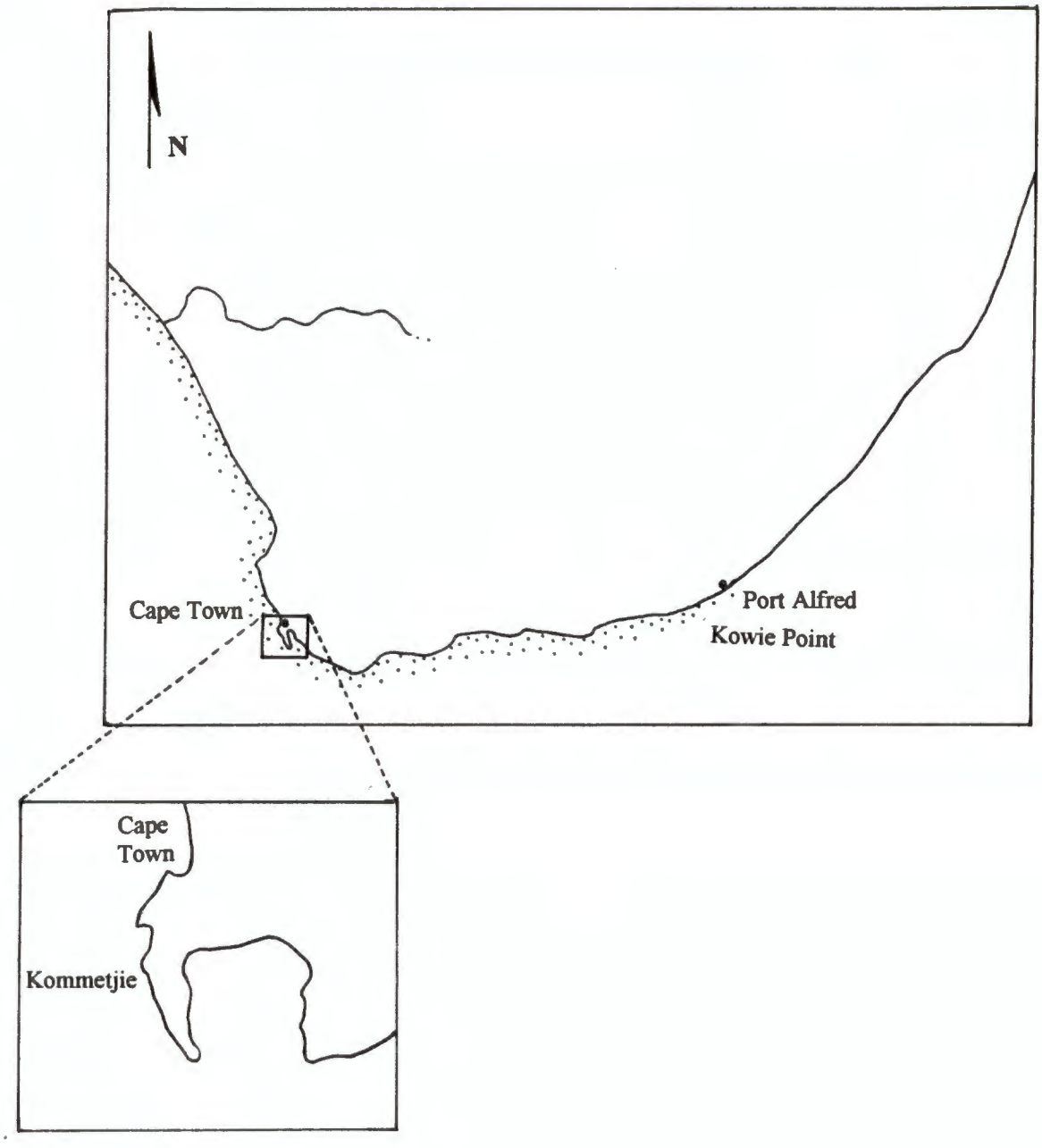



**Figure 5:** Map of South Africa showing the distribution of *G. filicina*. The study area is shown in the inset.

Treatment 2: Plants were immersed in tap water for 2 min (as in Anderson and Bolton 1985).

Treatment 3: Plants were left in seawater at 2°C for 2 h.

Treatment 4: Plants were left in seawater at 25°C for 45 min.

Control: These plants were retained in seawater at 15°C, at which temperature the experiment took place.

Treatments 1 and 2 were performed with tetrasporophytes and carposporophytes; for treatments 3, 4 and control, only carposporophytes were used.

After the stress treatments, plants were washed in a 0.5% solution of Povidone Iodine (a form if iodine commonly used as a disinfectant; see Hharitonidis 1992) to kill diatoms and epiphytes. For each treatment, pieces of the same six parent plants were used to exclude the possibility of genotypic variation confusing the results. Thallus segments of approximately 10 mm were then placed over cover slips in small culture dishes containing 20 - 30 ml of a third strength PES medium (Stein 1973). To inhibit diatom contamination, 1 $ml.l^{-1}$ of saturated $GeO_2$ solution was added to the medium. According to Markham and Hagmeier (1982, cited in Anderson and Bolton 1985), growth rates of red algae are unaffected by $GeO_2$ at concentrations of up to 2 $ml.l^{-1}$. The thallus pieces were left in the medium for 12 h (over night) at 15°C at a light intensity of 50 $\mu M.m^{-2}.s^{-1}$ in a cycle of 16 h light : 8 h dark. The thallus fragments were then removed from the medium, and the number of dishes in each treatment in which spore release had occurred, counted. The cultures were observed over the next four weeks, after which a few of the slides were kept to observe their further development, while further culture experiments under a variety of controlled conditions were initiated.

Culturing experiments

Two sets of experiments were carried out to determine the effects of light intensity, temperature and life history phase on growth rates. The first experiment was initiated on August 16. Spore release in this and the subsequent experiment was induced by placing thalli into tap water for 2 min. Carpospores and tetraspores were cultured at the same temperature under three different light intensities. The second experiment was carried out to examine the effect of temperature, and to obtain additional information on growth response to different light intensities. Only tetraspores were used for the second experiment, since no fertile carposporophytes were found in late September, when this experiment was set up. Photoperiod was 16 h light : 8 h dark for all experiments.

During Experiment 1, Light intensity was controlled by positioning the culture dishes at different distances from the light source (banks of cool-white fluorescent lamps), and was measured using a Li-Cor 190s quantum sensor connected to a Li-Cor 188 Integrator. Six replicates (each corresponding to the spores of one parent plant) of carposporophytes and tetrasporophytes were cultured at 10, 50 and 130 $\mu M.m^{-2}.s^{-1}$. Temperature was constant at 15°C. Measurements of 25 crust diameters per dish were taken under the light microscope at 6, 13, 20 and 28 days following spore release. The culture medium containing $GeO_2$ was changed weekly.

Experiment 2 was performed at 15 and 20°C at light intensities of 25, 50 and 75 $\mu M.m^{-2}.s^{-1}$. Four replicate dishes were prepared for this experiment, in each of which 20 crust diameters were measured. In all other respects, culturing methods were the same as in Experiment 1.

Regeneration from of crust and thallus fragments

To test whether fragmented basal crusts are able re-attach and give rise to new thalli, 10-week old plants that had grown and were retained from the first culturing attempt were used were used. The thalli of two young tetrasporophyte and two young carposporophyte plants were cut off and put into separate culturing dishes, and the crusts were carefully removed from the cover slips after they had been cut into several segments using a razor blade. The crust fragments were placed into culturing medium over microscope slides in two culturing dishes (one for each of the life history phases).

Statistical analysis

Differences in growth rates were compared and tested for significant differences using growth curves (box and whisker plots showing mean, standard error and STD) and two-way ANOVA with fixed effects.

For Experiment 1, separate sets of curves for tetrasporophytes and carposporophytes at different irradiance levels were plotted. To test for the influence of light, life history phase and parent plant, a 3-factor ANOVA was performed with the null hypothesis that growth was the same at all light intensities, life history phases and genotypes. The LSD test for planned comparisons (which, in procedure, is equivalent to the t-test) was used to test for the for the post-hoc significance of differences due to any of the above three factors.

For Experiment 2, curves were plotted separately for 15 and 20°C. Two-factor ANOVA followed by the LSD post-hoc test was performed to test whether the effects of temperature and light intensity were significant. Since only two or three dishes survived in each treatment, the data from different parent plants

was combined for the ANOVA after testing for normal distribution of the data values.

## Results

Shore phenology

Out of the 82 plants collected in mid -August, 55 were carposporophytes and 27 were tetrasporophytes. At the end of September, only three carposporophytes with relatively few and small carposporangia were found in a sample of 61 plants that were sectioned. No infertile or male plants were found in either sample. No data for other times of the year was obtained; specimens in the Bolus Herbarium (U.C.T.) did generally not specify the month of collection, and the life history phase was never given. During the first collection in mid-July, large numbers of juvenile plants (as well as some older plants) were observed. During the subsequent two collections the majority of the plants in the pools were large.

There was considerable phenotypic variation in the plants collected. Most were of similar size (about 5 - 15 cm), but while some thalli were relatively wide and flat, other were narrower and much more branched. Both phenotypes were observed in carposporophytes and tetrasporophytes.

Spore release

All the treatments applied, including the control, resulted in a high percentage of spore release. For a summary, see Table 1. It was therefore decided to use the method of immersing parent thalli in non-saline water for 2 min, as this is the quickest method (other than the control).

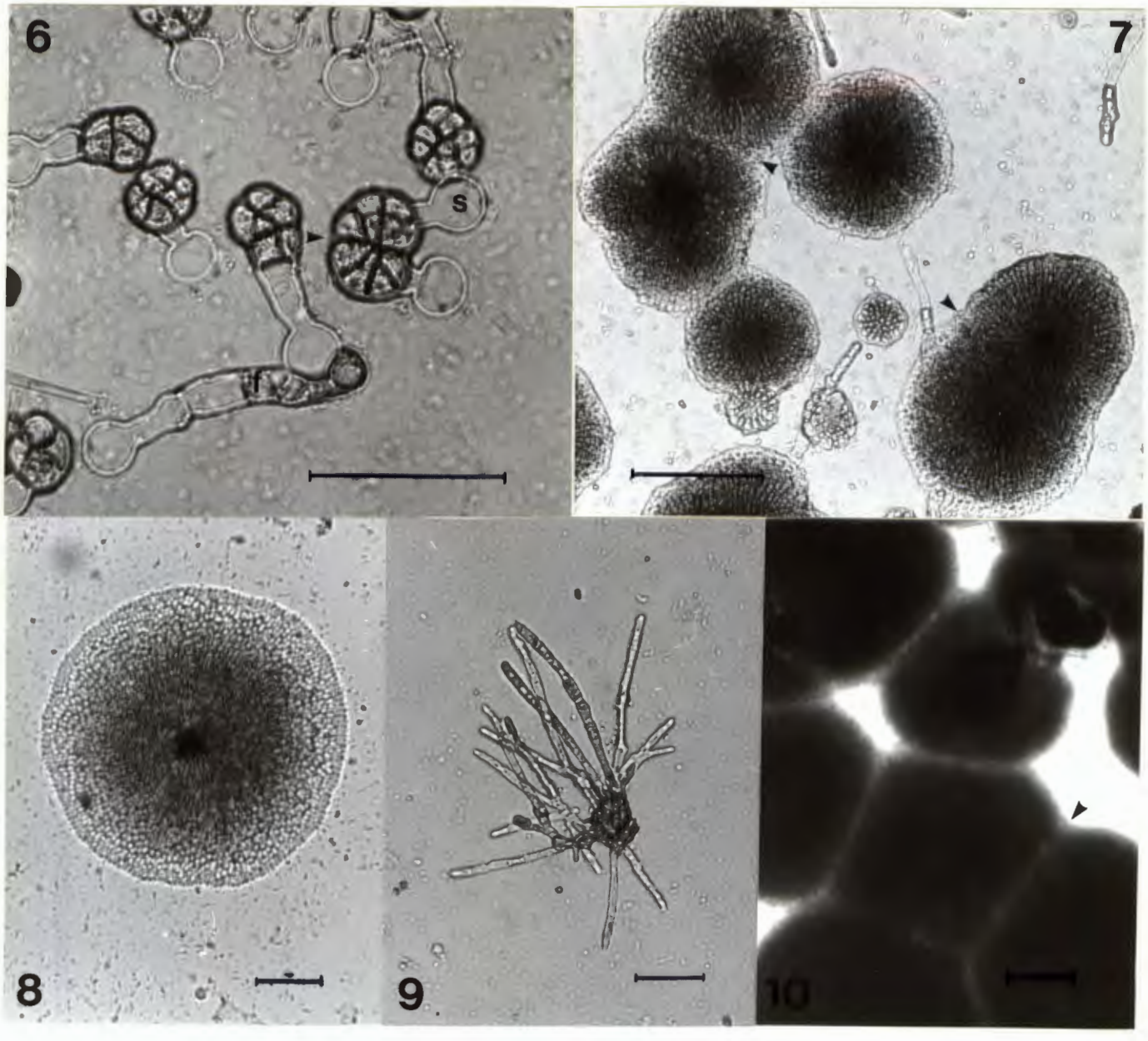
Tetraspores were scattered in the culture dish, while carpospores formed little clumps where the cystocarps were positioned.

**Table 1:** The number of dishes (out of 6) in which spore liberation had taken place.

| Treatment | Number of dishes with spore release |
|---|---|
| Temporary desiccation (carposporophyte) | 5 |
| Temporary desiccation (tetrasporophyte) | 6 |
| 2 min in tap water (carposporophyte) | 5 |
| 2 min in tap water (tetrasporophyte) | 5 |
| At 25°C for 45 min (carposporophyte) | 6 |
| At 2°C for 2 h (carposporophyte) | 6 |
| Control (carposporophyte) | 6 |

Spore development

Germination patterns were the same as previously described by lima et al (1995) and references therein. Spore development was identical in carpospores and tetraspores and is illustrated in Figures 6-10 (all cultured at 15°C at an irradiance of $\mu$50 $M.m^{-2}.s^{-1}$). Spores attached within 2-3 days of spore liberation and first developed a germ tube, into which the spore cytoplasm moved. Successive divisions of the germ tube then gave rise to circular crusts with a marginal meristem (Figs. 6 and 7). Where crusts touched, they appeared to fuse, although the separate centres could still be seen (Figs. 7, 8 and 10). Instead of forming circular crusts, some spores gave rise to filaments, which later branched (Fig. 9). These were particularly common in the first culture experiment, where cover slips with spores were agitated a day after release to test for attachment were transferred into clean




**Figs. 6-10:** Development of *Grateloupia filicina* sporelings.
6. Tetraspores after 5 days. Note the empty spore cases (s) that were left when the cytoplasm moved into the germ tube; two crusts touching and fusing (arrow), and filaments formed instead of crusts.
7. Crusts from carpospores after 2 weeks. Note where crusts have fused (arrow).
8. Tetraspore crust after 4 weeks. Note centre where upright thallus starts to form.
9. Filament after 4 weeks.
10. Carpospore crusts (young tetrasporophytes) after 8 weeks. Note that although crusts touch, each crust is discrete (arrow) and gives rise to its own thalli.
Scale bars: 100 μm in all figures.

culture dishes two days after release, and may be a response to agitation. They were not observed to give rise to crusts or upright thalli, and were rarely found in the subsequent experiments. After about one month, upright thalli started to upheave from the centre of each crust (Fig. 8). At 10 weeks after spore release, most thalli were between 1 and 3 cm in length with the exception of young carposporophytes in one particular dish, which grew much faster and reached an average thallus length of 6 cm. The thalli of these plants also started to branch at the tips after 10 weeks. None of the young plants were fertile after 10 weeks.

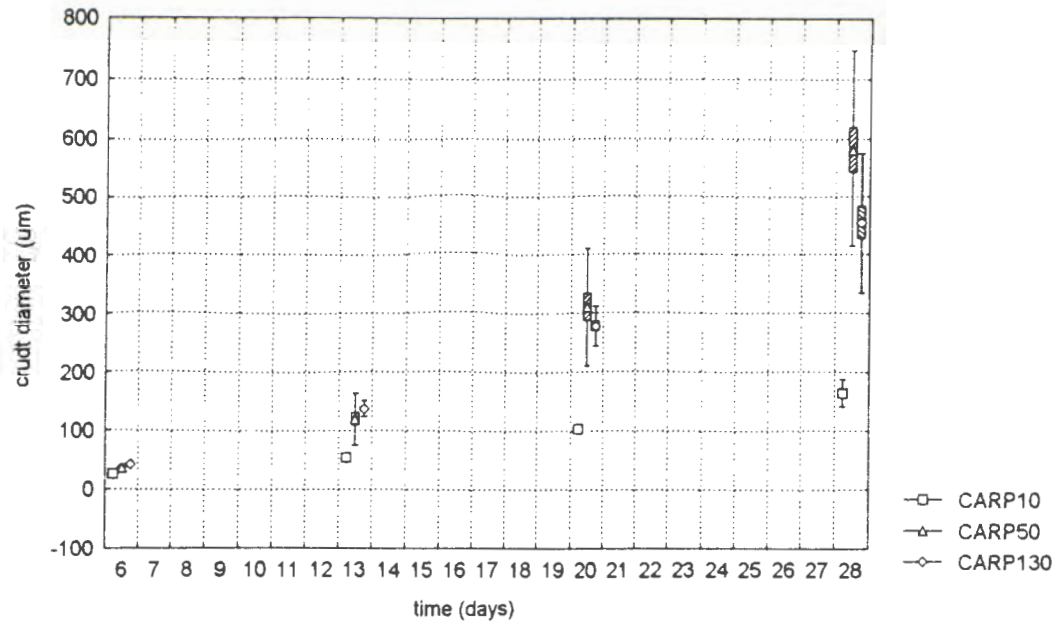
Growth of carpospores and tetraspores in different light and temperature conditions

*Experiment 1: Effects of light and life history phase*

Figure 11 shows growth of carpo- and tetrasporelings at 10, 50 and 130 $\mu M.m^{-2}.s^{-1}$. Differences in growth due to differences in light intensity is significant at the 1 % level. Growth is highest at 50 $\mu M.m^{-2}.s^{-1}$ Carposporelings grew slightly but significantly ($p<0.01$) faster than tetrasporelings. The LSD post-hoc test showed that in most cases, parent plant has a significant influence on growth.

*Experiment 2: Effects of light and temperature*

Figure 12 shows the growth of tetrasporelings under three different irradiance levels at 15 and 20°C. As in Experiment 1, sporelings grow fastest at 50 $\mu M.m^{-2}.s^{-1}$ ($p < 0.01$). Crusts grew faster at 20°C than at 15°C ($p < 0.01$). Almost all of the dishes kept at 20°C had developed fungal infestations after two weeks, and in the dishes that were kept at 25 $\mu M.m^{-2}.s^{-1}$, no sporelings survived. In some of the other dishes that were infected, sporelings also showed reduced growth. Whether the high incidence of fungal infections is a result of the higher temperature or whether the spores were present in the



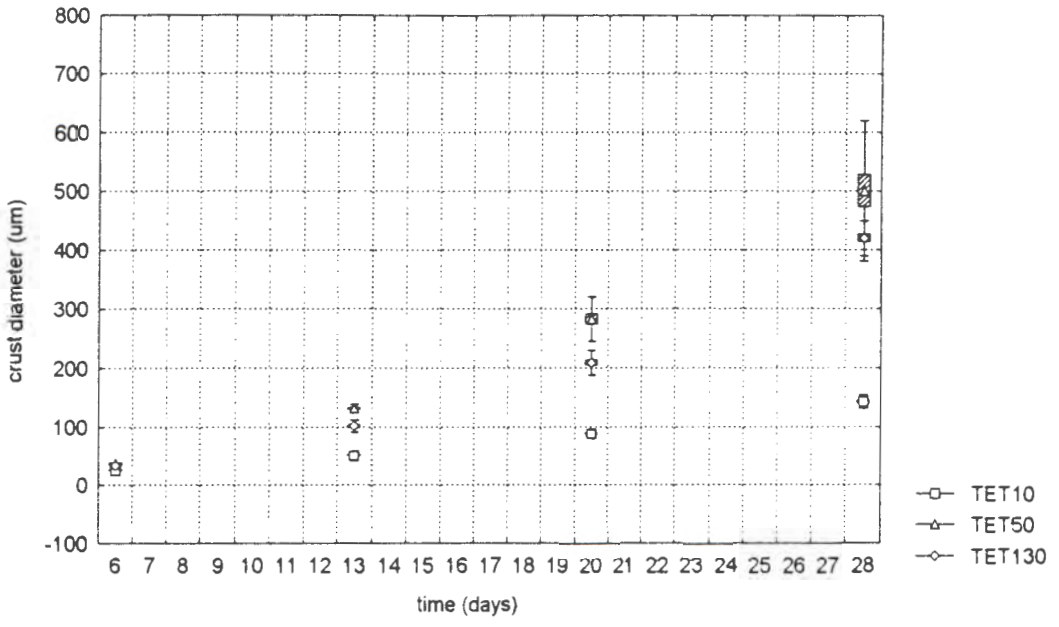
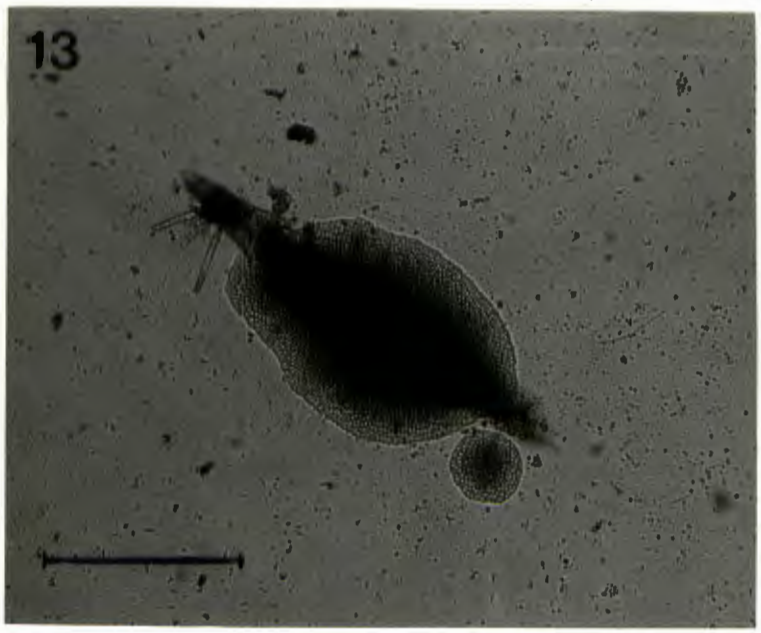
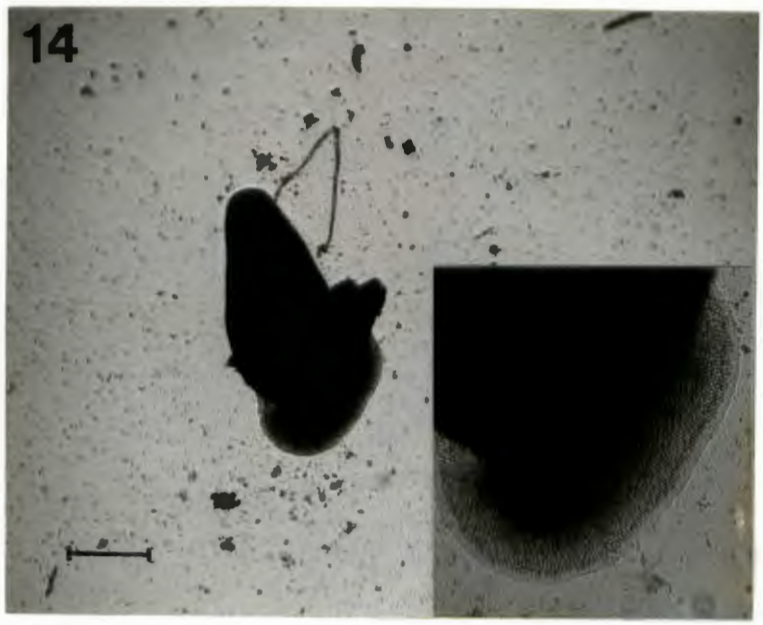




**Figure 11:** Diameters of carposporophytes (a) and tetrasporophytes (b) at 10, 50 and 130 $M.m^{-2}.s^{-1}$, showing means, standard errors and standard deviations. Experiment was conducted at 15°C




**Figs. 13 and 14:** Regeneration of crust fragments.
13. After one week, a crust fragment has attached and a new crust has started forming.
14. After 2 weeks, an upright thallus has begun to form from the re-attached crust. Inset shows the regenerated crust at higher magnification.
Scale bars: 500 μm in both figures.

## Discussion

Shore phenology

The results of this study do not cover enough time and nothing certain can therefore be deduced about whether *G. filicina* is an annual. The fact that the ratio of gametophytes to tetrasporophytes has shifted in just over one month shows that there are seasonal differences in the composition of *G. filicina* populations. The fact that there were no carposporophtytes found in September (spring) resembles the situation in Japan, where only tetrasporophytes of *G. acuminata* were found in April 1990 and March 1991(Iima et al 1995); in contrast, *G. filicina* in the British Isles has tetrasporophytes throughout the year (except during winter dormancy). Since *G. filicina* in South Africa is found on the shore throughout the year (Anderson and Bolton, pers. comm.), it seems more likely that due to seasonal differences in growing conditions, there are seasonal differences in growth and reproduction and hence in biomass and life history phase composition. A more intensive, long term study would be useful to clear up the evident confusion about the seasonality in this species. Knowledge about seasonal availability of *G. filicina* in the field is important for mariculture if parent plants are needed to obtain spores. As in other species, there may also be seasonal differences in carrageenan content, which should be explored for *G. filicina*. It is also of importance to ascertain what environmental or other factors cause seasonal differences in growth, as these will most likely affect any *G. filicina* crop on ropes in the sea; an understanding of the influence of these factors can also provide useful information for designing laboratory conditions.

Irvine (1983) describes that *G. filicina* var. *luxurians* regrows from its perennial attachment disc after the dormant winter. If *G. filicina* found in South Africa displayed this behaviour, it would benefit mariculture: it might be possible to harvest one crop, and instead of having to reseed the ropes with

culture dishes two days after release, and may be a response to agitation. They were not observed to give rise to crusts or upright thalli, and were rarely found in the subsequent experiments. After about one month, upright thalli started to upheave from the centre of each crust (Fig. 8). At 10 weeks after spore release, most thalli were between 1 and 3 cm in length with the exception of young carposporophytes in one particular dish, which grew much faster and reached an average thallus length of 6 cm. The thalli of these plants also started to branch at the tips after 10 weeks. None of the young plants were fertile after 10 weeks.

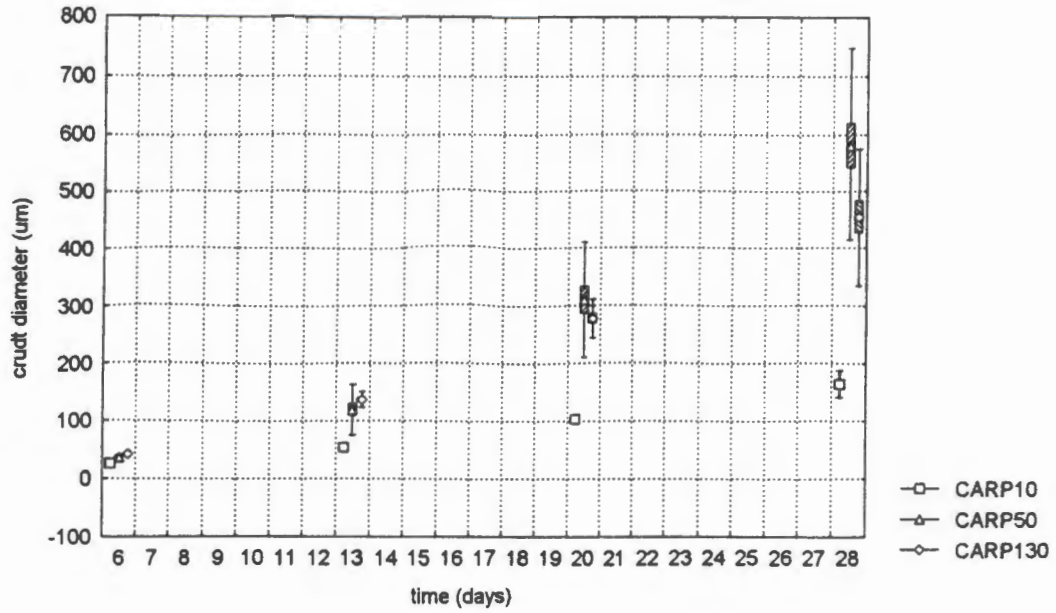
Growth of carpospores and tetraspores in different light and temperature conditions

*Experiment 1: Effects of light and life history phase*

Figure 11 shows growth of carpo- and tetrasporelings at 10, 50 and 130 $\mu M.m^{-2}.s^{-1}$. Differences in growth due to differences in light intensity is significant at the 1 % level. Growth is highest at 50 $\mu M.m^{-2}.s^{-1}$ Carposporelings grew slightly but significantly ($p<0.01$) faster than tetrasporelings. The LSD post-hoc test showed that in most cases, parent plant has a significant influence on growth.

*Experiment 2: Effects of light and temperature*

Figure 12 shows the growth of tetrasporelings under three different irradiance levels at 15 and 20°C. As in Experiment 1, sporelings grow fastest at 50 $\mu M.m^{-2}.s^{-1}$ ($p < 0.01$). Crusts grew faster at 20°C than at 15°C ($p < 0.01$). Almost all of the dishes kept at 20°C had developed fungal infestations after two weeks, and in the dishes that were kept at 25 $\mu M.m^{-2}.s^{-1}$, no sporelings survived. In some of the other dishes that were infected, sporelings also showed reduced growth. Whether the high incidence of fungal infections is a result of the higher temperature or whether the spores were present in the



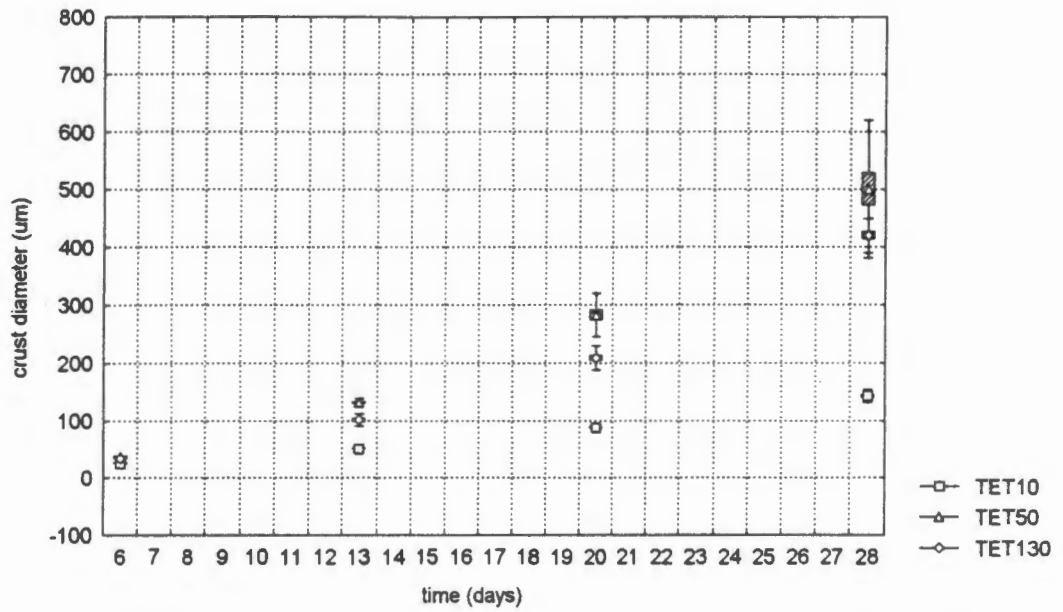
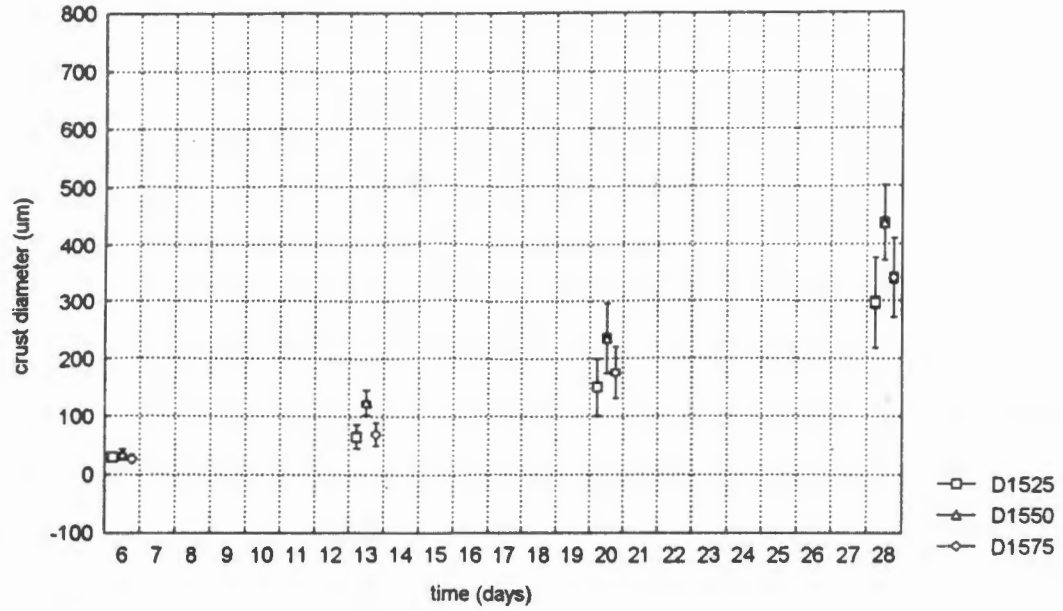




**Figure 11:** Diameters of carposporophytes (a) and tetrasporophytes (b) at 10, 50 and 130 $M.m^{-2}.s^{-1}$, showing means, standard errors and standard deviations. Experiment was conducted at 15°C



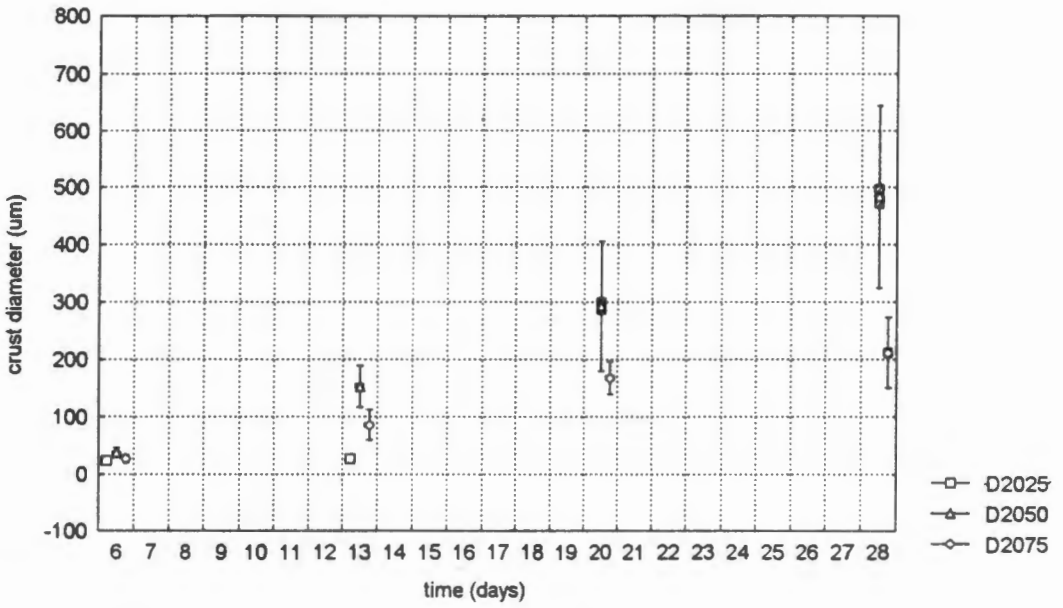




**Figure 12:** Diameters of tetrasporelings at 15°C (a) and 20°C (b) at light intensities of 25, 50 and 75 $M.m^{-2}.s^{-1}$. (D1525 = diameter at 15°C and 25 $\mu M.m^{-2}.s^{-2}$; and the same for the other curves)

20°C cold room and not in the 15°C room is not certain. A two-way ANOVA showed the effects of temperature, light and both factors combined to be significant at the 1 % level.

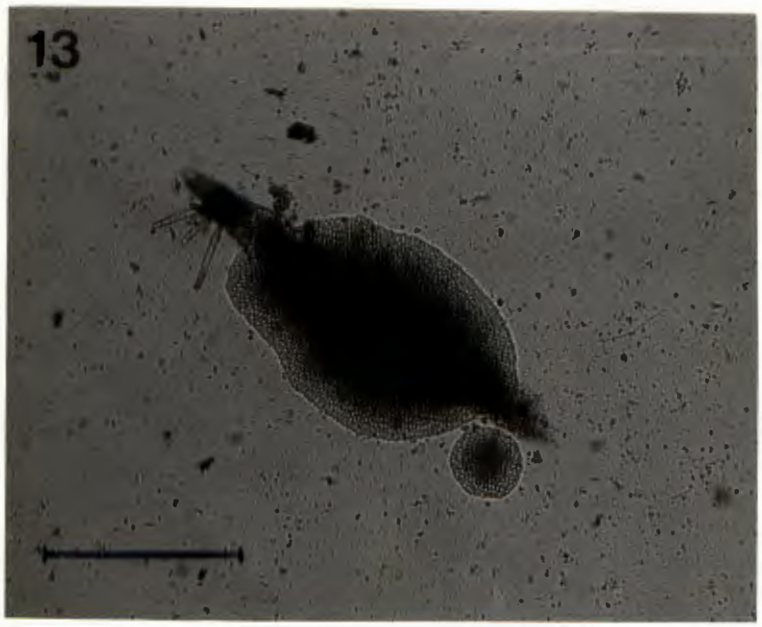
*Overall effects*

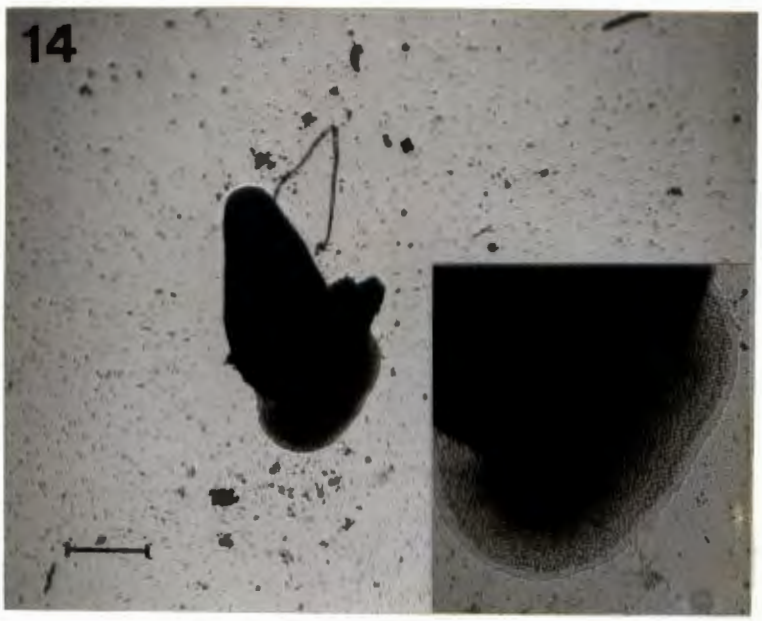
From both experiments, 50 $\mu M.m^{-2}.s^{-1}$ emerged as the irradiance under which growth is fastest. It was found (when comparing the means and standard deviations of the crust diameters after 28 days) that growth at 50 $\mu M.m^{-2}.s^{-1}$ was significantly slower in the second experiment than in the first experiment. The reasons for this are not clear. As a result, the data from both experiments could not be combined to deduce a light response curve with five different light intensities. Growth was faster at 20°C than at 15°C, and growth rates of carpospores (i.e. young tetrasporophytes) were slightly higher than those of tetraspores (i.e. young carposporophytes).

Regeneration from crust and thallus fragments

Of the crust fragments that were put into culture medium, only about 10-20 % re-attached. The re-attached fragments started to form new crusts which gave rise to 2-5 mm long upright thalli within 1-2 weeks (see Figs. 13 and 14). Attachment was, however, still weak after two weeks, and most of the re-established young plants were dislodged when unicellular epiphytes were gently removed from the culture with a soft paintbrush. In contrast, even after a few days, the crusts that grew from spores were extremely difficult to remove from the smooth glass coverslip, even when using a spatula. Only two out of 50-100 crust segments attached this firmly.

Thallus fragments did not regenerate, and most of them discoloured and died after two weeks.




**Figs. 13 and 14:** Regeneration of crust fragments.
13. After one week, a crust fragment has attached and a new crust has started forming.
14. After 2 weeks, an upright thallus has begun to form from the re-attached crust. Inset shows the regenerated crust at higher magnification.
Scale bars: 500 μm in both figures.

## Discussion

Shore phenology

The results of this study do not cover enough time and nothing certain can therefore be deduced about whether *G. filicina* is an annual. The fact that the ratio of gametophytes to tetrasporophytes has shifted in just over one month shows that there are seasonal differences in the composition of *G. filicina* populations. The fact that there were no carposporophtytes found in September (spring) resembles the situation in Japan, where only tetrasporophytes of *G. acuminata* were found in April 1990 and March 1991(lima et al 1995); in contrast, *G. filicina* in the British Isles has tetrasporophytes throughout the year (except during winter dormancy). Since *G. filicina* in South Africa is found on the shore throughout the year (Anderson and Bolton, pers. comm.), it seems more likely that due to seasonal differences in growing conditions, there are seasonal differences in growth and reproduction and hence in biomass and life history phase composition. A more intensive, long term study would be useful to clear up the evident confusion about the seasonality in this species. Knowledge about seasonal availability of *G. filicina* in the field is important for mariculture if parent plants are needed to obtain spores. As in other species, there may also be seasonal differences in carrageenan content, which should be explored for *G. filicina*. It is also of importance to ascertain what environmental or other factors cause seasonal differences in growth, as these will most likely affect any *G. filicina* crop on ropes in the sea; an understanding of the influence of these factors can also provide useful information for designing laboratory conditions.

Irvine (1983) describes that *G. filicina* var. *luxurians* regrows from its perennial attachment disc after the dormant winter. If *G. filicina* found in South Africa displayed this behaviour, it would benefit mariculture: it might be possible to harvest one crop, and instead of having to reseed the ropes with

spores, growth could start again from the persistent holdfast. Bolton and Joska (1993), however, point out that the crustose phase (in *Iridaea*) is often confusingly referred to as the perennating holdfast when the two are very different structures: the crustose phase can survive for months under sand and re-initiate growth under favourable conditions; the holdfast itself is not perennial. The details of this should be examined in *G. filicina*, as there are important implications for mariculture.

It was not clear from this study whether *G. filicina* is monoecious or dioecious. No male structures were found, and the following are possible reasons: *Grateloupia filicina* may be dioecious but the spermatangia are too inconspicuous to see on the gametophyte thalli; male gametophytes may be very rare in the population and did not emerge from the samples of around 80 and 60 individuals; or male gametophytes may not occur at the seasons when populations were sampled. It is difficult to draw a conclusion from this study, as so little of the year was covered in the sampling. If Womersley (1994) describes *G. filicina* as dioecious, it is presumably based on observations of both male and female structures on the same plant. In the present study, no plants were found that that were neither carposporophytes nor tetrasporophytes (and therefore possibly monoecious male plants). *In G. filicina* var. *filicina*, spermatangia are very small and are not found in sori. I therefore tentatively conclude that *G. filicina* is dioecious with inconspicuous male parts that are difficult to detect under the light microscope. A more careful and detailed study is need to resolve this question.

Spore release and development

Spore release was very easy to induce, which is a desirable characteristic for mariculture, as the risk of low / no spore release is low. The development of the sporelings was the same as described by lima et al. (1995); few problems were encountered in raising sporelings, the main one being fungal infections

at 20°C. Diatom growth could be controlled with $GeO_2$, and unicellular algae could be removed with a soft paintbrush, even after the first week following spore release. The growth of the young upright thalli seemed to slow down considerably after 6-8 weeks, with the exception of one fast-growing batch of carposporelings. Aeration culture may have been more suitable for the older sporelings

lima et al. (1995) report that it took 6 months to grow a harvest of *G. acuminata* from spores: 2-month old crusts were segmented and inoculated onto oyster shells and nori twine; the ropes and shells were kept in tanks for a further month, and plants were ready to harvest after 3 months in the sea. A similar time span for the development of adult thalli was observed by Anderson (pers. comm.): *G. filicina* thalli were tied to ropes on a culturing raft in Saldanha Bay on February 22, 1996; these decayed and disappeared, but young plants appeared on the ropes in early August, presumably from spores shed by the older thalli that were tied to the raft, as the nearest *G. filicina* populations are far away on rocky shores. By September 13, these little plants had grown into tufts with an average frond length of 8 cm (range: 5-13 cm). Growth seemed to slow down after this. Again, the development of an adult plant from spores took around half a year. The viability of growing *G. filicina* from spores over six months for carrageenan must be assessed, as work-intensive and time-consuming methods are often not repaid by the income from the carrageenan industry; seeding the ropes in the sea, as happened in Anderson's experiment, may be a way of circumventing laboratory work, but would be less reliable and possibly subject to seasonal differences in spore release and development. If the demand for *G. filicina* as food continues, laboratory methods and the long time span to maturity are not an obstacle because of the high prices obtained from this market.

Influence of light, temperature, life history phase and parent stock on growth

Optimal growth of crusts in this experiment occurred at 50 $\mu M.m^{-2}.s^{-1}$. Growth is more strongly reduced by low light levels than by higher ones in each experiment, and the optimum light level for growing *G. filicina* is probably between 50 and 70 $\mu M.m^{-2}.s^{-1}$. The light levels preferred by *G. filicina* are similar to the light levels at which growth is saturated in *Gelidium* spp. and *Devaleraea ramentaceum* in the eulittoral zone (references in Luning 1990). There is, however, considerable variation in growth saturating irradiance levels in eulittoral algae (from 28 $\mu M.m^{-2}.s^{-1}$ for *Codium fragile* to 300-350 $\mu M.m^{-2}.s^{-1}$ *for Fucus vesiculosus*;), and algae in the sublittoral zone show a similar range of growth saturating irradiance levels (references in Luning 1990). The light requirements of an alga reflect its ecological niche as well as its thallus anatomy and physiology, and it is therefore difficult to predict expected optimum light requirements simply from the habitat an alga occupies.

Increase in crust diameter was found to be fastest at 20°C. Iima et al (1995) reported that crusts of *G. acuminata* grew well at 15, 20 and 25°C with the best results at 20°C, but that that thallus growth was inhibited at 25°C. This suggests that optima for crust and thallus growth may differ, and since the thalli are the product of interest, ideal conditions for thallus growth for *G. filicina* should be investigated.

Carposporelings were found to grow slightly but significantly faster than tetrasporelings, although the biggest thallus produced was that of a tetrasporeling (i.e. a young carposporophyte). However, the carrageenan content of carposporophytes was found to be higher than that of tetrasporophytes (Levitt, unpubl. data). It is therefore necessary to investigate, over a longer experimental period, which life history phase produces more carrageenan during a culturing period.

The influence of the parent plant was found to have a significant influence on growth in most cases (Experiment 1). It is not recorded which variety *of G. filicina* is found on South African shores; from its size and ecological niche, stocks in Kommetjie would appear to belong to the smaller var. *filicina*. There is, however, considerable phenotypic variation in the standing stock in Kommetjie, and some fairly big, highly branched specimens can be found in the Bolus Herbarium. It would be useful to find out which variety is found in South Africa, or whether both are present (and what their habitats are). *G. filicina* var. *luxurians* sounds like a more suitable candidate for mariculture because of its greater size, and the suggestion that it regrows from its attachment disc. If algae are grown for the food industry, however, size *per se* may not be as important as texture, colour and flavour. It is therefore apparent that a better assessment of the natural stocks in South Africa and their characteristics and variation is needed, and that strain selection should play an important role in obtaining high quantity and quality *G. filicina*, depending on its proposed use.

Crust and thallus regeneration

Iima et al (1995) found that both crust and thallus regeneration worked well in propagating *G. acuminata* sporelings. Thallus fragments gave rise to filamentous regrowth, and where these filaments touched the substrate, they formed new circular crusts. The procedure for mariculture using propagation from crust and thallus fragments proposed by Iima et al. (1995) is illustrated in Figure 15. In the present study, crust re-attachment was found to be inefficient (only 10-20 % of crust fragments re-attached) and weak. It was, however, found that a few crusts were able to attach very firmly, and that thallus regrowth from crust fragments was rapid (even where attachment was weak). On the other hand, this procedure can reduce the total culturing time by about two months if stock cultures of crust fragments are maintained, and stock cultures of crusts, which could be maintained throughout the year by

lima et al. (1995), may be important if parent sources of spores are found to be seasonally unavailable. It is therefore worth attempting to refine the technique. It is particularly important to keep growing conditions as sterile as possible, as at the crust re-attachment stage, these presented the greatest problem. Ensuring firm attachment on ropes is also important, and ways of achieving this should be devised.

Thallus fragments did not regrow in this experiment, but it is worth experimenting with culturing methods to achieve this.

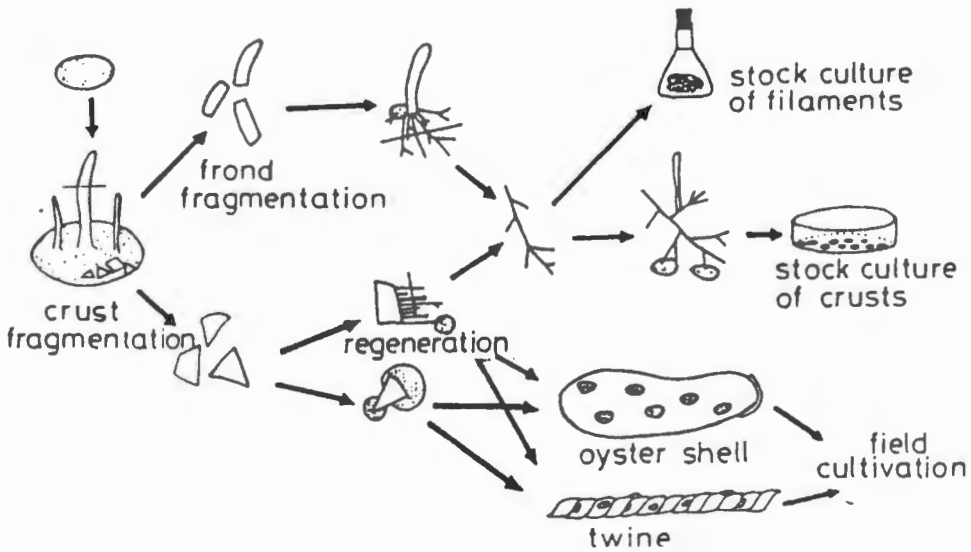



**Figure 15:** The procedure proposed by lima *et al.* (1995) for cutting and regenerating stock cultures of crusts in the laboratory. (From lima *et al.* 1995)

The alternative to using crust fragments is to release spores directly onto ropes and placing the ropes into the sea after a month or two in a tank. This method may take somewhat longer, but is less work intensive, and if it proves possible to grow adult plants at all seasons, crops can be staggered to achieve a regular harvest. If it turns out that the seasonal growth and biomass production of *Grateloupia* species cannot be overcome in the sea (here or in the Far East), there should be a great demand for South African *G. filicina* since the *Grateloupia* harvest from plants placed into the sea after laboratory culture peaked between December and March when the water temperatures were low, while growth was very slow between September and November (Iima et al. 1995), while *Grateloupia filicina* was found to be abundant in Kommetjie in September.

In conclusion, *G. filicina* appears to be a suitable candidate for mariculture involving growth from spores. The best technique, in terms of cost and productivity, still needs to be established: seeding ropes directly with spores, or maintaining stock cultures of crust fragments that can be inoculated into ropes are two options. It is also essential to obtain a better understanding of the phenology and seasonality of *G. filicina*, and which processes drive these. This understanding is vital if parent plants are to be collected in the field and later put into the sea on ropes, where they experience the same environmental influences as natural populations.

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