Phenology and Growth in Culture of the Carrageenophytes *Sarcothalia scutellata* and *Grateloupia filicina*

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Abstract

Sarcothalia scutellata (Hering) Leister and *Grateloupia filicina* (Lamouroux) C. Agardh are abundant in the intertidal zone along the west coast of South Africa. *S. scutellata*, a Southern African endemic, is a member of the Gigartinaceae, a family with a number of species from which carrageenan is extracted on a commercial basis. Carrageenan has also been extracted in other parts of the world from *G. filicina*, a species with a world wide warm temperate distribution. This species may also have potential for the food industry as it is used in Oriental cuisine.

The first aim of this study was to investigate various factors characteristic of these two species in the natural environment over the course of a year. This included carrageenan content, phase composition, frond fresh mass and percentage dry mass. As *S. scutellata* tended to grow in clumps of seaweed, the mass of the entire clump was measured in addition to individual frond mass. The number of fronds comprising each clump was also determined and each frond was recorded as fertile or infertile. Gametophytes of *S. scutellata* produced a dry mass carrageenan content of 33 to 58% whereas the tetrasporophytes only produced 14 to 24%. Both phases of *G. filicina* produced low quantities of carrageenan with gametophytes possessing 3 to 7% and tetrasporophytes 3 to 15%. Gametophytes were the dominant phase in *S. scutellata* (54 to 78% of population gametophytic) whereas *G. filicina* was highly dominated by tetrasporophytes throughout the year (60 to 94% tetrasporic). Fresh frond mass in both species was highly variable with the largest fronds occurring in summer for both species (mean of 4.43g for tetrasporic and 2.30g for cystocarpic fronds of *S. scutellata* and 9.84g for tetrasporic and 7.82g for carposporic fronds of *G. filicina*). Lowest mean frond masses occurred in winter for *S. scutellata* (1.35g for tetrasporic and 1.34g for cystocarpic fronds) whereas mean masses were not significantly different in autumn, winter and spring for *G. filicina* (means ranging from 4.66g to 6.48g for tetrasporic and 3.86g to 5.52g for carposporic fronds). Comparisons between the phases indicated that the tetrasporic fronds of *S. scutellata* were significantly larger than cystocarpic fronds in spring and summer. Tetrasporic fronds of *G. filicina* were significantly larger than carposporic fronds in winter. The clumps of *S. scutellata* were composed of mixed fertile and infertile fronds of varying numbers but clumps containing both fertile tetrasporic and cystocarpic fronds were not encountered. Clumps appeared to be of three types: female gametophytic, male gametophytic and tetrasporic. Dry mass percentages of *S. scutellata* showed little seasonal variation and averaged from 21 to 24%. *G. filicina* showed slightly higher dry mass percentages from 22 to 28% with lowest values occurring in spring.

The second aim of the study was to investigate seasonal fertility and fecundity within a natural population. Investigation of fertility involved determining the seasonal production of reproductive structures. Fecundity was determined by counting the number of spores released per reproductive structure in *S. scutellata* and per 4mm^2 fertile thallus section in *G. filicina*. The germination success rate of the released spores was then examined.

Both species were fertile throughout the year. *S. scutellata* produced greatest numbers of reproductive structures in summer (a mean of 44.8 tetrasporangial sori and 48.3 cystocarps per gram of fresh mass), which was also the period of greatest mean frond mass. Fewest reproductive structures were produced in winter (a mean of 30.3 tetrasporangial sori and 28.9 cystocarps per gram of fresh mass), the period of lowest mean frond mass. Tetrasporic fronds did not show significant differences in fecundity over the course of the year whereas cystocarpic fronds showed significantly greater fecundity in spring. The mean number of tetraspores released from tetrasporangial sori ranged from a mean of 4.4×10^4 per sorus in winter to 7.3×10^4 per sorus in spring. Cystocarps released significantly more carpospores in spring (mean of 1.1×10^5 carpospores) than in autumn (mean of 3.8×10^4 carpospores). Most cystocarps used in the summer experiment failed to release spores resulting in a mean of 6.0×10^3 spores. Differences in fertility did not appear to contribute to gametophyte dominance, as there were no significant differences in the number of reproductive structures produced between the two phases. In terms of fecundity, enhanced production of tetraspores would contribute to gametophyte dominance. In the case of *S. scutellata*, more tetraspores may have been released in summer when many cystocarps failed to release spores, but fewer tetraspores than carpospores were released per reproductive structure in spring (mean of 7.3×10^4 tetraspores released per tetrasporangial sorus compared to mean of 1.1×10^4 carpospores released per cystocarp). However, larger frond sizes in tetrasporic fronds compared to cystocarpic fronds in spring and summer may compensate for decreased numbers of tetraspores released per sorus. An approximation of the mean number of spores released per entire frond each season was obtained using mean frond mass, mean number of reproductive structures produced per gram of fresh mass and mean number of spores released per sorus. This reveals that tetrasporic fronds released more spores in summer than cystocarpic fronds (1.4×10^7 compared to 8.8×10^6 spores released per frond) but similar number of spores were released during the rest of the year (in the region of 1 to 8 million spores per frond).

Tetrasporic fronds of *G. filicina* exhibited greatest fertility in spring whereas carposporic fronds showed no significant differences in fertility. The tetrasporic fronds produced most tetrasporangia (note that tetrasporangia are not grouped in sori) in spring (mean of 1.6×10^6 tetrasporangia per gram of fresh mass) and fewest in winter (mean of 2.0×10^5 tetrasporangia per gram of fresh mass). Carposporic fronds ranged from a mean of 42% of thallus that was fertile in autumn to a mean of 75% in spring. The tetrasporic fronds did not show significant differences in fecundity whereas that of the carposporic fronds was significantly increased in spring. The maximum mean for numbers of tetraspores released occurred in spring (5.2×10^3 per $4mm^2$ sections of fertile thallus). Significantly more carpospores were released in spring (means of 1.2×10^4 per $4mm^2$ sections of fertile thallus in October 1997and 1.1×10^4 in September 1998) than in summer (mean of 3.3×10^3 in December 1997) and carposporophytes failed to release spores in autumn. Comparisons between the two phases indicated that greater numbers of carpospores than tetraspores were released per $4mm^2$ sections of fertile thallus. This might contribute to tetrasporophyte dominance. However, overall frond size needs to be considered. Carposporic fronds tended to be smaller than tetrasporic fronds but only significantly so in June 1998. A rough estimation of mean numbers of spores released per entire frond was obtained using the mean frond mass, the number of spores released per $4mm^2$ section and the mass of the $4mm^2$ sections. This indicated that the carposporic fronds released more spores each season (except autumn when fertile carposporic sections failed to release spores) than the tetrasporic fronds (1.2×10^7 to 2.9×10^7 carpospores compared to 5.5×10^6 to 1.8×10^7 tetraspores).

Both species had high germination success rates with spores that settled in clumped positions showing enhanced germination (88 to 94% for both tetraspores and carpospores of *S. scutellata* and 54 to 83% for tetraspores and 44 to 90% for carpospores of *G. filicina*) compared to isolated spores (60 to 70% for tetraspores and 36 to 86% for carpospores of *S. scutellata* and 25 to 70% for tetraspores and 19 to 57% for carpospores of *G. filicina*). As carpospores of *G. filicina* show a more clumped settling pattern than tetraspores, this enhances germination of the diploid phase. This might be a further factor contributing to the tetrasporophyte dominance found in this species. Germination of both carpospores and tetraspores of *G. filicina* was also enhanced at 15°C compared to 20°C.

The third main aim was to determine the optimum conditions for growth from spores in terms of temperature and irradiance. Maximum sporeling crust diameters of both species occurred at 18°C (to 21°C for *S. scutellata*) and sporelings did not survive at 24°C. Light saturation occurred around 30 to 60 μ mol photons $m^{-2} s^{-1}$.

Chapter 1

General Introduction

This study is concerned with two rhodophytic seaweeds, *Sarcothalia scutellata* (Hering) Leister and *Grateloupia filicina* (Lamouroux) C. Agardh, that occur in South Africa and belong to a group classified as carrageenophytic seaweeds.

Most members of the Rhodophyta (red seaweeds) possess sulfated cell wall polysaccharides known as phycocolloids. These are structural polymers that provide flexible support suitable in a marine environment. The two main groups of these phycocolloids have been termed agars and carrageenans. They consist of polymers of galactose (galactans) of which the hydroxyl groups may be sulfated, methylated or glycosylated (Craigie 1990). The basic difference between the two groups is in stereochemical properties with agar possessing the L-form of 4-linked α-galactose residues and carrageenan the D-form (Anderson *et al.* 1965; Craigie 1990; Knutsen *et al.* 1994).

Several different types of carrageenan have been characterized which differ in chemical properties such as the degree and pattern of sulfation (Craigie 1990). The most common have been classified as κ-, λ-, and ι-type carrageenans. Many others have also been characterized and are denoted by Greek symbols. This classification is felt to be somewhat arbitrary and attempts have been made at a more systematic method (Knutsen *et al.* 1994). Fourier transform infrared diffuse reflectance (FT-IR) spectrometry has been used to identify 15 carrageenan-types (Chopin *et al.* 1999).

Carrageenan is extracted from certain seaweeds in order to be used in a variety of commercial applications. The major uses are as stabilizers or gelling agents in milk-based products, processed meat products, pharmaceuticals and cosmetics (Abbott 1996; Bixler 1996). Certain carrageenans have also been found to be suitable as bacteriological media (Abbott 1996).

The type of carrageenan that is extracted in the largest quantities is κ-carrageenan (Bixler 1996). This is principally extracted from cultivated species of *Kappaphycus cottonii* (Weber-van Bosse) Doty ex P. Silva (previously *Eucheuma cottonii*) occurring in the Philippines and Indonesia (McHugh 1991). Iota-type is largely from cultivated *Eucheuma denticulatum* (Burman) Collins *et* Harvey (previously *Eucheuma spinosum*) in Indonesia (McHugh 1991). Other areas that cultivate species of *Eucheuma* include Fiji, China, (McHugh 1991) and Tanzania (Bixler 1996). Cultivation of these species has allowed considerable expansion of the carrageenan industry (McHugh 1991).

The tropical species are good sources of κ- and ι-carrageenan but not of λ-carrageenan. This type of carrageenan is largely from seaweeds that occur in temperate waters. It is mainly obtained from species within the Gigartinaceae, such as *Chondrus crispus* Stackhouse from France and Canada and species of *Mazzaella, Sarcothalia, Gigartina* and *Mastocarpus* from South America (Oliveira de 1989; Santelices 1989; McHugh 1991; Santelices 1996). Most of the colder water carrageenophytes, however, are harvested from natural populations. None of these have been commercially maricultured although experimental cultivation has begun in certain areas, for example, *Sarcothalia crispata* (Bory) Leister in Chile (Santelices 1996). Carrageenan from natural populations provides a more unpredictable supply, is often insufficient and has potentially adverse effects on the environment due to over-harvesting. Resulting shortages lead to higher prices. Thus interest has been expressed in new supplies of these carrageenans. Multinational corporations involved in the processing of carrageenan have expressed interest in obtaining material from South Africa (J.J. Bolton and R.J. Anderson, pers. comm.).

There is no carrageenan industry at present in South Africa. Between 1956 and 1978 sporadic harvesting of *Gigartina polycarpa* (Kützing) Setchell *et* Gardner (formerly *Gigartina radula* (Esper) J. Agardh – Hommersand 1993) and *Sarcothalia stiriata* (Turner) Leister (formerly *Gigartina stiriata* (Turner) J. Agardh – Hommersand *et al.* 1993) occurred and was exported as dried raw material (Anderson *et al.* 1989). Other species that are mentioned as having commercial potential include *Hypnea spicifera* (Suhr) Harvey (Anderson *et al.* 1989), *Aeodes orbitosa* (Suhr) Schmitz and *Mazzaella capensis* (J. Agardh) Fredericq (formerly *Iridaea capensis* J. Agardh – Hommersand *et al.* 1993) (Bolton and Levitt 1992).

Previous research on carrageenophytic seaweeds in South Africa includes an assessment of carrageenan content and biomass of species occurring on the west coast that found *Gigartina polycarpa*, *Sarcothalia stiriata*, *Aeodes orbitosa* and *Mazzaella capensis* to be the most abundant potential carrageenophytes (Bolton and Levitt 1992). The carrageenophyte *Gigartina pistillata* (Gmelin) Stackhouse, which occurs along the south to south-east coast of South Africa, was investigated by Gierdien (1992) who researched carrageenan content, relative abundance of life history phases, reproductive phenology, effects of sand burial on growth and floridean starch content, and temperature tolerances in culture. A study was performed on an intertidal population of *Mazzaella capensis* that investigated biomass, plant density, individual plant size, ratio of gametophytes to tetrasporophytes and carrageenan content (Bolton and Joska 1993). Investigation of the harvestable biomass of *Gigartina polycarpa*, *Sarcothalia stiriata*, *Aeodes orbitosa* and *Mazzaella capensis* found the former three to occur in sufficient quantities to be harvested (Levitt *et al.* 1995). The carrageenan content of *Gigartina polycarpa* and *Sarcothalia stiriata* was also investigated. A study by Levitt (1998) included observations on natural populations of *Gigartina polycarpa* and *Sarcothalia stiriata* such as ratios of life history phases, thallus and reproductive weight, plant density and fecundity. Other factors related to these two species explored in the latter study included: the effects of wave exposure on transplanted gametophytes and tetrasporophytes; the effects of harvesting; and light and temperature tolerances in culture.

The two carrageenophytic species investigated in this study, *Sarcothalia scutellata* and *Grateloupia filicina* were chosen because they are common enough, particularly in rock pools along the west coast, to provide seed stock for mariculture, but are not abundant enough to be harvested commercially (Bolton and Levitt 1992). *Sarcothalia scutellata*, a southern African endemic, has been little studied and *Grateloupia filicina*, a species that occurs worldwide, has received little attention in southern Africa.

Grateloupia filicina is also of interest as it may have potential for use in the food industry as the species is used in oriental cuisine. Higher prices are obtained for seaweed that is used as food compared to those used for colloid extraction. Edible seaweeds are mostly (94%) obtained by cultivation in eastern countries with the majority being cultivated and consumed in China, Japan and Korea (McHugh 1991). Seaweed is also used as a food source in Hawaii where it is collectively known as 'limu' and includes *Grateloupia filicina* which is also called 'limu hulu hulu waena' (Chapman and Chapman 1980; Zablackis and Perez 1990). Japanese *Grateloupia filicina*, called 'Mukade-nori', is prepared in various ways for human consumption (Chapman and Chapman 1980).

Sarcothalia scutellata, a member of the Gigartinaceae, was previously named *Gigartina scutellata* Hering. Transferal to the genus *Sarcothalia* was based on cystocarpic and tetrasporangial development and morphology (Hommersand *et al.* 1993). A subsequent study using sequence analysis of *rbc*L, the gene that codes for the large subunit of RuBisCO, questioned its inclusion in the genus *Sarcothalia*, as there was some separation from the true *Sarcothalia* clade (Hommersand *et al.* 1994). The species exhibits alternation of isomorphic gametophytes and tetrasporophytes. Few to many upright fronds that consist of terete, dichotomously branching axes arise from a discoid holdfast (Stegenga *et al.* 1997). Mature tetrasporic and cystocarpic fronds are easily differentiated by the naked eye due to the presence of dark brown soral patches on the tetrasporophytes and rounded papillae on the female gametophytes (Fig. 1.1). It is suspected that this species is dioecious but spermatangia were not positively identified.

The species is endemic to the west coast of southern Africa where it occurs from Namibia to Cape Hangklip (Stegenga *et al.* 1997). It is largely found in the lower intertidal to shallow subtidal although it may sometimes be found in high-intertidal rock pools.

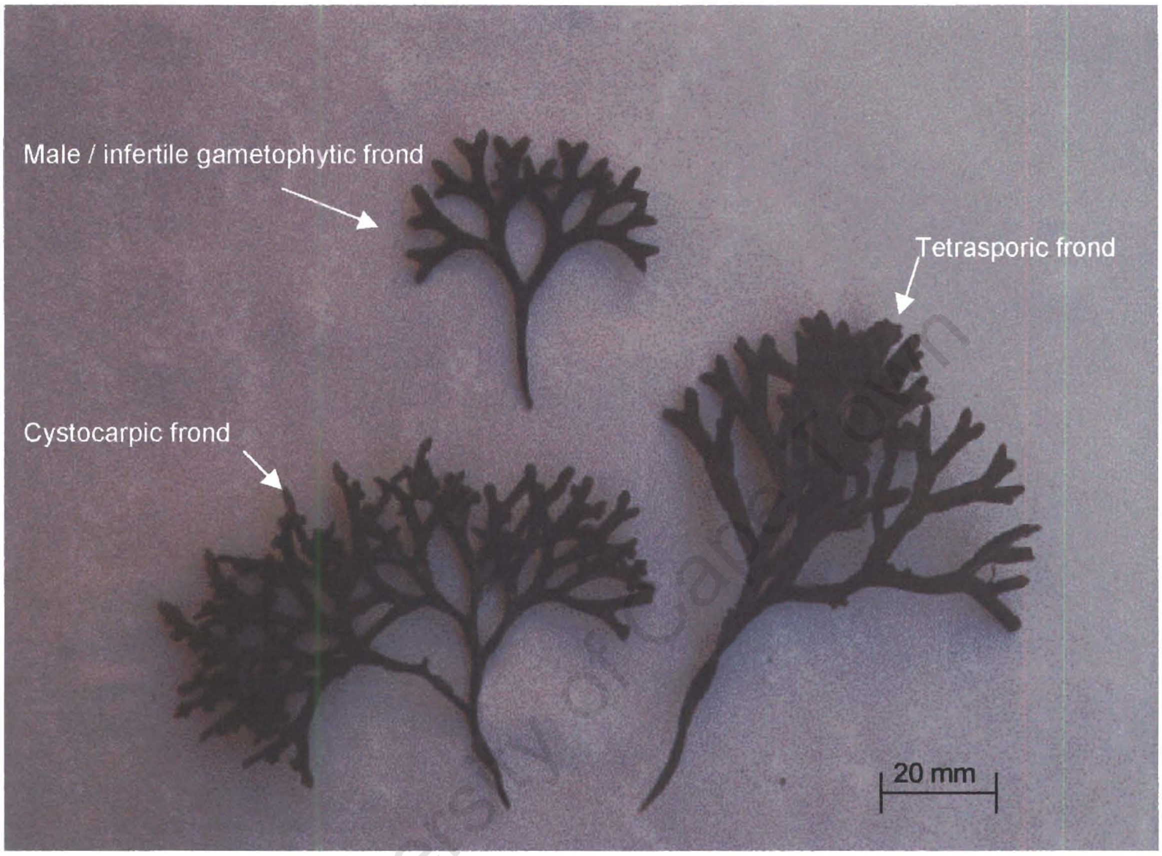

Fig. 1.1: Cystocarpic, tetrasporic, and male or infertile gametophytic fronds of *Sarcothalia scutellata*.

Grateloupia filicina is a member of the family Halymeniaceae and the order Cryptonemiales. Recognition of this order is not universal and it is often merged with the Gigartinales (Garbary and Gabrielson 1990). The species has an isomorphic alternation of generations. One to many compressed, pinnately branched fronds arise from discoid holdfasts. Tetrasporic and carposporic fronds are indistinguishable without the aid of a microscope (Fig. 1.2). It is uncertain whether the South African populations are monoecious or dioecious as no spermatangia were identified in the fronds that were collected.

The species is described as occurring worldwide in warm temperate seas (Stegenga *et al.* 1997). In southern Africa it extends along the Cape province west coast to the Kowie area near Port Alfred on the South coast. It occurs in intertidal rock pools as high as the supralittoral. Subtidal specimens have also been collected (F. Leliaert, pers.comm.).

Fig. 1.2: Tetrasporic and gametophytic fronds of the seaweed *Grateloupia filicina* that are not easily distinguishable without the aid of a microscope.

Two varieties are described for the British isles (Irvine 1983). *Grateloupia filicina* var. *filicina* occurs in intertidal pools as well as being sublittoral up to 10m. High-intertidal pools possessed some plants in summer and late autumn. *Grateloupia filicina* var. *luxurians* occurs in lagoons, harbours, estuaries and sublittorally to 6 m. The species occurring in southern Australia has been described as *Grateloupia filicina* var. *luxurians* and can be abundant in shallow water or just above the low tide level in harbours and sheltered coasts (Womersley 1994). Three varieties are also mentioned in Silva *et al.* (1996) in the Indian ocean, namely *G. filicina* var. *filicina* in India and Sri Lanka, *G. filicina* var. *elongata* in Java and *G. filicina* var. *luxurians* in Australia. Such a wide distribution in both tropical and temperate waters appears unusual as species are usually confined to biogeographical regions where water temperature is the main factor governing seaweed distribution (Lüning 1990).

The differences in the two varieties described by Irvine (1983) include greater length, breadth and thickness in the variety *luxurians* compared to *filicina*. The former is described as being up to 700mm long by 10mm wide and 1 to 3 mm thick whereas the latter is up to 40 (120) mm long by 2 to 5mm wide by 150 to 200 µm thick. The cortex of the *luxurians* variety is described as 10 to 14 cells thick with outermost cells radially elongated, whereas the cortex of the *filicina* variety is only 3 to 8 cells thick. The variety *Luxurians* is described as possessing a mucilaginous layer up to 40 µm thick. Cystocarps of the variety *luxurians* are larger, over 300 µm in diameter, whereas those of *filicina* are less than 210 µm. Both species are described as occurring in South Africa. The population sampled from the west coast rock pools did not neatly fit the description of either variety. Length was intermediate as many fronds were about 150mm long. They were mostly 2mm wide, buy there were also 10mm wide fronds. Frond thickness was about 300 to 800 µm. The cortex was mostly about 8 cells thick but could be up to 10 cells in the main axis and was often only 4 cells thick in the pinnate branches. The outermost cortical cells were radially elongated, but no mucilaginous layer was apparent. The cystocarps were about 300 µm in diameter.

A number of taxonomic questions are thus raised with regard to this species such as which anatomical features are reliable indicators of the variety or even the species involved. The extent to which anatomical features vary in response to the environment possibly also needs to be ascertained. The wide geographical distribution in both temperate and tropical waters suggests the involvement of more than one species. Molecular systematics could be performed on the species from these different geographical locations to determine what taxonomic separation exists.

The first main objective of this study was to gain a greater understanding of these two species in the natural environment as a preliminary to any attempts at mariculture. This included determining the amount of carrageenan possessed by each species and by each phase and whether this varied with season. As different phases may contain different types of carrageenan the ratio of tetrasporophytes to gametophytes within the populations was examined to see if there were any imbalances and whether this changed over time. Frond size was also examined to determine if there was a season of optimal growth and to compare sizes between phases. In addition, the composition of clumps of *Sarcothalia scutellata* was examined in terms of numbers of fertile and infertile fronds making up each clump.

The second main objective was to investigate seasonal variation in frond fertility and fecundity. Estimation of fertility involved determining the number of reproductive structures (tetrasporangial sori or cystocarps) that were formed by *Sarcothalia scutellata*, and the number of tetrasporangia that were produced or the percentage of the thallus that contained carposporophytes in *Grateloupia filicina*. Fecundity has been defined as the number of eggs, or seeds, or generally offspring produced by an individual (Begon *et al.* 1990). Fecundity was thus determined by the number of spores that were released per reproductive structure for *Sarcothalia scutellata* and per section of fertile thallus for *Grateloupia filicina*. The viability of the released spores was also examined to see if this varied with phase or season. The influence of temperature on spore germination was also investigated for spores of *Grateloupia filicina* which were germinated under two different temperature regimes, 15°C and 20°C .

The third main objective was to examine the growth of the species in culture. Attempts at mariculturing *Grateloupia filicina* from vegetative thalli have been unsuccessful due to biofouling whereas plants that germinated from spores were noted to be free of epibionts (Bula-Meyer 1989). A preliminary attempt in this project at vegetative laboratory cultivation of *Sarcothalia scutellata* was unsuccessful as the pieces soon disintegrated. However, there has been some success in cultivation from spores of *Grateloupia filicina* (Ishikawa 1984 & Migita 1988 cited in Iima *et al.* 1995). These two species were thus grown from spores to determine the optimal conditions for growth in terms of temperature and light. This would provide guidelines for suitable conditions for culturing the species. It would also determine whether the different phases have differing tolerances which may account for differences in abundance. Finally, this would provide insight into how well the temperature optima correspond to the seawater temperatures in the regions in which the species grow.

Chapter 2

Seasonal variation in carrageenan content, phase composition, frond mass and number of fronds within clumps of *Sarcothalia scutellata* (Hering) Leister (Gigartinaceae, Rhodophyta) on the west coast of South Africa

Introduction

Sarcothalia scutellata (Hering) Leister belongs to the Gigartinales, an order in which carrageenophytes have been most widely studied as these species tend to have high yields of phycocolloids as well as high biomass in nature (Craigie 1990). Many studies found seasonal variation in the quantity and quality of carrageenan but since phases were usually collected simultaneously this was often a result of seasonal variation in the phase composition (Piriz and Cerezo 1991) as the tetrasporophyte (diploid) and gametophyte (haploid) life-history stages produce different carrageenans in the Gigartinaceae. The type of carrageenan extracted from tetrasporophytes is largely λ-carrageenan and from gametophytes κ-carrageenan (Pickmere *et al.* 1973; Waaland 1975). *Sarcothalia scutellata* conformed to this pattern as FT – IR spectra characterized the gametophytic carrageenan as κ/ι/μ and the sporophytic as λ/θ/α (Chopin *et al.* 1999). This difference in carrageenan between the two phases has been frequently utilized to differentiate them in the absence of easily discernible fertile structures as the two types stain differently when subjected to the acetal-resorcinol reaction (Garbary and DeWreede 1988).

The carrageenophytic species in South Africa that have been most researched are *Gigartina polycarpa* (Kützing) Setchell *et* Gardner, *Sarcothalia stiriata* (Turner) Leister, *Aeodes orbitosa* (Suhr) Schmitz and *Mazzaella capensis* (J. Agardh) Fredericq as they are the most abundant. Carrageenan yields from these species are in the region of 39 to 41% for tetrasporophytes and 44 to 49% for gametophytes of *Gigartina polycarpa* and *Sarcothalia stiriata* (Levitt 1998) and 30 to 42% for *Mazzaella capensis* (Bolton and Joska 1993). This compares favourably with the economically important seaweed *Chondrus crispus* Stackhouse that showed a seasonal variation of 31.4 to 69.8% (Chopin *et al.* 1999). The polysaccharide from *Aeodes orbitosa* is unusual and has been termed "aeodan" (Bolton and Levitt 1992).

The present work examines the carrageenan yield from tetrasporic and cystocarpic fronds of *Sarcothalia scutellata* over the course of a year. The relative proportions of the life-history phases was examined to see if either phase dominated, and whether this showed any seasonal variation.

Sarcothalia scutellata exhibits an isomorphic alternation of generations, as is common for species within the Gigartinaceae. The functional-form model of Littler and Littler (1980) states that macro-morphological attributes are related to functional responses to the environment. This implies that the similar macro-morphologies of the of the gametophyte and tetrasporophyte phases of isomorphic species undergo similar environmental selective forces. However, it appears that the isomorphic generations within the Gigartinaceae differ in some respect in their response to the environment as the majority of studies found populations of species within this family to be gametophyte dominated (Bhattacharya 1985; Braga 1990; Scrosati *et al.* 1994; Levitt *et al.* 1995; Piriz 1996; Westermeier and Sigel 1997; Scrosati 1998a). Equal representation of the two phases has also been reported (Laura Lazo *et al.* 1989). A seasonal changeover in life-history phase dominance has been recorded for *Mazzaella splendens* (Setchell *et* Gardner) Fredericq (formerly *Iridaea splendens* (Setchell *et* Gardner) Papenfuss – Hommersand *et al.* 1993) with gametophyte dominance in summer and tetrasporophyte dominance in winter (Ang *et al.* 1990; Dyck and DeWreede 1995). This changeover in dominance was also recorded for *Mazzaella capensis* but did not appear to be directly related to season (Bolton and Joska 1993).

However, the assumption that the stable ratio between gametophytes and tetrasporophytes would be one if reproductive phases are ecologically similar is not necessarily valid as indicated by demographic models performed by Scrosati and DeWreede (1999). Models that defined ecological similarity as the same number of spores produced per thallus for both phases (four spores per tetrasporophyte and eight spores per male-female gametophyte pair) found that annual thalli that did not have an initial G:T (gametophyte to tetrasporophyte) ratio of one exhibited a continual alternation in phase dominance. The G:T ratio of triennial and perennial thalli, on the other hand, did stabilize at one regardless of the initial ratio. Scrosati and DeWreede (1999) then investigated another possible scenario for spore production, where the lowest possible spore output per phase was used, namely four spores per tetrasporophyte and one spore per male and female gametophyte pair. Annual thalli now showed alternation of dominance even if the initial ratio was one. The G:T ratio for both triennial and perennial thalli this time stabilized at 2.8 regardless of initial ratio. Thus ecologically similar species may not necessarily have G:T ratios of one.

Thallus size is another factor that may show seasonal variation. Carrageenophytic seaweeds from the west coast appear to attain their largest size during summer months and smallest size during winter months. Average plant fresh weight was greatest at 10g in February and lowest at 2g in June for *Mazzaella capensis* (Bolton and Joska 1993). *Gigartina polycarpa* and *Sarcothalia stiriata* were reported to show increased thallus weight in summer and decreased thallus weight in winter (Levitt 1998).

Thallus weight of *Sarcothalia scutellata* was examined to determine whether there was a season in which optimal growth occurred. A problem was encountered in determining the individual unit to be measured. The seaweed is comprised of more or less discrete clumps consisting of a prostrate circular crust from which few to many upright dichotomously branching fronds or ramets arise. A similar phenomenon occurs with the species *Chondrus crispus* in which the genet has been described as consisting of a perennating disc-like holdfast from which arise numerous fronds in varying stages of ontogenetic development (Bhattacharya 1985). Individual fronds are commonly referred to as ramets, which are modules with the potential for separate existence, as opposed to the genet which is the product of the zygote (Begon *et al.* 1990). However, identification of the genet is further confused within the red algae as sporeling coalescence has been described for several species including *Chondrus crispus*, *Mastocarpus stellatus* (Stackhouse) Guiry (previously *Gigartina stellata* (Stackhouse) Batters) (Tveter and Mathieson 1976; Tveter-Gallagher and Mathieson 1980) and *Gracilaria gracilis* (Stackhouse) Steentoft (formerly *Gracilaria verrucosa* (Hudson) Papenfuss) (Jones 1957). Coalesced sporelings of *Chondrus crispus* had enhanced growth of upright fronds compared to non-coalesced sporelings (Tveter and Mathieson 1976). The coalesced crusts were characterized by vertical and horizontal alignment of cells, cuticular continuity and secondary pit connections (Maggs and Cheney 1990). Gametophyte coalescence was noted to occur to a lesser degree than that for tetrasporophytes and coalescence that occurred early in sporeling development had less distinct boundaries than that of later coalescence (Tveter-Gallagher and Mathieson 1980). Carpospores, being the product of mitosis would be genetically identical if originating from the same cystocarp and may be the reason for enhanced coalescence observed in the diploid phase. However, carpospores from different cystocarps on the same plant may not be genetically identical as each carpogonium has the potential to be fertilized by a different spermatium. Coalescence between genetically different individuals, such as would happen between crusts originating from meiotically derived tetraspores and from carpospores from different cystocarps, complicates the concepts of genotype and individual.

The composition of the seaweed clumps was examined to determine whether the clumps consisted of mixed phases or not; the total number of fronds within clumps and whether this varies with season; the proportion of the fronds that are fertile and at what size fronds of the different phases become fertile.

A relationship between frond number and frond mass within clumps was also investigated. Frond crowding has a cost in that self-shading may reduce photosynthetic rates. However, experiments on *Mazzaella cornucopiae* (Postels *et* Ruprecht) Hommersand found it to have important adaptive significance as it protected the fronds from desiccation, bleaching and tissue loss (Scrosati and DeWreede 1998). The latter species occurs in the high-intertidal and so these factors would be significant stresses. *Sarcothalia scutellata* tends to occur in rock pools of the mid- to low-intertidal and would thus be less exposed to such factors. Large fronds, however, have a more bushy appearance due to much dichotomous branching, which might confer the same benefits as frond crowding. Large fronds could be predicted to survive in small clumps whereas fronds of small mass would need to occur in large clumps. Thus a negative correlation between frond mass and the number of fronds within a clump would be predicted

Method

Sarcothalia scutellata was collected from rock pools of the mid- to low-intertidal at Olifantsbos (lat. 34° 16' S, long. 18° 23' E) on the Atlantic coast of the Cape Peninsula. Collections were made in April, July, August, October and December 1997 and March, June and September 1998. Ten clumps of seaweed were removed from each of five rock pools. To sample the pools objectively the knife used to remove the clumps was first suspended above the rock pool and, whilst eyes were closed, positioned vertically on the rock surface amongst the seaweed and the clump that was closest to the knife was removed. Subsequent collections were never from the same rock pools, unless they were very large, to avoid effects resulting from removal of the seaweed. Thus fifty clumps consisting of varying numbers of both fertile and infertile fronds or ramets were collected on each occasion. Each clump was placed in a separate plastic bag and transported to the laboratory in an insulated container.

Carrageenan content

Carrageenan content was measured in October and December 1997 and March, June and September 1998. The method used to extract carrageenan was based on that used by Santos and Doty (1975) with modifications similar to Levitt *et al.* (1995). Fronds were washed in filtered seawater and oven dried to constant weight at 60°C. After all observations pertaining to phase composition and mass had been performed, as described below, all fronds bearing cystocarps were then ground together as were all fronds bearing tetrasporangial sori using a Wiley mill with a 20 mesh screen. 4g of this ground material was then used for the extraction procedure. It was not feasible to use individual fronds as the individual dry frond masses rarely exceeded the 4g required in the extraction procedure and there was also loss of material during grinding, thus some information was lost. Carrageenan was also extracted form those clumps that did not possess fertile structures in December 1997 and September 1998. The crushed dried seaweed (4g) was placed in distilled water (200ml) adjusted to pH 9 using 2% NaOH and placed in a waterbath at 85°C for 12 hours. The mixture was centrifuged at 10 000 G for 40 minutes and inverted over flasks to allow the supernatant to drain off. A 10% NaCl solution (20ml) was added and the mixture poured into twice its volume of 85% isopropanol. The contents were stirred, and the precipitate containing the carrageenan removed by straining the contents of the flask through a muslin cloth. The precipitate was washed twice in 200ml of 85% isopropanol and oven dried to constant weight at 60°C.

Phase Composition

Phase composition was determined in April, July, August, October and December 1997 and March, June and September 1998. Clumps of *Sarcothalia scutellata* were divided by eye into three types: tetrasporic, cystocarpic and those in which no obvious fertile structures could be observed. A clump was denoted as tetrasporic or cystocarpic if it had any fertile fronds bearing the relevant fertile structures. Each of these clumps consisted of fertile and infertile fronds but a mixture of fertile cystocarpic and tetrasporic fronds was not encountered. An attempt was made to search for male spermatangia. Fronds were taken from those clumps lacking obvious fertile structures, particularly those fronds which were of comparable size to the fertile cystocarpic and tetrasporic fronds. Transverse sections were cut through the thallus in the region where the other fertile structures were found, namely toward the distal ends of the frond. A freezing microtome was used to cut the sections, which were then viewed under the light microscope with and without staining with fast green. The fast green was used to stain cortical cells possessing chloroplasts green whereas the spermatangia, which lack chloroplasts, should remain clear. The sections were immersed in the stain for one minute and then rinsed in filtered seawater to remove excess stain.

The Resorcinol test, which separates tissue containing κ-carrageenan from those containing λ carrageenan (Garbary and DeWreede 1988), was then performed in an attempt to ascertain the phase of the 'infertile' group of fronds. A dry mass of 0.1g was placed in the reagents, in a water bath at 85°C for 60 seconds and then placed into a container of ice. A variety of modifications to the test were attempted to improve its reliability (as suggested by G.J. Levitt, pers. comm.). This included using firstly, 0.5g of fresh material, then 0.05g of dry material and lastly 0.5g of dry material. The test was also repeated using 0.1g of dry material but placing the test tubes in the water bath for 30 seconds only and then by omitting the acetal. To determine how known phases of this species react with the reagents controls were formed that consisted of thallus pieces removed from fronds of a known phase.

Clump composition (mass and number of fronds) and dry to wet mass ratios

Frond masses and number of fronds per clump were determined every second or third month from October 1997 to September 1998. Surface organisms were scraped off and the fresh mass of entire clumps and individual fertile and infertile fronds determined. The number of each type of frond (cystocarpic, tetrasporic and 'infertile') was noted. The fronds were then oven dried to constant weight at 60°C. The ratio of wet to dry mass was then determined.

To determine a relationship between mass and density of fronds within clumps a problem arises similar to that in attempting to determine whether self-thinning occurs. Plotting mean frond mass against density results in a spurious relationship as mean frond mass is calculated using the number of fronds within the clump which is also the second variable to be used in the correlation (Weller 1987; Scrosati 1996). To avoid this problem, each individual frond mass was plotted against the number of fronds in the clump from which it originated. A non-parametric test was used to test for correlation as the data were not normally distributed. The Gamma statistic was used as this test is preferable when the data contains many tied observations (Statistica manual) as occurred in this data set.

Statistics

Kruskal-Wallis ANOVA by ranks was used to determine significant differences using the statistical package "STATISTICA" version 5.1 (StatSoft, Inc. 1996).

Results

Carrageenan content

Cystocarpic fronds of *Sarcothalia scutellata* had much higher carrageenan contents than the tetrasporic fronds throughout the study period. The tetrasporic fronds had 14 to 24% of the dry mass composed of carrageenan whereas the cystocarpic fronds had 33 to 58% (Fig. 2.1). Carrageenan was extracted from 'infertile' clumps in December 1997 and September 1998. The quantity extracted was virtually the same as that extracted from the cystocarpic clumps on the respective months. The carrageenan also had a similar texture to that of the cystocarpic clumps (jelly-like as opposed to stringy in the tetrasporophytes). The highest carrageenan content in the tetrasporophytes occurred in October 1997 and June 1998. The cystocarpic fronds exhibited highest carrageenan content in June 1998.

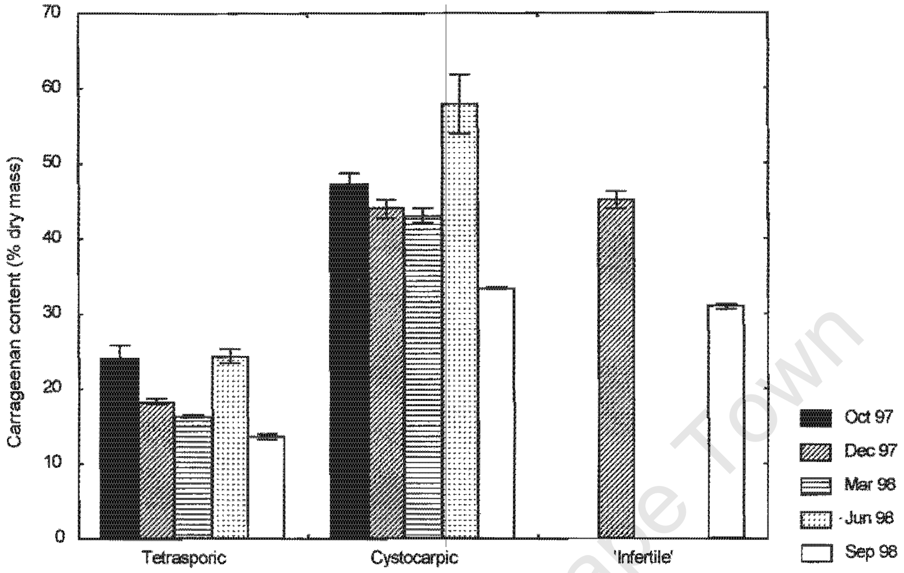

Fig. 2.1: Carrageenan content of tetrasporic, cystocarpic and 'infertile' clumps of *Sarcothalia scutellata*. Mean ± SE; n = 6.

Phase Composition

The transverse sections of fronds from infertile clumps did not reveal any spermatangia. Resorcinol test reagents immediately stained a deep red in the presence of cystocarpic fronds. However, tetrasporic fronds also resulted in a red colour, which, although of a paler red initially, soon also turned deep red. Most of the fronds from clumps with no fertile structures displayed a similar reaction to that of the cystocarpic fronds, but this test did not provide clear distinctions as reported in other species of the Gigartinaceae. Variations to the method, such as using fresh instead of dry material, larger or smaller thallus pieces, omitting the acetal and a shorter period in the water bath, all produced the same result.

The division of the clumps into three types (tetrasporic, cystocarpic and those with no obvious fertile structures) exhibited no obvious pattern throughout the study period (Fig. 2.2). Each type of clump is well represented throughout the year. However, as the carrageenan extracted from the fronds belonging to the 'infertile' clumps is of a similar quantity and texture, as well as the similar reaction in the Resorcinol test, to that of the cystocarpic fronds, it is probable that they are male gametophytic fronds or monoecious gametophytic fronds prior to carposporophyte formation. Thus, if the cystocarpic and 'infertile' gametophytic fronds are combined the population was dominated by the gametophytic phase throughout the study period (Fig. 2.3). The ratio of gametophytes to tetrasporophytes showed a clear pattern with a gradual decrease in gametophyte dominance from 2.7:1 in April 1997 down to 1.2:1 in December 1997 followed by a steady increase up to 3.5:1 in September 1998.

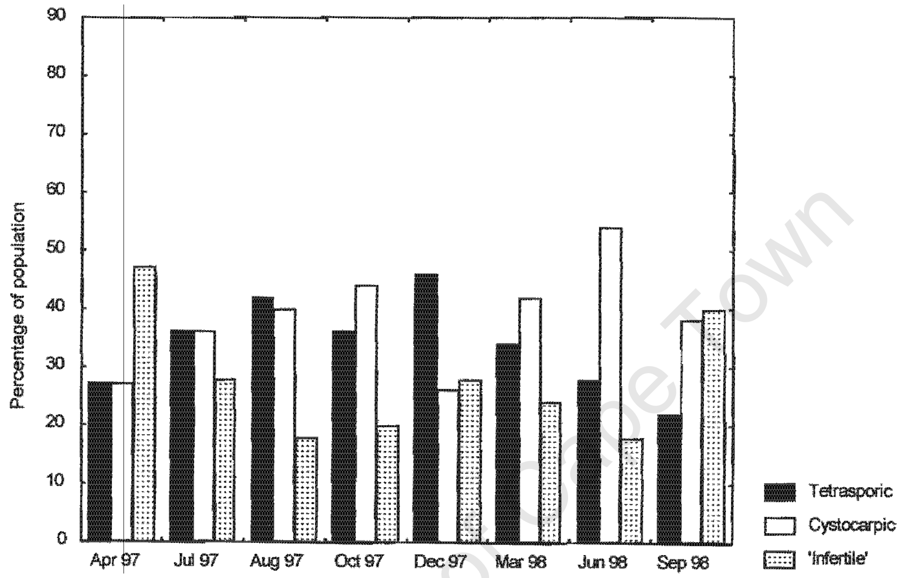

Fig. 2.2: Percentage of population of *Sarcothalia scutellata* composed of tetrasporic, cystocarpic and 'infertile' clumps from April 1997 to September 1998. (n = 50).

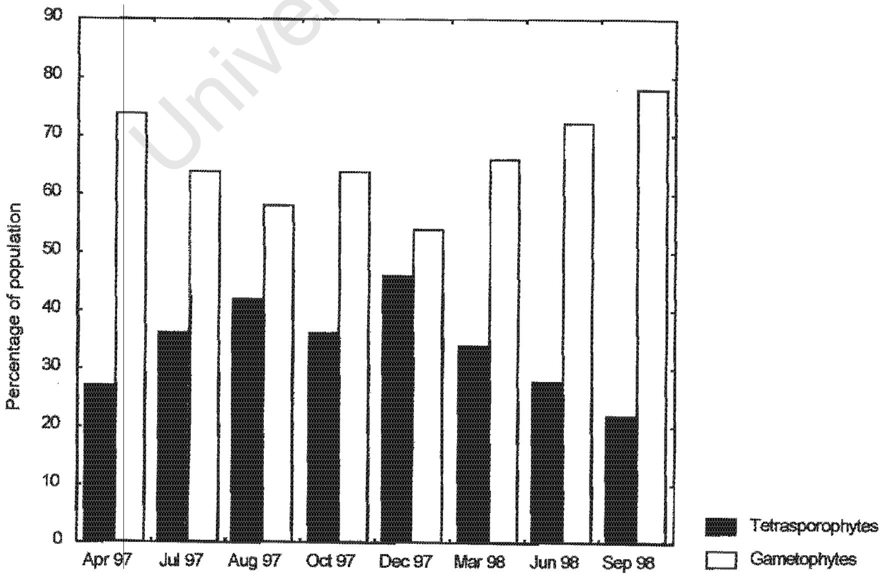

Fig. 2.3: Percentage of population of *Sarcothalia scutellata* composed of tetrasporic and gametophytic clumps from April 1997 to September 1998. Gametophytes consist of cystocarpic and 'infertile' clumps. (n=50).

Clump composition (mass and number of fronds) and dry to wet mass ratios

Lowest mean mass of entire clumps occurred in June 1998 for all three types of clump (Fig. 2.4). According to Kruskal-Wallis ANOVA by ranks the fresh mass of the tetrasporic clumps in December 1997 is significantly different to tetrasporic clumps of October 1997 ($p = 0.011$) and June 1998 ($p = 0.001$). Cystocarpic clumps of June 1998 were significantly different to cystocarpic clumps of December 1997 ($p = 0.003$) and September 1998 ($p = 0.001$). Those clumps that did not possess obvious fertile structures ('infertile' clumps) had significantly different masses between December 1997 and June 1998 ($p = 0.044$). Comparisons between the different phases indicated that the tetrasporic clumps were significantly larger than the cystocarpic clumps in March 1998 ($p = 0.048$) and significantly larger than the 'infertile' clumps in September 1998 ($p = 0.023$).

The fresh masses of the individual fronds are indicated in Figure 2.5 where the mean values represent the mean fresh mass of all fronds collected in each category for that season. Tetrasporic and cystocarpic clumps contained both fertile and infertile fronds. Infertile gametophytic fronds refer to all the fronds within the 'infertile' clumps as those bearing spermatangia could not be distinguished. The fertile fronds from tetrasporic and cystocarpic clumps tended to have higher masses in December 1997 and lower masses in June 1998. The infertile frond masses from these two types of clump, although also possessing higher masses in December 1997, do not show as much variation. Fertile tetrasporic fronds had higher masses than fertile carposporic fronds in October 1997 ($p = 0.028$), December 1997 ($p = 0.017$) and September 1998 ($p = 0.020$) but were not significantly different in March 1998 and June 1998. The individual fronds from gametophytic / 'infertile' clumps had higher average masses than infertile fronds of tetrasporic and cystocarpic clumps.

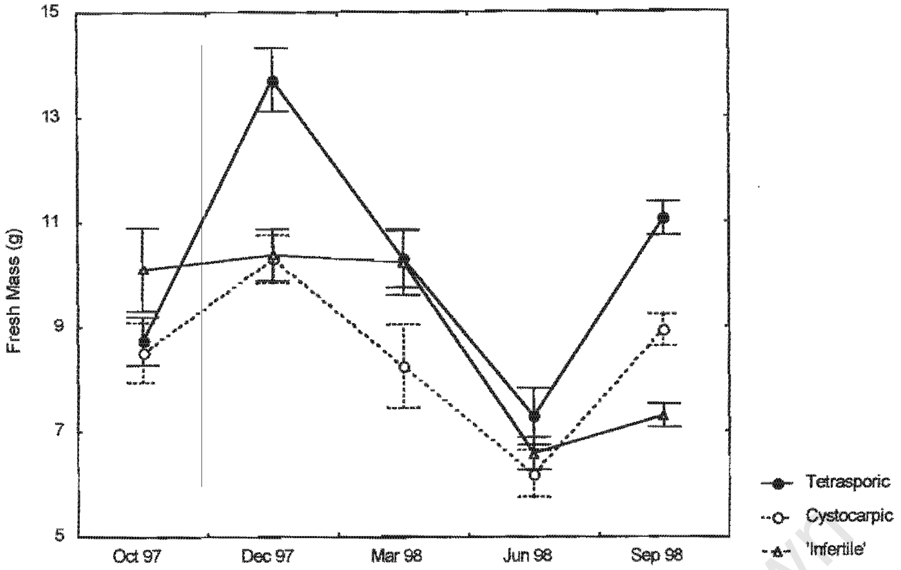

Fig. 2.4: Fresh mass in grams of entire clumps of the seaweed *Sarcothalia scutellata*. Three types of clumps are depicted: tetrasporic, cystocarpic and 'infertile'. Mean ± SE.

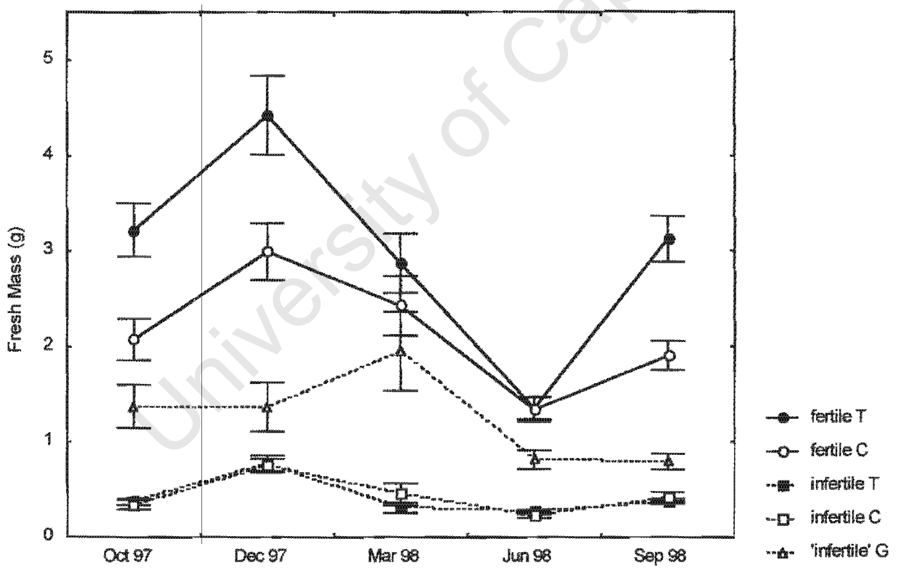

Fig. 2.5: Fresh mass in grams of individual fronds of *Sarcothalia scutellata*. Fertile T = fertile fronds from tetrasporic clumps; fertile C = fertile fronds from cystocarpic clumps; infertile T = infertile fronds from tetrasporic clumps; infertile C = infertile fronds from cystocarpic clumps; infertile G = all fronds from gametophytic/ 'infertile' clumps. Mean ± SE.

An examination of the distribution of individual frond masses within tetrasporic clumps indicated different patterns between fertile and infertile fronds (Fig 2.6). The fertile fronds had a wide distribution in size with a minimum mass of 0.14g and a maximum of 22.96g. No particular size class predominated. The greatest number of infertile fronds however occurred in the size class of less than and equal to 0.2g. A closer examination of mass distributions in the lower range (Fig.

2.7) further emphasized the wide distribution of fertile frond masses with few less than 0.32g. Most infertile fronds fell within the 0.04g to 0.08g mass class with few larger than 0.52g, although a maximum mass of 3.64g was recorded.

A similar pattern emerged for fertile and infertile fronds of cystocarpic clumps. Fertile cystocarpic fronds had a wide range in mass values from a minimum of 0.14g to a maximum of 17.8g and no size class predominated (Fig. 2.8). The infertile cystocarpic fronds occurred largely within the size class of less than or equal to 0.2g and ranged from 0.01g to 7.8g. A closer look at mass distribution at the lower end of the scale (Fig. 2.9) again indicated that most of the infertile fronds occurred within the classes below 0.24g. The fertile fronds had a widespread range in mass with few below 0.2g.

The 'infertile' gametophytic fronds showed a mixed pattern that combines the features of fertile and infertile fronds from the former two types of clumps (Figs. 2.8 & 2.9). There was a predominance of frond masses in the 0.2g and below size class as occurred for infertile fronds of the other two types of clump. However, there was also a very wide distribution amongst the higher mass classes as occurred for fertile fronds. Mass values ranged from 0.01g to 20.1g. This suggests that two types of fronds, fertile and infertile, were present, as was the case with the other clump types.

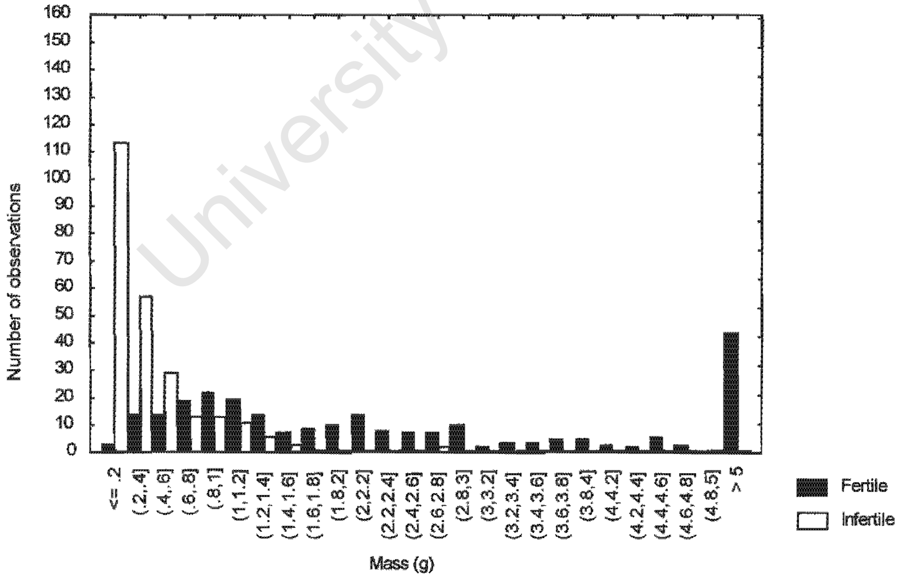

Fig. 2.6: Mass distribution of individual fertile and infertile fronds from tetrasporic clumps of *Sarcothalia scutellata*. Fronds are grouped within 0.2g mass classes with the exception of the last class, which contains all fronds over 5g. (n = 257 for fertile fronds; n = 254 for infertile fronds).

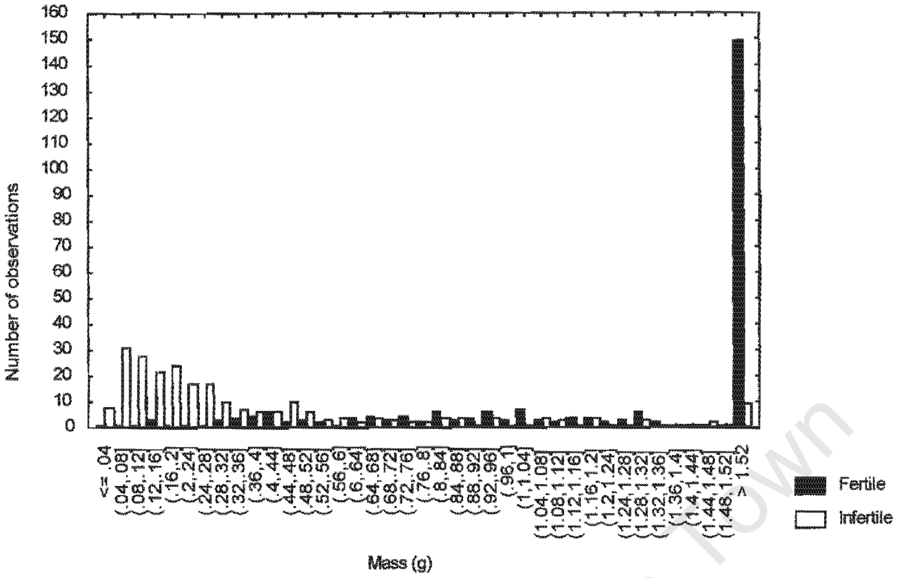

Fig. 2.7: Mass distribution of individual fertile and infertile fronds from tetrasporic clumps emphasizing distribution within the lower mass classes. Fronds are grouped within 0.04g mass classes except for the last class, which contained all fronds over 1.52g. (n = 257 for fertile fronds; n = 254 for infertile fronds).

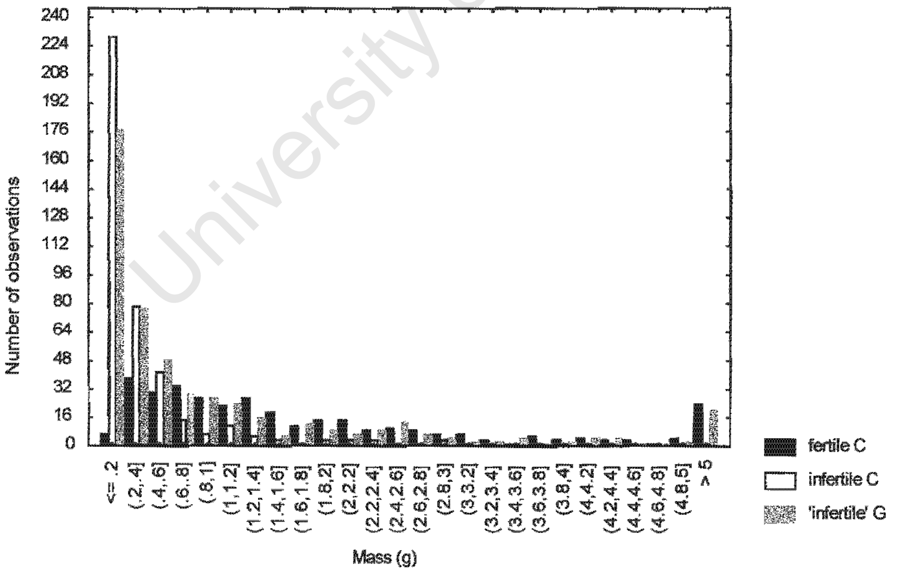

Fig. 2.8: Mass distribution of individual fertile and infertile fronds from cystocarpic (C) clumps and 'infertile' fronds from 'infertile'/gametophytic (G) clumps. Fronds are grouped within 0.2g mass classes with the exception of the last class, which contains all fronds over 5g. (n = 339 for fertile cystocarpic fronds; n = 406 for infertile cystocarpic fronds; n = 508 for infertile gametophytic fronds).

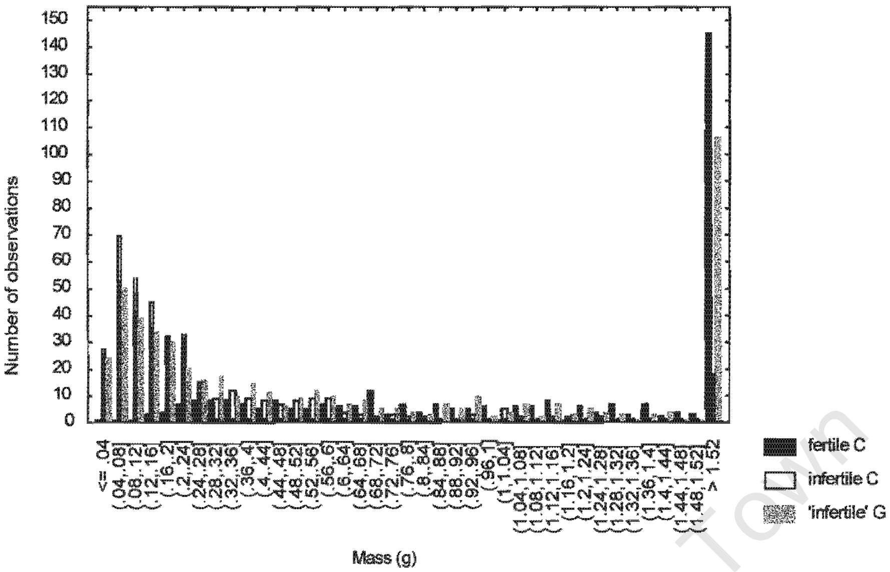

Fig. 2.9: Mass distribution of individual fertile and infertile fronds from cystocarpic (C) clumps and 'infertile' fronds from 'infertile'/gametophytic (G) clumps emphasizing distribution in the lower mass classes. Fronds are grouped within 0.04g mass classes except for the last class which contains all fronds over 1.52g. (n = 339 for fertile cystocarpic fronds; n = 406 for infertile fronds from cystocarpic clumps; n = 508 for 'Infertile' fronds from 'infertile'/gametophytic clumps).

The ratio of dry to wet mass varied little throughout the study period with the dry mass being on average 21% to 24% of the wet mass (Fig. 2.10).

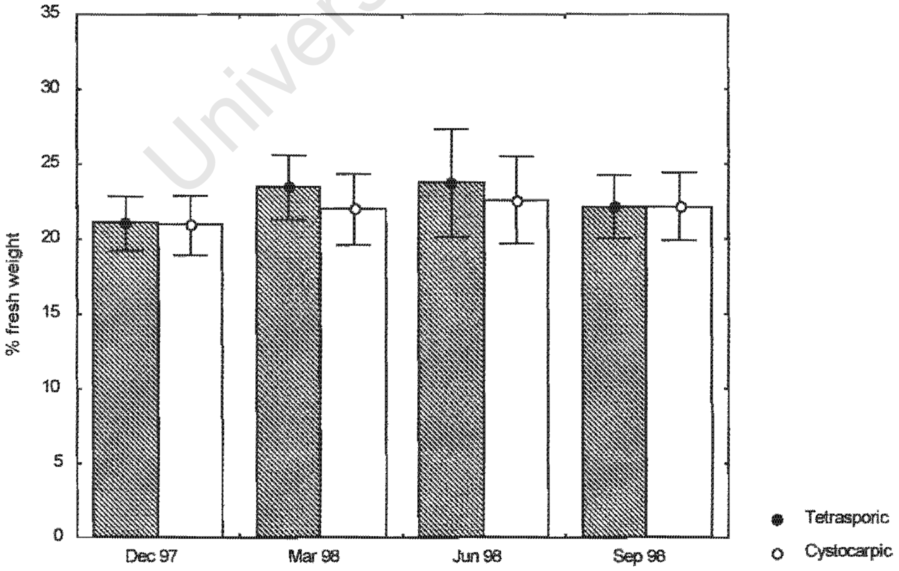

Figure 2.10: Seasonal variation of dry matter content of *Sarcothalia scutellata*. Results are expressed in % fresh weight. Mean ± SE.

Numbers of fronds within clumps

As previously mentioned, clumps were noted to consist of fertile and infertile fronds or only infertile fronds. Mixed phases were not noted. The average number of total fronds (fertile plus infertile) per clump over the entire study period was around 6 to 8 for all three types of clumps (Table 2.1). All three also displayed a wide range in number from only one frond to as many as 31 to 52.

Phase	Mean	Minimum	Maximum	Std. Dev.
Tetrasporic	6.1	1	31	5.40
Cystocarpic	7.3	1	52	6.82
Gametophytic	7.7	1	36	6.02

Table 2.1: Number of fronds per tetrasporic, cystocarpic or gametophytic/'infertile' clump of the seaweed *Sarcothalia scutellata*.

Seasonal variation in the number of total fronds per clump is shown in Figure 2.11. The three types of clump possessed similar numbers of fronds per clump with a wide range in number occurring on each date. Tetrasporic clumps tended to have more fronds in June 1998 otherwise no obvious seasonal pattern was detected. Kruskal-Wallis ANOVA by ranks indicated no significant seasonal differences on each date for all three phases with the exception of tetrasporic clumps in September 1998 which had less fronds than those of June 1998 ($p = 0.031$). The phases had similar numbers of total fronds to each other with the exception of September 1998 when the tetrasporic clumps had fewer fronds than both the cystocarpic ($p = 0.019$) and gametophytic clumps ($p = 0.015$).

An examination of the number of fertile and infertile fronds per clump is indicated in Figure 2.12. Kruskal-Wallis ANOVA by ranks indicated that the tetrasporic clumps of September 1998 had fewer infertile fronds than those of June 1998 ($p = 0.045$) but did not show significant variation in the number of fertile or infertile fronds per clump on the other dates. Cystocarpic clumps had fewer infertile fronds in December 1997 than in September 1998 ($p = 0.009$) but did not show seasonal variation in the number of fertile and infertile fronds per clump otherwise. The two phases did not differ in number of fronds compared to each other with the exception of fewer infertile tetrasporic than infertile cystocarpic fronds in September 1998 ($p = 0.015$).

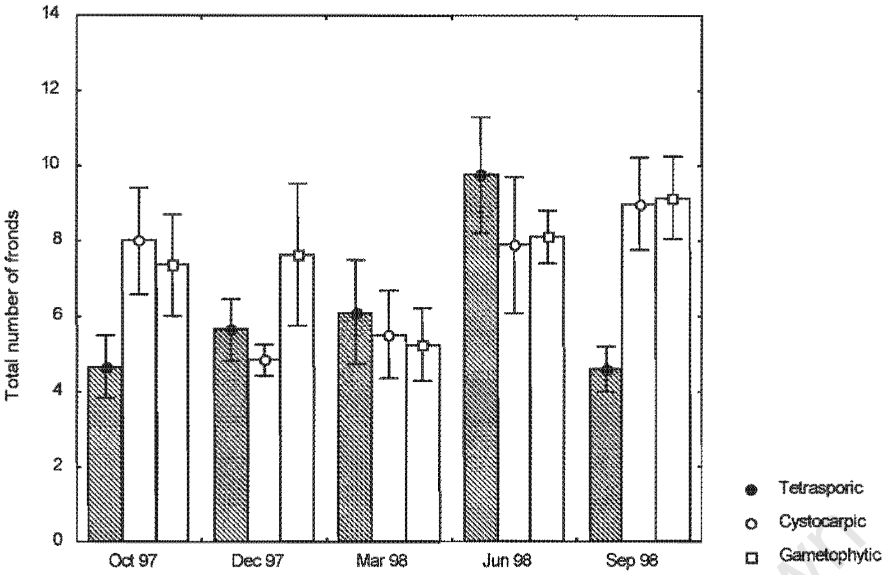

Fig. 2.11: Total number of fronds (fertile plus infertile) per tetrasporic, cystocarpic and gametophytic/ 'infertile' clump of *Sarcothalia scutellata* from October 1997 to September 1998. Mean ± SE.

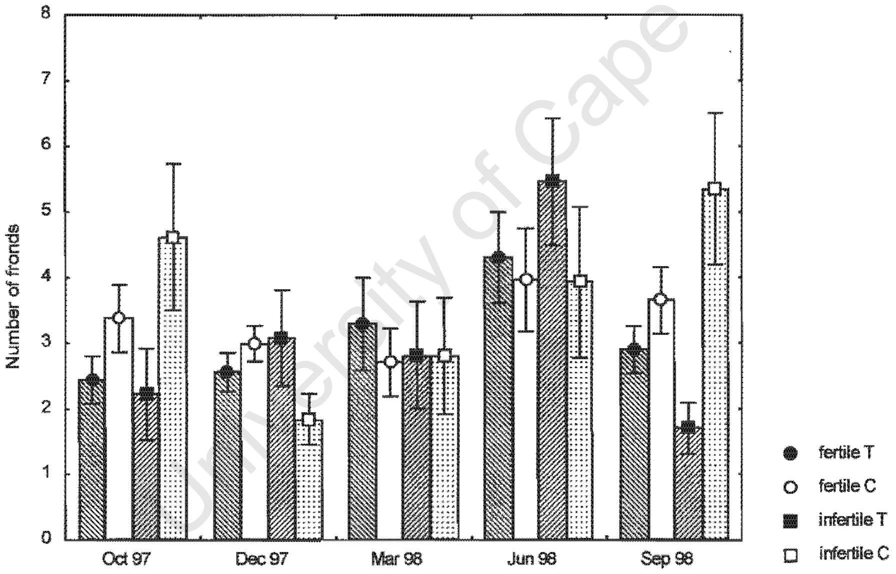

Fig. 2.12: Number of fertile and infertile fronds per tetrasporic (T) and cystocarpic (C) clump of the seaweed *Sarcothalia scutellata* from October 1997 to September 1998. Mean ± SE.

Investigation of a relationship between frond mass and the number of fronds within the clump from which the frond originated revealed a weak negative correlation with the Gamma statistic of -0.266 (Fig. 2.13). The skewed nature of the data due to the presence of more small than large fronds and more small than large clumps caused a problematic correlation, but examination of the scatter-plot provides some insights. The mass of fronds that occurred as isolated individuals (clump size = 1) was rarely less than 1g. However, fronds of less than 1g did occur in clumps with as few as 2 fronds per clump. Clumps with large numbers of fronds, for example, those with as many as 24 fronds, possessed relatively large fronds with masses a high as 8g, although the largest clumps of more than 30 fronds tended to possess small fronds. Thus there was a tendency only for very large clumps to possess smaller fronds but small fronds were able to occur in small clumps too. These observations do not therefore support the idea that small fronds would occur in large clumps and vice versa.

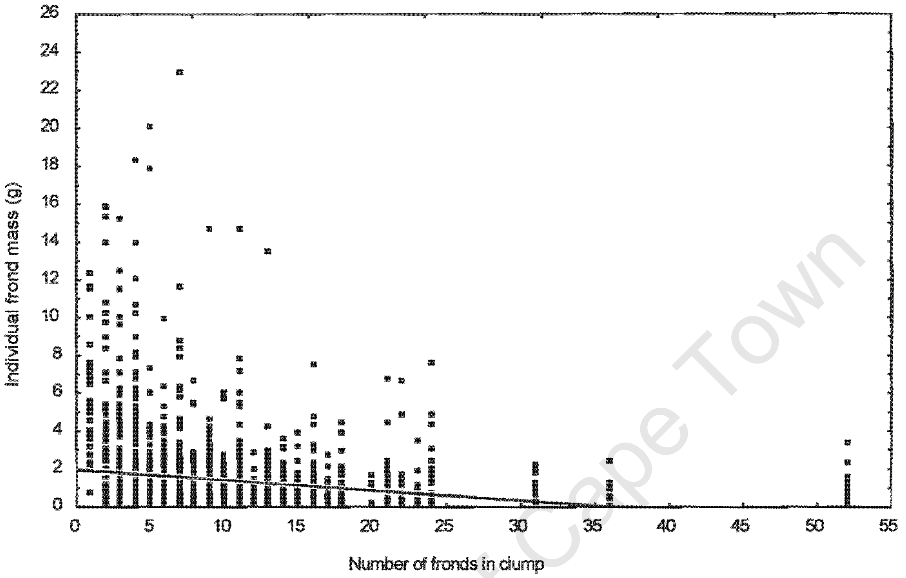

Fig. 2.13: Relationship between clump size (number of fronds within a clump) and mass of fronds comprising clumps. Individual fronds masses were plotted against the number of fronds within the clump from which the frond originated. (Gamma statistic = -0.266)

Discussion

Carrageenan content

In most members of the Gigartinaceae, the two life history phases are reported to have similar carrageenan contents (Pickmere *et al.* 1973; Waaland 1975; Bolton and Joska 1993; Levitt *et al.* 1995; Molteno 1996). Where differences are reported, the tetrasporic phases had a lower yield, as occurred in *Sarcothalia scutellata*, where the tetrasporic fronds had a carrageenan content of 14 to 24% and cystocarpic fronds 33 to 58%. Tetrasporophytes of *Sarcothalia decipiens* (J.D.Hooker *et* Harvey) Hommersand (formerly *Gigartina decipiens* – Hommersand *et al.* 1993) from New Zealand had a slightly lower carrageenan yield of 39 to 46% compared to gametophytes with 62 to 73 % (Pickmere *et al.* 1973). Tetrasporophytes of *Gigartina skottsbergii* Setchell *et* Gardner had a yield of 10.1 to 34.0 %, whereas the cystocarpic and 'sterile' plants had yields of 34.1 to 64.7% and 32.3 to 71.8% respectively (Piriz and Cerezo 1991). Monthly extractions from *Gigartina pistillata* (Gmelin) Stackhouse found tetrasporophytes to always produce less carrageenan, with an average of 30% whereas female gametophytes yielded, on average, 46% (Gierdien 1992).

The yield from commercially utilized carrageenophytes (Pickmere *et al.* 1973; McCandless and Craigie 1974; Waaland 1975; Piriz and Cerezo 1991; Chopin and Floc'h 1992; Zinoun *et al.* 1993) or those considered to have potential (Bolton and Joska 1993; Levitt *et al.* 1995; Molteno 1996) is usually in the region of 30 to 70% of the dry weight. Thus only the gametophytic fronds of *Sarcothalia scutellata* compared favorably.

Different methods of extraction make direct comparisons between yields difficult. Two types of extraction are commonly used, namely hexadecyltrimethylammonium bromide (CTAB) and alcohol precipitation. In the latter, variations in the method used by different authors can have significant effects on yield. An investigation on extraction procedures by Lai and Lii (1998) indicated that the temperature used for extraction has the most significant effect followed by pH and extraction time. Carrageenan yield against extraction time was determined by Levitt *et al.* (1995) for *Gigartina polycarpa* and extraction periods of four hours or less yielded significantly less carrageenan than those of five to twelve hours.

Similar quantities of carrageenan were produced throughout the year from *Sarcothalia scutellata*, except for September, when there was a decrease. This corresponds to a period of increasing frond size after a decline in frond mass occurred in June. An inverse relationship between growth rate and phycocolloid accumulation has frequently been reported (Gordon-Guist *et al.* 1982; Zinoun *et al.* 1993; Zinoun and Cosson 1996). Nitrogen limitation has been associated with decreased photosynthetic activity and growth, whilst phycocolloid content increases, a phenomenon referred to as the Neish effect (Fuller and Mathieson 1972; Mathieson and Tveter 1975; Neish *et al.* 1977). A similar effect has been reported for phosphorus nutrition (Chopin and Gallant 1995). Reani *et al.* (1998) reported the opposite for *Cystoclonium purpureum* (Hudson) Batters of the Cystocloniaceae, an ι-carrageenan producer, in which total carrageenan yield and growth were positively related. No seasonal variation occurred in yields of cystocarpic, 'sterile' and tetrasporic stages of *Gigartina skottsbergii* in Argentina (Piriz and Cerezo 1991) or in *Gigartina polycarpa* and *Sarcothalia stiriata* in South Africa (Levitt *et al.* 1995).

Phase Composition

Assuming that the haploid and the diploid phases are equally represented and that the sex ratios are 1:1, a ratio of 2:1 for tetrasporophytes to female gametophytes would be expected for dioecious species and of 1:1 for monoecious species. This does not occur in *Sarcothalia scutellata* (Fig. 2.2) where cystocarpic clumps frequently occurred in greater numbers than the tetrasporic clumps or were equal in number. December 1997 was the only period when tetrasporic clumps outnumbered cystocarpic ones.

The clumps in which no obvious fertile structures were found could contain juvenile fronds of either phase or male gametophytes. An attempt to identify male structures microscopically was unsuccessful. Spermatangia are very small, for example, those of *Chondrus crispus* measured 2.5 µm in diameter (Tveter-Gallagher *et al.* 1980). If they are dispersed throughout the thallus rather than grouped into sori they would be difficult to find. They also appear to be short-lived. Spermatangial sori disappeared after about 8 weeks whereas cystocarps persisted for over 6 months in *Chondrus crispus* (Tveter-Gallagher *et al.* 1980). The duration of reproduction was also noted to be greater in subtidal than in intertidal populations of this species. Spermatangia were only found during one month of the year on some of the large 'vegetative' plants of *Gigartina skottsbergii* (Piriz 1996).

The Resorcinol test was not reliable in distinguishing phases for this species, as there was also a reaction in tetrasporic fronds. An investigation into the reliability of the test (Shaughnessy and DeWreede 1991) found it to be very reliable in the case of species in the genera *Iridaea* and *Rhodoglossum* but not for *Gigartina exasperata* Harvey and Bailey, although modifications to the test did lower the percentage of misidentifications quite significantly. Reducing the sample weight and allowing more time for the reaction to stabilize appears to have had some benefit. Further modifications may be required to enable this test to be used for *Sarcothalia scutellata*.

Some species have been reported to possess low ratios κ- to λ-carrageenan in the tetrasporophytes. *Gigartina decipiens* was reported to have a ratio of 0.12, *Gigartina angulata* : 0.07 to 0.23 (Pickmere *et al.* 1973) and *Iridaea lineare* 0.24 (Waaland 1976). Failure of the test may thus be due to the presence of κ-carrageenan in the tetrasporophyte. However, there has since been advances made in the identification of carrageenan-types and recent reviews make no mention of κ-carrageenan in the tetrasporophyte but other carrageenan-types are introduced. For example, the type of carrageenan extracted form the tetrasporophytes of *Sarcothalia scutellata* was λ/θ/α (Chopin *et al.* 1999). The reliability of the resorcinol test when applied to such hybrid carrageenan-types compared to the more pure λ-carrageenan needs to be investigated.

The colour change of the 'infertile' fronds was mostly similar to that of the cystocarpic fronds but unreliability of the test probably lead to some misidentifications. However, in *Gigartina* *skottsbergii*, for which the test worked well, most vegetative plants were Resorcinol positive as well (Piriz 1996). Carrageenan yield from the 'infertile' clumps of *Sarcothalia scutellata* was very similar to that of the cystocarpic clumps and the carrageenen was jelly-like rather than stringy as in the tetrasporic fronds. The gametophytes of the Gigartinaceae are described as largely dioecious, although monoecious species also exist (Hommersand *et al.* 1993). The mass distributions also showed that fronds occurred in the 'infertile' clumps that were of a similar size to that of fertile tetrasporic and cystocarpic fronds. This indicates that they are possibly mature male gametophytes. Thus, gametophytic dominance occurred for the entire study period (Fig. 2.3).

How does this compare with other members within the Rhodophyta? Phase dominance is very common but the diploid phase usually dominates. Sporophytic dominance has been reported in a wide range of red algae including *Iridaea cordata* (Turner) Bory from California (Hansen and Doyle 1976), *Gelidium pristoides* (Turner) Kützing (Carter 1985); *Plocamium cartilagineum*, *Cyptopleura ramosa* and *Palmaria palmata* (Kain 1986); *Gelidium robustum* (Gardner) Hollenberg *et* Abbott (Melo and Neushul 1993); *Gracilaria* species (Kain and Destombe 1995) and *Grateloupia filicina* (see Chapter 2).

However, in many studies of various species within the Gigartinaceae a different pattern emerges as gametophytic dominance is frequently reported. An extremely high proportion of fronds (91%) were found to be gametophytic in intertidal populations of *Chondrus crispus* in Nova Scotia (Bhattacharya 1985). Scrosati *et al.* (1994) found *Chondrus crispus* in Nova Scotia to be gametophyte dominated by a ratio of 3:1. The phases of vegetative fronds, of which the populations were largely composed, were not ascertained. A study conducted in Brittany, also on *Chondrus crispus*, found that mature female gametophytes represented 18.9 and 15.2% of infra- and mid-littoral populations respectively, whereas mature tetrasporophytes represented only 10.3 and 5.6% respectively (Chopin and Floc'h 1992). Gametophyte dominance (83%) occurred in *Iridaea cordata* (Bory) Turner from San Juan Island in Washington (May 1986). Intertidal *Mazzaella cornucopiae* occurring on the Pacific coast of Canada was dominated by gametophytic vegetative fronds regardless of site and season (Scrosati 1998a). In Central Chile, the upper intertidal species, *Mazzaella laminarioides* (Bory) Fredericq (formerly *Iridaea laminarioides* Bory - Hommersand *et al.* 1993), was dominated by cystocarpic fronds (80 to 100%), as was the low-intertidal to shallow subtidal species *Iridaea ciliata* Kützing (65 to 100%) (Hannach and Santelices 1985). *Chondracanthus teedii* (Roth) Kützing (formerly *Gigartina teedii* (Roth) Lamouroux – Hommersand *et al.* 1993) in Brazil was predominantly non-reproductive, with cystocarpic ramets representing 0.5 to 14.5% whereas tetrasporangial sori were rare (Braga 1990). *Gigartina skottsbergii* in Argentina had many vegetative/male plants as well as a number of cystocarpic plants but few tetrasporic ones (Piriz 1996). The Resorcinol test indicated most of the vegetative plants to be gametophytic. *Gigartina skottsbergii* in central -southern Chile was dominated by the haploid phase with tetrasporophytes never representing more than 30% of the population (Westermeier and Sigel 1997).

In certain species it was found that phase dominance may switch periodically. *Mazzaella capensis* in intertidal populations in South Africa was 70% sporophytic in April 1990 but was 80% gametophytic by October and remained gametophyte dominated until the end of the study period in April of the following year (Bolton and Joska 1993). In Spring, gametophytes of *Mazzaella splendens* in Vancouver, increased more rapidly in density than the tetrasporophytes attaining higher summer densities, but then decreased more rapidly in the Autumn resulting in winter predominance of tetrasporophytes (Ang *et al.* 1990; Dyck and DeWreede 1995).

More or less equal representation of phases in the Gigartinaceae has also been reported. *Chondrus crispus* had an overall ratio of ≈1:1 in a survey of a number of populations around Prince Edward Island in Canada (Laura Lazo *et al.* 1989).

The G:T ratios obtained for *Sarcothalia scutellata* were close to the 2.8 value obtained in demographic models for triennial and perennial thalli that followed the simplest life-history sequence (Scrosati and DeWreede 1999). This might indicate that fronds of this species survive for at least three years with tetrasporic fronds producing four tetraspores for every one carpospore produced per male-female gametophyte pair and there is in fact no ecological differences between the fronds.

The reason(s) for one stage of a life cycle to predominate is uncertain. Numerical dominance may be caused by ecological differences between the life-history phases but, as the assumption that ecologically similar phases should have a stable ratio of one is not necessarily valid, numerical dominance is also possible in ecologically similar phases. A general discussion of phase dominance is presented in chapter 7 as the topic is pertinent to both species.

Clump composition (mass and number of fronds) and dry to wet mass ratios

Sarcothalia scutellata consists of crusts from which few to many upright fronds arise. The species was noted to consist of discrete clumps that could be classified as tetrasporic, cystocarpic or 'infertile'. The occurrence of highest masses in summer and lowest in winter is similar to other carrageenophytic species in South Africa. *Gigartina polycarpa*, *Sarcothalia stiriata*, *Mazzaella capensis* and *Aeodes orbitosa* have distinct seasonal patterns of growth with many juveniles produced in winter, and greatest size achieved in summer (Bolton and Levitt 1992; Bolton and Joska 1993; Levitt 1998).

Fertile fronds were generally heavier than infertile fronds in tetrasporic and cystocarpic clumps. Fronds of both phases became fertile at masses as low as 0.14g but were usually more than 0.2 to 0.3g. The infertile fronds were mostly less than 0.2 to 0.3g. There were however, a few surprisingly heavy infertile fronds with masses as high as 3.62g in tetrasporic clumps and 7.81g in cystocarpic clumps. As tetrasporophytes would be expected to release both male and female tetraspores, it would seem unlikely that the spores separate out into male and female clumps. If the species is dioecious, as are most of the Gigartinaceae (Hommersand *et al.* 1993), it would seem more likely that male and female fronds would be found intermixed in gametophytic clumps. The cystocarpic clumps would then be expected to consist of heavier cystocarpic fronds as well as fertile male gametophytic fronds that would not be easily distinguished from the smaller infertile fronds. Thus there should not be a clear-cut pattern of heavy fertile cystocarpic fronds and light infertile fronds as the latter would include the heavier fertile male fronds too. However, this did not occur as the cystocarpic clumps possessed few large 'infertile' fronds (Figs. 2.8 &2.9). The 'infertile' clumps however, did possess large fronds with the distribution pattern indicating a mixture of fertile and infertile fronds. Thus either the clumps, rather than just the fronds, are somehow separated sexually or the fronds are monoecious with the 'infertile' clumps containing fronds that have yet to form cystocarps. Tveter and Mathieson (1976) discovered different degrees of sporeling coalescence between tetrasporophytes and gametophytes as the former showed a higher proportion of coalescence than the latter. This led them to speculate that only gametophytic crusts of the same gender coalesce. This could explain the existence of separate female and male clumps.

Dry mass content did not vary seasonally and was similar in the two phases. Values ranged from 21 to 24% and were similar to those found for *Chondrus crispus* (Chopin and Floc'h 1992) and *Grateloupia filicina* (See Chapter 3). Some seasonal variation occurred in *Chondrus crispus* but it was generally around 22 to 28% with the infralittoral form exhibiting a lower dry mass content than the midlittoral form.

The clumps had a similar structure to each other in that they generally consisted of larger central fronds surrounded by smaller ones. Each clump consisted of similar numbers of fertile and infertile fronds. The number of fronds making up the clumps did not show much variation seasonally except for a tendency for the number of fronds in tetrasporic clumps to increase in June 1998. This corresponded to the period in which the fronds had lowest masses.

This leads to the question of whether there is an inverse relationship between the number of fronds per clump and the mass of the fronds comprising the clumps. However, no strong correlation was found in this study. The mid- to low-intertidal rock pools in which *Sarcothalia scutellata* was sampled may not experience severe enough stresses to prevent the survival of small clumps of low massed fronds.

Chapter 3

Seasonal variation in carrageenan content, phase composition and frond mass of *Grateloupia filicina* (Lamouroux) C. Agardh (Halymeniaceae; Rhodophyta)

Introduction

There is no history in South Africa of *Grateloupia filicina* (Lamouroux) C. Agardh being used as a source of carrageenan. There have also been no studies conducted on the yield or type of carrageenan produced by this species in South Africa. In other parts of the world the type of carrageenan produced by this species, as well as other members of the Halymeniaceae, has been characterized, although much less attention has been paid to the Cryptonemiales compared to the Gigartinales (Craigie 1990), a closely related order (Bold and Wynne 1985). Carrageenan extracted from *Grateloupia filicina* from Taiwan was described as λ-type carrageenan (Lai and Lii 1998). A review of phycocolloids from the Cryptonemiales (Chopin *et al.* 1994) indicated that many other members of the Halymeniaceae also produced λ-type carrageenan. *Grateloupia filicina* from Hawaii possessed carrageenan very similar to λ-carrageenan but IR spectra and polyacrylamide gel electrophoresis indicated slight differences (Zablackis and Perez 1990). The presence of pyruvate acid residues lead to its being characterized as π-carrageenan. The phycocolloid industry has specific applications for λ- carrageenan which is not produced by the genus *Eucheuma* which dominates the industry otherwise (McHugh 1991).

The Cryptonemiales generally have a lower carrageenan yield compared to the Gigartinales as indicated in a review of 26 species within the Cryptonemiales (Chopin *et al.* 1994). The carrageenan content of *Grateloupia filicina* from other parts of the world ranged from 34 to 37% (Chopin *et al.* 1994, Zablackis and Perez 1990) and could thus be a commercially valuable carrageenophytic species. It does, however, have a very widespread geographical distribution and taxonomic work is required to ensure that the South African species is the same as those occurring elsewhere.

A number of studies on various species of *Grateloupia* have been conducted including cultivation from fragments of *Grateloupia acuminata* Holmes (Iima *et al.* 1995) and *Grateloupia filicina* (Migita cited in Iima *et al.* 1995); protoplast isolation and culture of the previous two species (Chen and Chiang 1994); regeneration of plantlets from segments of *Grateloupia filiformis*

Kützing (Yokoya *et al.* 1993) and effects of plant growth regulators (auxins and cytokinins) on callus formation, growth and morphogenesis of *Grateloupia dichotoma* J. Agardh (Yokoya and Handro 1996).

Pilot studies of commercial cultivation of *Grateloupia filicina* have been attempted on the Caribbean coast of Colombia (Bula-Meyer 1989). Attempts at vegetative propagation were unsuccessful largely due to biofouling. Greater success was obtained by cultivating from spores and by increasing the available substratum within the environment.

In South Africa the species is abundant in rock pools along the west coast but not of sufficient biomass to make harvesting practical. It is abundant in high-intertidal rock pools, sometimes as high as the supralittoral, although subtidal specimens have also been collected. A physically stressed environment would be experienced in these high-intertidal rock pools with temperature and salinity fluctuations and high light intensities.

The first aim of this study was to determine the yield of carrageenan from South African *Grateloupia filicina* and whether any seasonal variation in yield occurs. Dominance by one of the life history phases has frequently been reported in various seaweed species with diploid (tetrasporophyte) dominance most frequently reported, except in the Gigartinaceae where haploid (gametophyte) dominance is more frequently reported (see Chapter 2). The second aim of this study was thus to determine if *Grateloupia filicina* exhibited phase dominance and whether this showed any seasonal variation. The third aim was to determine if there was a season of optimal growth. The pattern for other carrageenophytic species in South Africa is for maximum frond mass to occur in summer and minimum frond mass to occur in winter (Bolton and Levitt 1992; Bolton and Joska 1993; Levitt 1998). Finally, the seasonal variation in dry mass content was examined to see how this compares to other seaweeds and to see if there is any relationship with seasonal patterns of carrageenan content and fresh mass. Seaweeds in general have a water content that ranges from 80 to 90% of the fresh mass (Lüning 1990). Vertical location on the shore influenced dry matter content in *Chondrus crispus* as the infralittoral form had a lower dry matter content than the midlittoral form. There was also some seasonal variation in this factor, but maximum values occurred in both summer and winter. Dry matter content values ranged from about 24 to 28% in the midlittoral form of *Chondrus crispus* and from about 22 to 26% in the infralittoral form. *Sarcothalia scutellata*, which occurred in the mid- to low-intertidal zone, had a dry matter content that ranged from 21 to 24% (see Chapter 2). There was no seasonal variation, nor did it vary according to life history phase. *Grateloupia filicina* might be expected to have a higher dry matter content as it occurs in the high-intertidal.

Method

Fronds of *Grateloupia filicina* were collected from high-intertidal rock pools at Olifantsbos (lat. 34° 16' S, long. 18°23' E) on the Atlantic coast of the Cape Peninsula. Collections were made in April, July, August, October and December 1997 and March, June and September 1998. Fifty fronds were collected on each occasion (ten fronds were from five different rock pools) using the same method as that used for *Sarcothalia scutellata* to achieve objective sampling. Collections were never from the same rock pools as those of the latter species as they tended to predominate in different pools. Fronds were transported back to the laboratory in plastic bags in an insulated container. As the fronds often occurred in dense stands from which it was not possible to distinguish separate clumps, only single fronds rather than clumps were examined.

Carrageenan content

Carrageenan extractions were performed in October and December 1997 and March, June and September 1998. The same method was used as for *Sarcothalia scutellata* (see Chapter 2).

Phase Composition

The proportion of the fifty fronds that were collected in April, July, August, October and December 1997 and March, June and September 1998 that were tetrasporic, infertile or carposporic was determined. The phase of the fronds was determined by sectioning the thallus and examining under the light microscope. Tetrasporic fronds were identified by the presence of dispersed tetrasporangia within the cortex (see Fig. 5.1 of Chapter 5) and carposporic fronds were identified by the presence of carposporophytes immersed within the thallus (see Fig. 5.2 of Chapter 5). The Resorcinol test could not be used for this species as both phases possess λ-type carrageenan and so neither react with the reagents.

Frond Mass

Frond mass was measured every second or third month from October 1997 to September 1998. Epiphytic organisms were removed and the fresh mass of each frond determined. Fronds were dried in an oven at 60°C until constant weight and the dry mass determined.

Statistics

Significant differences were determined using Kruskal-Wallis ANOVA by ranks using the software package STATISTICA version 5.1 (StatSoft 1996).

Results

Carrageenan Content

Both the tetrasporic and the carposporic fronds possessed little carrageenan for the entire period in which the study was conducted (Fig. 3.1). Tetrasporic fronds had only 3 to 15% of their dry mass composed of carrageenan and the carposporic fronds only 3 to 7%. Very few of the fronds collected in December 1997 were carposporic and were of insufficient mass to perform the carrageenan extractions.

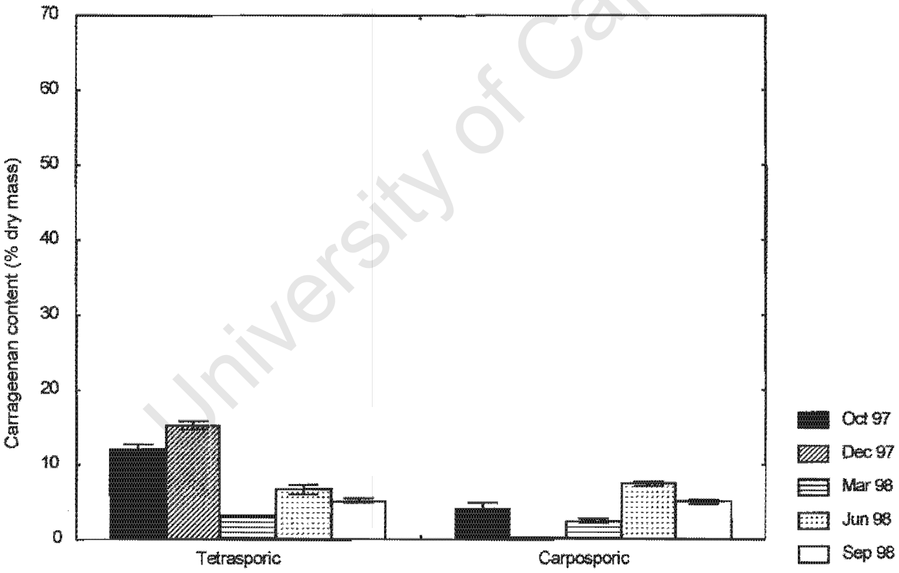

Fig. 3.1: Carrageenan content of tetrasporic and carposporic fronds of *Grateloupia filicina* from October 1997 to September 1998. There was insufficient carposporic frond mass to perform the extraction in December 1997. Mean ± SE; n = 3.

Phase Composition

Using the light microscope to identify fertile structures, the fronds were divided into three different types: tetrasporic, carposporic and infertile. The rock pools were highly dominated by tetrasporic fronds over the entire study period (Fig. 3.2).

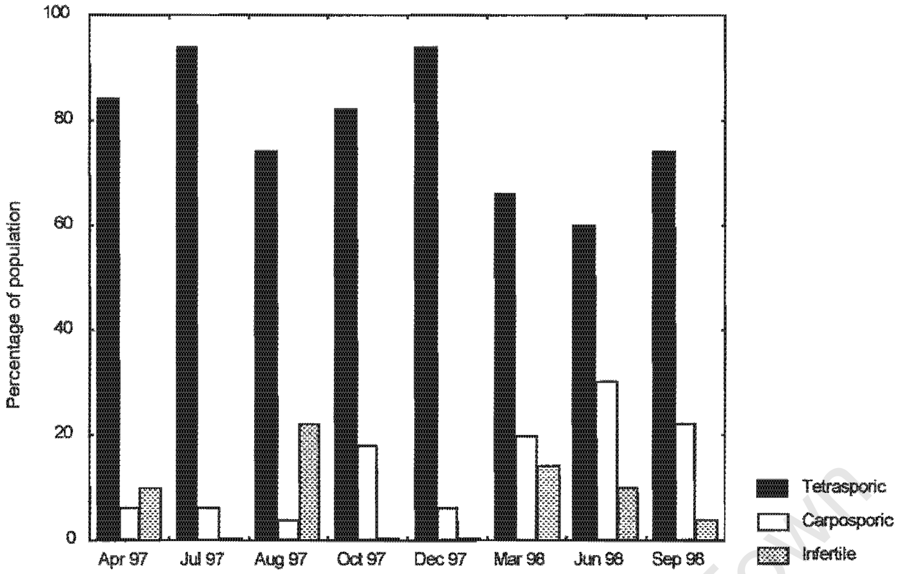

Fig. 3.2: Percentage of population of *Grateloupia filicina* composed of tetrasporic, carposporic and infertile fronds from April 1997 to September 1998. (n = 50).

Individual Frond Mass

Tetrasporic fronds had an average fresh mass of 5 to 10 g (Fig. 3.3). Tetrasporic fronds of December 1997 were significantly heavier than tetrasporic fronds of October 1997 ($p < 0.0001$), March 1998 ($p = 0.003$) and September 1998 ($p = 0.017$). Tetrasporic fronds in October 1997 were also smaller than those of June 1998 ($p = 0.014$). Carposporic fronds also appeared to have larger fronds in December 1997 compared to the other months but too few fronds appeared in the December sample to allow any conclusions to be made. All fronds collected were fertile in October 1997 and December 1997. Infertile fronds collected in March 1998 to September 1998 were of comparable size to the fertile fronds, although too few infertile fronds occurred in September 1998 to allow much comparison.

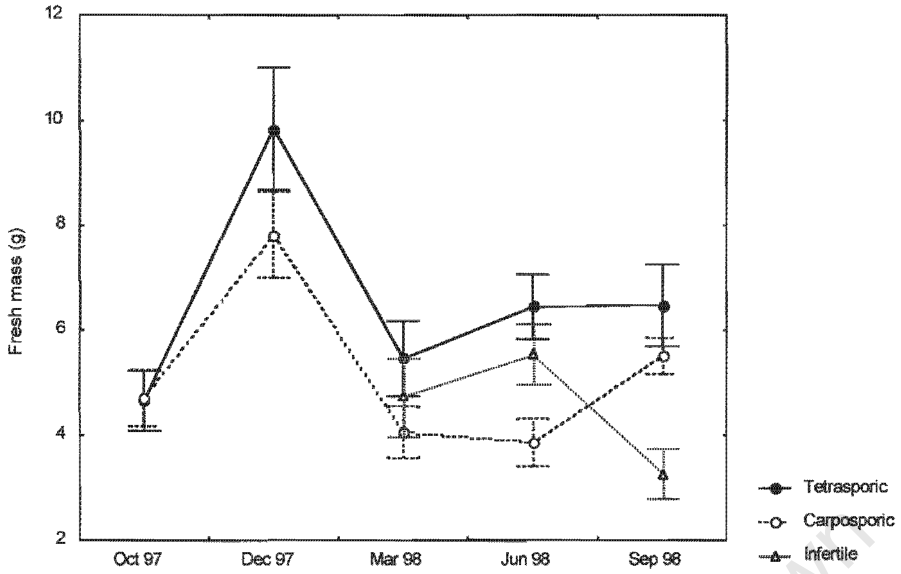

Fig. 3.3: Fresh mass in grams of fronds of *Grateloupia filicina* from Oct 97 to Sep 98. Three types of frond are depicted: tetrasporic, carposporic and infertile. Mean ± SE. Values of n for T (tetrasporic), C (Carposporic) and I (Infertile) fronds: Oct 97 T = 40 C = 10 I = 0; Dec 97 T = 47 C = 3 I = 0; Mar 98 T = 33 C = 10 I = 7; Jun 98 T = 30 C = 15 I = 5; Sep 98 T = 37 C = 11 I = 2.

The percentage of dry to wet mass was around 22 to 26% for tetrasporic fronds and 24 to 28% for carposporic fronds (Fig. 3.4). Values were constant for most of the year but decreased in September 1998 for both phases. Tetrasporic fronds of September 1998 had significantly lower dry mass percentages than tetrasporic fronds of December 1997 to June 1998 ($p < 0.0001$). Carposporic fronds of September 1998 had significantly lower dry mass percentages than carposporic fronds of March 1998 ($p = 0.024$) and June 1998 ($p = 0.012$). The two phases showed similar values to each other at each season, with the exception of September 1998 when tetrasporic fronds had significantly lower values than carposporic fronds ($p = 0.007$).

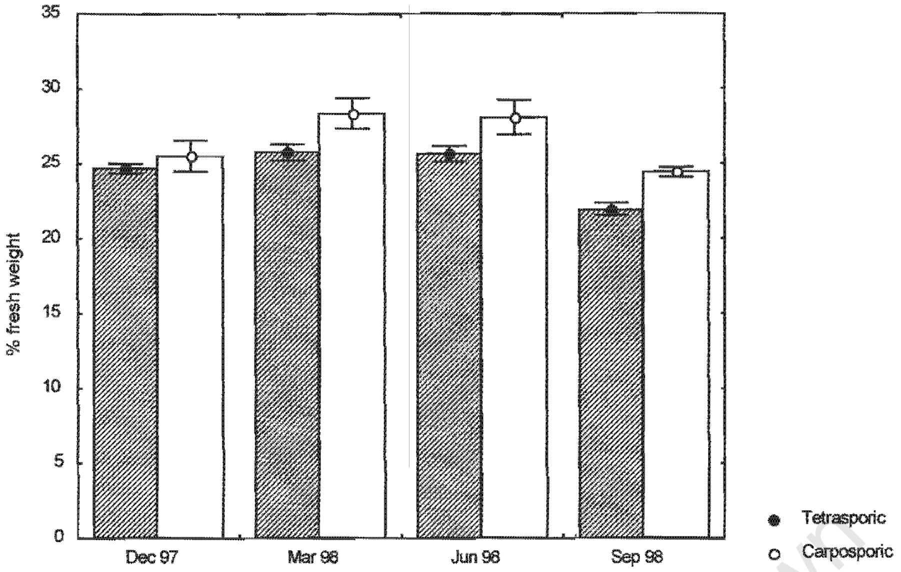

Fig. 3.4: Seasonal variation in dry matter content of *Grateloupia filicina* from December 1997 to September 1998. Results are expressed in % fresh weight. Mean ± SE. Values of n for T (tetrasporic) and C (Carposporic) fronds: Dec 97 T = 47 C = 3. Mar 98 T = 33 C = 10. Jun 98 T = 30 C = 15;. Sep 98 T = 37 C = 11.

Discussion

The very low yield of carrageenan from both life phases of *Grateloupia filicina* from the west coast of South Africa differs from that found for the species growing elsewhere. *Grateloupia filicina* from Korea had a yield of 35% (Park *et al.* cited in Chopin *et al.* 1994) and from Hawaii of 34 to 37% of dry mass (Zablackis and Perez 1990). Extraction procedures also involved alcohol precipitation. A review of 26 species within the Cryptonemiales indicated a lower carrageenan yield in general compared to the Gigartinales (Chopin *et al.* 1994). Yields ranged from 4.8 to 73.8% of the dry weight. Although disappointing in terms of phycocolloid yield this species may still have potential for use in the food industry.

Grateloupia filicina was highly dominated by the tetrasporophytic phase throughout the study period. The seaweed was collected from pools that occurred in the high-intertidal zone which probably experience physical stresses such as temperature and salinity fluctuations and high light intensities. Large numbers of the cushion star (*Patiriella exigua* (Lamarck), which feeds on diatoms and algal sporelings (Branch and Branch 1980), were often found on the fronds. The diploid phase is possibly more capable of withstanding such environmental stresses.

Few studies report on phase distribution within *Grateloupia* so the frequency of tetrasporophyte dominance is uncertain. *Grateloupia acuminata* was collected in April 1990 and March 1991 in

Central Japan and only tetrasporophytes were encountered (Iima *et al.* 1995). However, this species was collected as drift plants from a subtidal population so phases comprising the attached population were not investigated. *Aeodes orbitosa* (Suhr) Schmitz, a member of the Halymeniaceae on the South African west coast, had similar representations of gametophytes and tetrasporophytes in late summer when most plants are reproductive (Bolton and Levitt 1992).

It is usually the diploid phase that dominates implying that diploidy has inherent advantages over haploidy. Examples of diploid dominance include: *Eucheuma isiforme* (C. Agardh) J. Agardh (Dawes *et al.* 1974); *Iridaea cordata* from California (Turner) Bory (Hansen and Doyle 1976) *Gelidium pristoides* from the south coast of South Africa (Carter 1985); the subtidal species *Plocamium cartilagineum* and *Cryptopleura ramosa* from Isle of Man (Kain 1986); *Gelidium robustum* from the inter- and sub-tidal in California (Melo and Neushul 1993) and certain populations of *Gracilaria* species from various parts of the world (Kain and Destombe 1995).

This species complies with the general pattern of greatest mass in summer for many rhodophytes in general (Kain and Norton 1990), and those on the South African west coast in particular (Bolton and Levitt 1992; Bolton and Joska 1993; Levitt 1998). The few fronds in which no fertile structures could be detected were not much different in size to fertile fronds. The main axes of these fronds were generally well developed and it appeared that the distal portions of the thalli might have been lost. As fertile structures of tetrasporic fronds tended to occur predominantly in the ultimate branchlets (see Chapter 4) it is possible that these were tetrasporic fronds whose ultimate branches had deteriorated after spore release. It is also possible that these are male gametophytes as this species has been described as both monoecious (Irvine 1983) and dioecious (Womersley 1994).

The dry mass content, at 22 to 28%, is comparable to that found for *Sarcothalia scutellata* (See Chapter 2) and *Chondrus crispus* (Chopin and Floc'h 1992). As expected, the values are somewhat higher than those obtained for the mid- to low-intertidal *Sarcothalia scutellata* (21 to 24%). It thus appears that seaweed high on the shore have a higher dry matter content which may be related to physical stresses encountered here. Chopin and Floc'h (1992) found seasonal variation in dry matter content but with maxima occurring in both winter and summer whereas no seasonal variation was apparent for *Sarcothalia scutellata*. The decrease in dry matter in September 1998 might be related to the initiation of a growth spurt in spring as greatest frond mass was achieved in the summer. Higher growth rates have been noted to correlate with lower dry matter and carrageenan content in *Chondrus crispus* (Neish *et al.* 1977). There was no clear relationship between carrageenan content and dry mass content in this study as, although a lowered carrageenan content occurred in the tetrasporophytes of *Grateloupia filicina* in

September, it was also low in March and June. The carposporic fronds had comparably low contents at all periods measured. The carposporic phase had a higher dry mass content than the tetrasporic phase in September 1998 otherwise there was little difference between the phases, as occurred for *Sarcothalia scutellata*.

Chapter 4

Seasonal fertility, fecundity and germination of *Sarcothalia scutellata* (Hering) Leister (Gigartinaceae, Rhodophyta) from the west coast of South Africa

Introduction

This study investigated reproductive characteristics of a population of *Sarcothalia scutellata* (Hering) Leister on the west coast of South Africa. Seasonal variation in reproductive characteristics were examined to determine if fronds were fertile year round and if there is a period of maximum fertility and fecundity. Such knowledge is important if a species is to be cultured from spores. Investigation of fertility involved determining the number of reproductive structures produced by each phase, namely, the number of tetrasporangial sori or cystocarps produced. The tetrasporangial sori (Fig. 4.1) occur in the inner cortex and outer medulla and consist of circular clusters of numerous cruciately divided spores (Bold and Wynne 1978; Hommersand *et al.* 1993; Stegenga *et al.* 1997). The cystocarps (Fig. 4.2) are rounded projections occurring towards the terminal portions of the fronds (Bold and Wynne 1978; Hommersand and Fredericq 1990; Hommersand *et al.* 1993; Stegenga *et al.* 1997). Both these structures can be easily seen by the naked eye (Fig. 4.3). Investigation of fecundity involved determining the number of spores released per reproductive structure.

Environmental temperature changes may affect reproduction (Lüning 1990). A variety of warm temperate species listed in Lüning (1990) indicated that spore formation occurred largely between 10 to 20°C. Gamete formation showed greater variation but was also in the region of 10 to 20°C. The west coast of South Africa falls within the Benguela Marine Province, which is intermediate between warm temperate and cold temperate, as mean monthly temperatures never fall below 11°C and in the warmest month are as low as 14°C in certain areas and up to 20°C in northern Namibia (Bolton and Anderson 1997). It has also been demonstrated that reproduction is stimulated by short days (Dring 1982). However, greatest numbers of reproductive structures have also been reported in summer (Melo and Neushul 1993). Two other carrageenophytes that occur in the mid- to low-intertidal zone of the west coast of South Africa, *Sarcothalia stiriata* (Turner) Leister and *Gigartina polycarpa* Kützing were fertile throughout the year and did not show any seasonal variation in fertility (number of tetrasporangial sori or cystocarps produced) (Levitt 1998). *Gigartina polycarpa* produced greater numbers of carpospores in summer whereas

Sarcothalia stiriata did not show seasonal variation in carpospore production and neither species showed seasonal variation in the number of tetraspores produced.

The presence of cystocarps and tetrasporangial sori does not necessarily guarantee that spores will be released. At certain times of the year in Indian populations of *Gracilaria* and *Gracilariopsis*, although reproductive structures were present, spores were not shed in the laboratory (Kain and Destombe 1995). It has also been reported that the period of greatest spore release did not always coincide to the period in which the greatest numbers of reproductive structures were produced (Melo and Neushul 1993).This confounds the attempt to indicate an optimal period for the collection of fertile fronds. Spore release should be timed for when conditions are favourable for settling and sporeling development as most algal spores do not appear to possess reserves to allow for long quiescent periods such as occurs in seeds of terrestrial plants.

The population of *Sarcothalia scutellata* was numerically dominated by fronds of the gametophyte life-history phase (see Chapter 2). Differences in fertility and fecundity between the two phases were examined to determine if these factors could be contributing to numerical dominance of the gametophyte phase. The G:T ratio for this species was fairly close to the 2.8 ratio obtained in demographic models of triennial and perennial thalli that followed the simplest life-history sequence, namely four tetraspores per tetrasporophyte and one carpospore per male-female gametophyte pair (Scrosati and DeWreede 1999). It was thus noted whether tetrasporangial sori produced four times as many spores as did cystocarps.

Red algal spores are non-motile and symmetrical with diameters of 15 to 120 µm (Lobban and Harrison 1994). Various methods have been evolved by seaweeds to improve spore settling. The presence of a loosely binding mucilage in many Gigartinales may aid settling (Jones 1957; Coon *et al.* 1971; Tveter and Mathieson 1976; Boney 1978; Pacheco-Ruiz *et al.* 1989). Such aggregated spores would sink faster than single spores. Five types of spore germination have been described for the Rhodophyta but intermediate types prevent clear-cut categories (Guiry 1990). The spores of *Sarcothalia scutellata* corresponded most closely to that described as *Dumontia* type. Environment is thought to have little influence on the rate of spore germination as a result of their small size, low content of food reserves and mostly lacking resistant walls in contrast to that found in terrestrial seeds which can time germination to favourable conditions (Dring 1982). Thus it appears that germination of algal spores cannot be delayed. However, germinated zoospores of brown algae have been noted to enter a long period of stasis during periods of darkness which implies that they must have some form of food reserves. It has been speculated that the rapid germination may be to ensure secure attachment to the substratum (Dring 1982). Differences in germination success have been recorded for red algae, with seasonal variation occurring in *Gelidium robustum* (Gardner) Hollenberg *et* Abbott (Melo and Neushul 1993), higher germination rates in tetraspores than in carpospores of *Chondrus crispus* Stackhouse (Scrosati *et al.* 1994) and with spores that settled in clumped positions having higher germination success rates than those that had fallen in isolated positions (Carter 1985).

In this study, germination of the released spores was studied in culture to see if a variation exists between the different seasons, between the tetrasporic and carposporic phases and between spores that had settled in clumped or isolated positions.

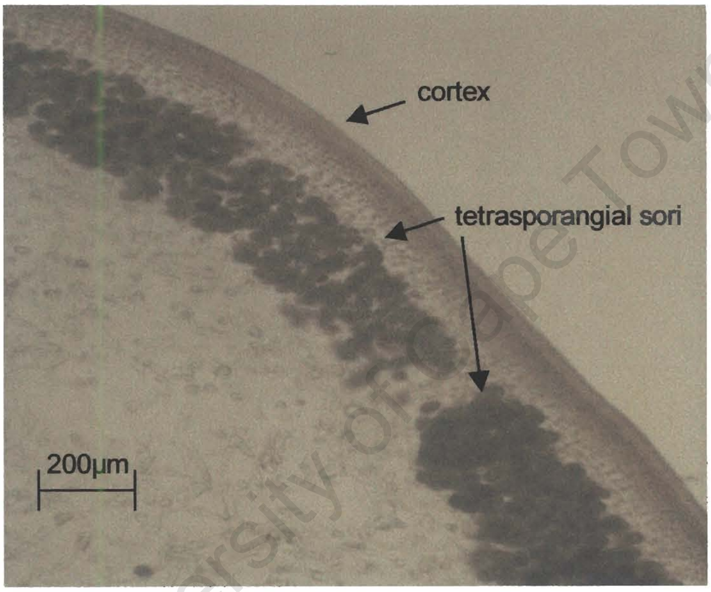

Fig. 4.1: Cross-section through tetrasporangial sori of *Sarcothalia scutellata.*

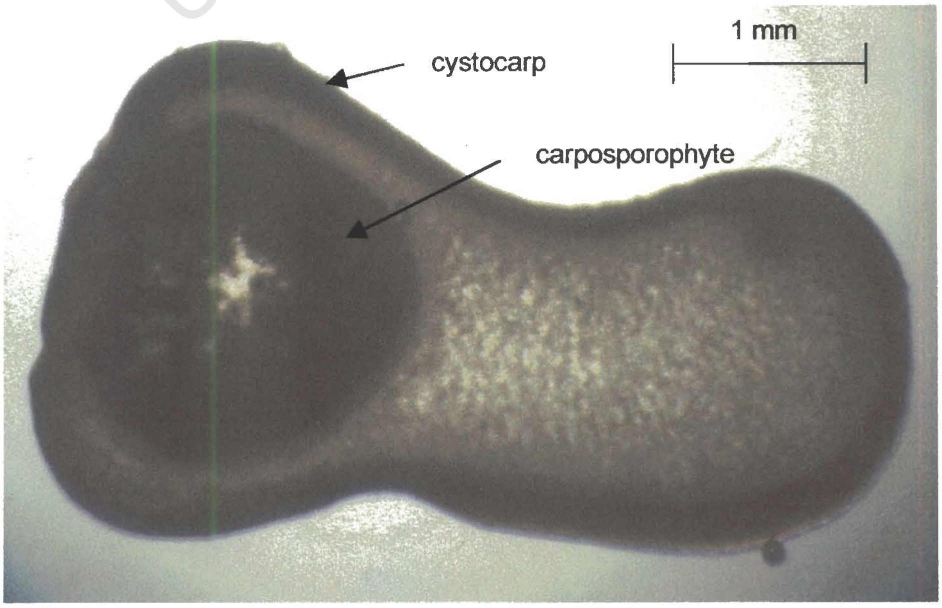

Fig. 4.2: Cross section through frond and cystocarp of *Sarcothalia scutellata.*

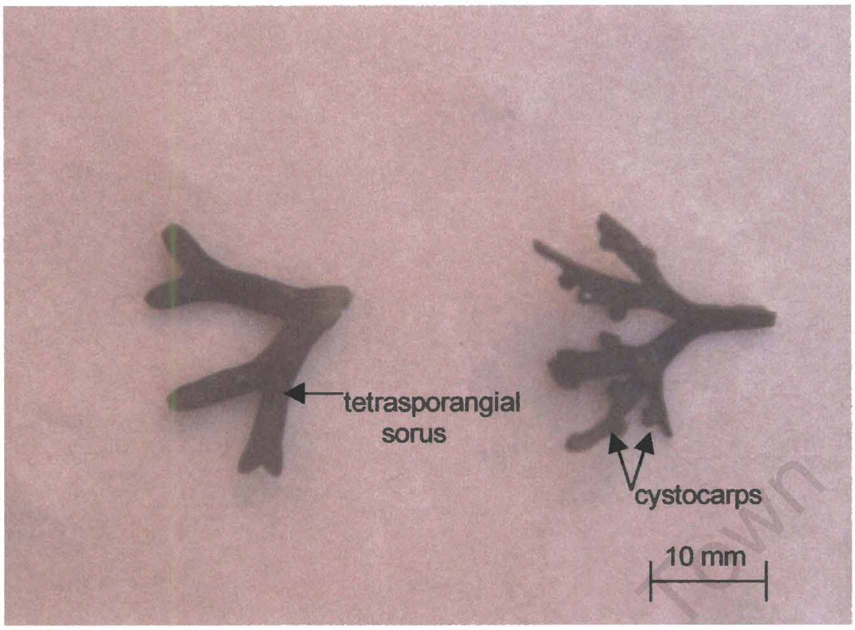

Fig.4.3: Fertile frond tips of *Sarcothalia scutellata*

Method

Clumps of the seaweed *Sarcothalia scutellata* that were collected as described in the methods of Chapter 2 in December 1997, March 1998, June 1998 and September 1998 were utilized for the investigation of reproductive characteristics. Spore discharge has been noted to be related to the tidal cycle in intertidal tropical red algae where it occurred when thalli were immersed (Ngan and Price 1983). Thus to prevent spore release during transportation no seawater was enclosed in the bags. Time constraints prevented the assessments of reproduction to be performed on the same day as collection thus the seaweed was stored overnight in sealed plastic bags in the dark at 0°C. This maintained the fronds in a healthy condition.

The number of reproductive structures produced was determined by counting the number of tetrasporangial sori or cystocarps on fertile tetrasporic and cystocarpic blades. The fresh mass of each blade was measured to determine the number of fertile structures per gram of fresh mass. The random collection of 50 clumps per sample period and the varying number of fertile fronds within clumps prevented equal sample sizes for fertile tetrasporic and cystocarpic fronds. All fertile fronds that were collected were included in the analysis.

To determine the number and pattern of spores released, ten frond tips of each phase bearing either a tetrasporangial sorus or cystocarp were placed in two plastic culture dishes (repli-dishes) per phase. Each of the four culture dishes (two per phase) consisted of five rows of five chambers making a total of 25 × $6cm^3$ chambers per dish (Fig. 4.4). Each thallus section was placed in a separate $6cm^3$ chamber in the first row. The media placed in each chamber was 4ml of third strength (6 $ml.l^{-1}$) Provasoli Enriched Seawater (Provasoli 1968) and GeO_2 (1ml of saturated solution. l^{-1}) to inhibit diatom contamination (Markham and Hagmeier 1982). The culture dishes were placed in a temperature controlled room set to 15°C, under a light intensity of 60µ mol photons m^{-2} s^{-1} and a day: night cycle of 16:8 hours. After 24 hours, the thallus sections were transferred to the next row of five chambers containing 4ml of fresh medium and the number of spores released in the first row of chambers was counted using an inverted microscope. The parent thalli were transferred to new chambers containing fresh medium and the spores released each day counted for five consecutive days. For the first and sometimes second day of spore release dense clusters of spores were released. In order to count these spores the eyepiece scale was positioned over a section of the clumped spores with the 20× objective in place and all spores within the area demarcated by the eyepiece scale (an area of 4 by 100 eye piece units or 20 µm × 500 µm) were counted. The area of the entire spore cluster was then used to estimate the total number of spores that were released. The third to fourth days simply involved counting any spores that could be found within the chambers.

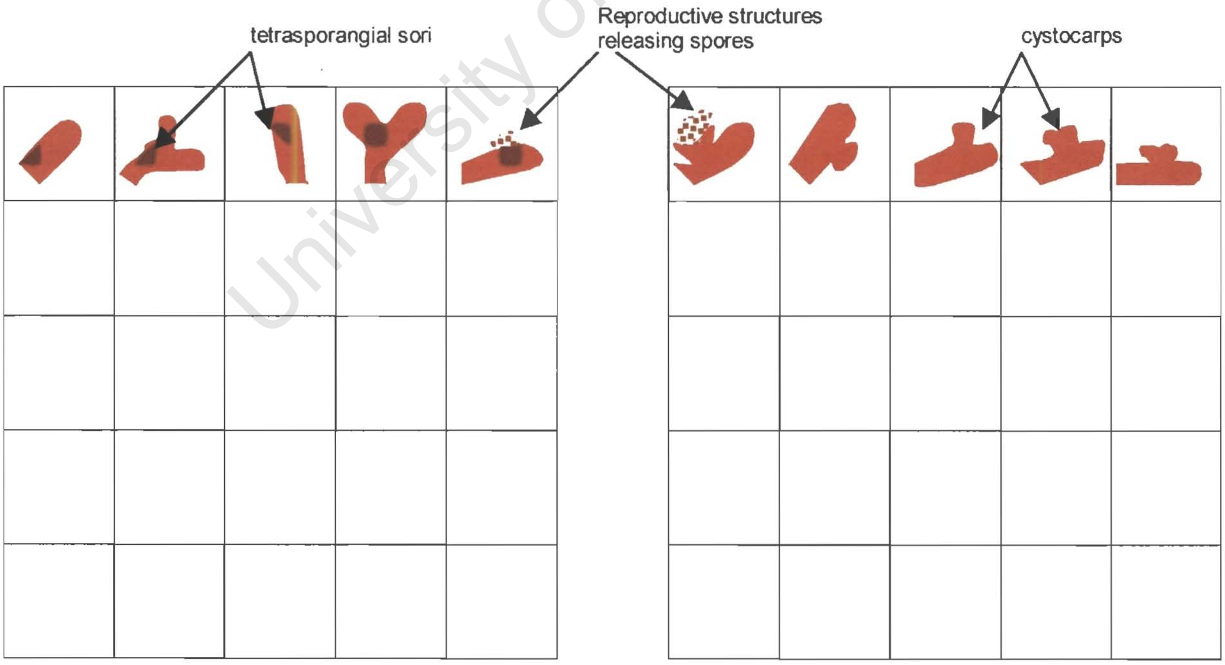

Fig. 4.4: Experimental setup for determining number of spores released per tetrasporangial sorus or cystocarp of *Sarcothalia scutellata* over five consecutive days. A second set of dishes was set up as diagrammed above providing a sample size of ten tetrasporangial sori and ten cystocarps.

A rough idea of the number of spores released per entire thallus was achieved by utilizing mean frond mass (see Chapter 2) and mean number of spores released per reproductive structure. The number of reproductive structures per gram was used to determine the number of reproductive structures per mean frond mass. The number of spores per reproductive structure was then used to determine the number of spores per mean frond mass.

To determine germination success rate, ten fronds bearing tetrasporangial sori and ten bearing cystocarps were rinsed in a surface sterilant, 0.5% Povidone Iodine in seawater, for 20 to 30 seconds (Haritonidis 1992). Fertile frond tips from each of the ten tetrasporic and ten cystocarpic fronds bearing a number of tetrasporangial sori or cystocarps were placed in separate 6cm^3 chambers of plastic culture dishes as used in the previous experiment and which contained the same culture medium. The containers were placed overnight under the same conditions as those in the previous experiment. The fertile frond tips were removed after 24 hours. The percentage of spores that had germinated on the third day after spores were released was calculated. Spores were considered as germinated when cell divisions were noted to occur. Germination success rate was compared between spores from fronds that were collected in different seasons and that had originated from different life history phases. Comparisons were also made between clumped spores, which were those that had settled in dense groupings, and isolated spores, which were those that had settled away from dense groupings with no physical contact with any other spores. Frond tips bearing many reproductive structures were used in this experiment (as opposed to the single reproductive structure per chamber used in the previous experiment) to ensure that each chamber would receive an adequate sampling of clumped and isolated spores.

Results

Number of fertile structures produced

Year-round fertility was encountered for both phases of *Sarcothalia scutellata*. The number of fertile structures per gram of fresh mass varied widely, with raw data values ranging from a minimum of 0.3 to a maximum of 383 tetrasporangial sori, and from 0.7 to 150 cystocarps per gram of fresh mass. A similar seasonal variation occurred for mean number of tetrasporangial sori and cystocarps despite the very different structures (Fig. 4.5). The number of reproductive structures produced was highest in summer (44.8 tetrasporangial sori and 48.3 cystocarps per gram of fresh mass in December 1997) and lowest in winter (30.3 tetrasporangial sori and 28.9 cystocarps per gram of fresh mass in June 1998). Kruskal-Wallis ANOVA by ranks indicated that the number of tetrasporangial sori produced was not significantly different for each season (Table

4.1(A)). Significantly fewer cystocarps were produced in June 1998 than in December 1997 and September 1998. Significantly fewer cystocarps were also produced in March 1998 compared to December 1997. In comparing the phases to each other at each month there is no significant difference in the number of reproductive structures produced (Table 4.1(B)).

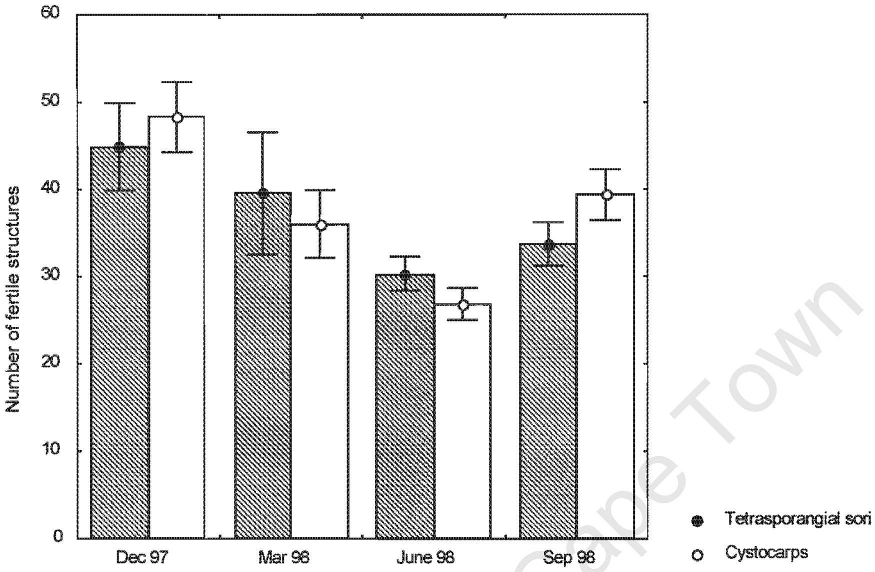

Fig. 4.5: Seasonal variation in number of fertile structures (tetrasporangial sori or cystocarps) per gram of fresh mass of fronds of *Sarcothalia scutellata* from December 1997 to September 1998. Mean ± SE. Values of n for tetrasporic (T) and cystocarpic (C) fronds: Dec 97 T = 59 C = 39; Mar 98 T = 55 C = 55; Jun 98 T = 61 C = 106; Sep 98 T = 37 C = 66.

(A)	**Tetrasporangial sori**				**Cystocarps**			
	Dec 97	**Mar 98**	**Jun 98**	**Sep 98**	**Dec 97**	**Mar 98**	**Jun 98**	**Sep 98**
Dec 97		0.400	0.110	0.360		0.036*	0.000***	0.188
Mar 98	0.400		0.599	0.821	0.036*		0.095	0.223
Jun 98	0.110	0.599		0.344	0.000***	0.095		0.001***
Sep 98	0.360	0.821	0.344		0.188	0.223	0.001***	

(B)		**Tetrasporic**			
		Dec 97	**Mar 98**	**Jun 98**	**Sep 98**
Cystocarpic	**Dec 97**	0.386			
	Mar 98		0.921		
	Jun 98			0.263	
	Sep 98				0.312

Table 4.1: Significant differences in numbers of fertile structures produced **(A)** between different seasons and **(B)** between the two phases. (Kruskal-Wallis ANOVA by ranks). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

Number and pattern of spore release

Both the tetrasporangial sori and the cystocarps released numerous spores (in the tens of thousands) simultaneously (Fig. 4.6; Fig. 4.7). As spores were released, they formed a continuous stream that was loosely bound in mucilage. Soon after spore release (within 12 to 24 hours), the spores would attach to the substratum and very few loose spores were noted. Thallus sections remained reddish-brown and firm until all sori were released; thereafter, they became green and soft.

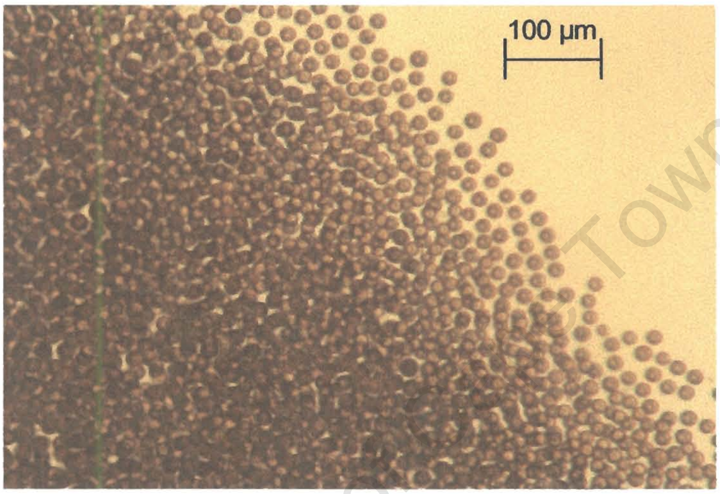

Fig. 4.6: Tetraspores of *Sarcothalia scutellata* soon after spores were released from the sorus.

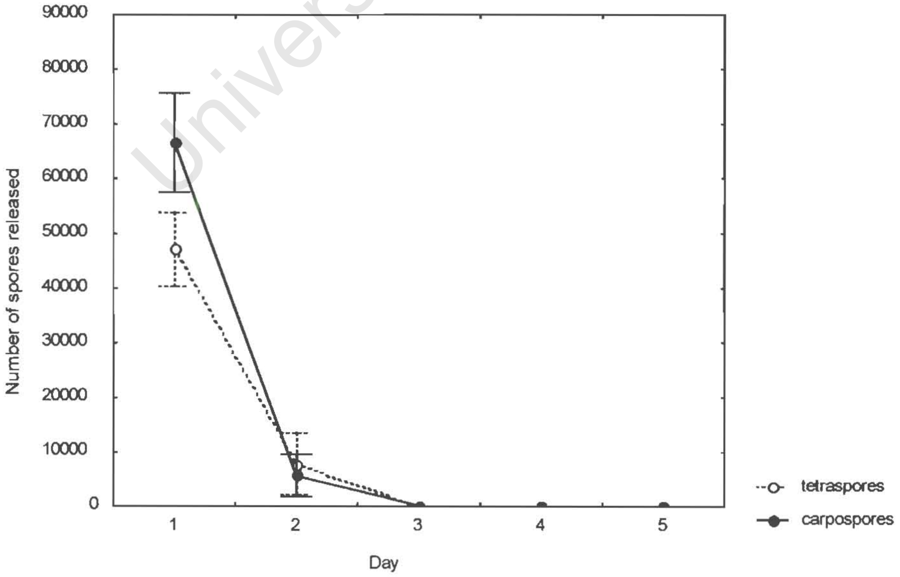

Fig. 4.7: Number of spores released on a daily basis from individual tetrasporangial sori and cystocarps of *Sarcothalia scutellata*. Combined data from March 1998, June 1998 and September 1998. Data of December 1997 excluded as cystocarps failed to release spores. Mean ± SE; n = 30.

The mean number of tetraspores released per tetrasporangial sorus ranged from 44000 spores in June 1998 to 73000 spores in September 1998 but did not show significant seasonal variation (Fig.4.8). The mean number of carpospores released per cystocarp increased from March 1998 (38000 carpospores) to September 1998 (109000 carpospores). The low mean in December 1997 (6000 carpospores) was due to all but one of the cystocarps used in this experiment failing to release carpospores whereas all ten cystocarps released spores in the other three months tested. In comparing those months in which all ten cystocarps released carpospores, significant differences occurred between the number of carpospores released in March 1998 and those released in September 1998 ($p = 0.005$). In comparing the number of spores released between the two phases, fewer tetraspores were released than carpospores in September 1998 ($p = 0.041$), whereas fewer carpospores than tetraspores were released in December 1997 ($p = 0.001$) due to only one cystocarp releasing spores. Ratios obtained for the mean number of carpospores to mean number of tetraspores were 0.09 in December 1997, 0.80 in March 1998, 1.59 in June 1998 and 1.49 in September 1998. Therefore the reproductive structures did not appear to produce four tetraspores for each carpospore. The mean number of spores produced per frond each season is indicated in Table 4.2.

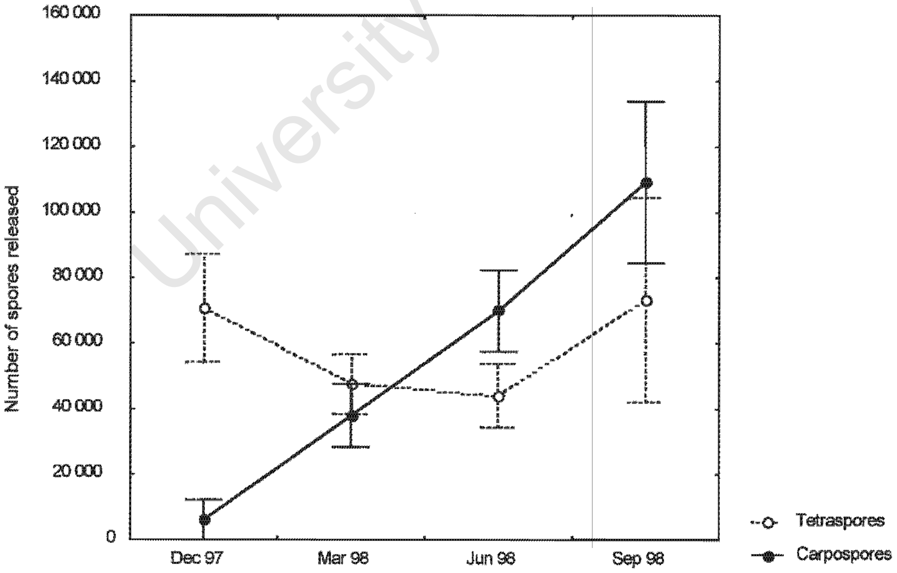

Fig. 4.8: Total number of spores released per tetrasporangial sorus and cystocarp of *Sarcothalia scutellata* from December 1997 to September 1998. Only one of the cystocarps released carpospores in December 1997. Mean ± SE; $n = 10$.

Month	Tetrasporic	Cystocarpic
Dec 97	13 986 522	883 021
Mar 98	5 409 266	3 332 684
Jun 98	1 805 138	2 524 229
Sep 98	7 726 800	8 177 108

Table 4.2: Mean number of spores per tetrasporic and cystocarpic frond of from *Sarcothalia scutellata* from December 1997 to September 1998.

Germination success rate

Germination of spores was indicated when the spore contents began to divide (Fig. 4.9). Both tetraspores and carpospores had very high germination rates for clumped spores throughout the study period. Mean values for clumped tetraspores ranged from 88 to 93% and for clumped carpospores from 88 to 95% (Fig. 4.10). In both phases, the isolated spores had much more variable germination rates ranging from means of 61 to 70% for the tetraspores and 36 to 86% for the carpospores (Fig. 4.10). The isolated tetraspores did not show significant seasonal differences in germination rates. The isolated carpospores of December 1997 had significantly lower germination rates than those of March 1998 ($p = 0.009$), June 1998 ($p = 0.019$) and September 1998 ($p < 0.0001$). Those of March 1998 were also significantly different to those of September 1998 ($p = 0.041$).

A comparison of germination rates between the phases showed no differences in germination rates between clumped tetraspores and carpospores or between isolated tetraspores and carpospores on each date. A comparison of settling position indicated that fewer isolated spores germinated than did clumped spores on each date with the exception of carpospores in June 1998 when isolated carpospores did not show significantly lower germination rates than did the clumped carpospores ($p = 0.069$).

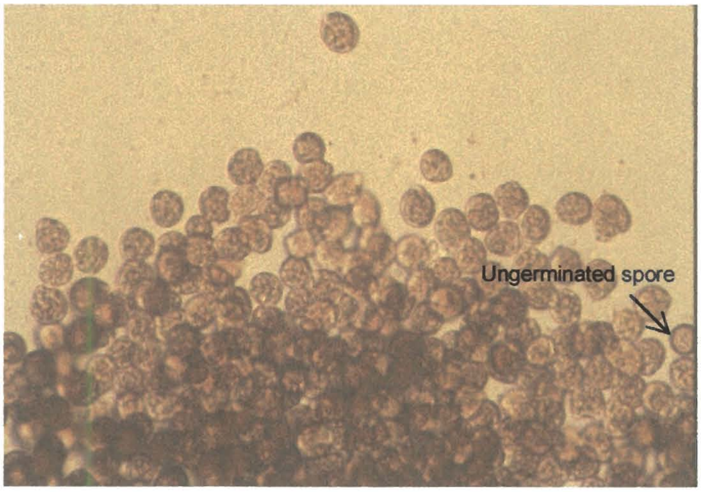

Fig. 4.9 Tetrasporelings of *Sarcothalia scutellata* 24 hours after spore release. Most spores have germinated as indicated by divisions within spore. Ungerminated spore indicated by arrow.

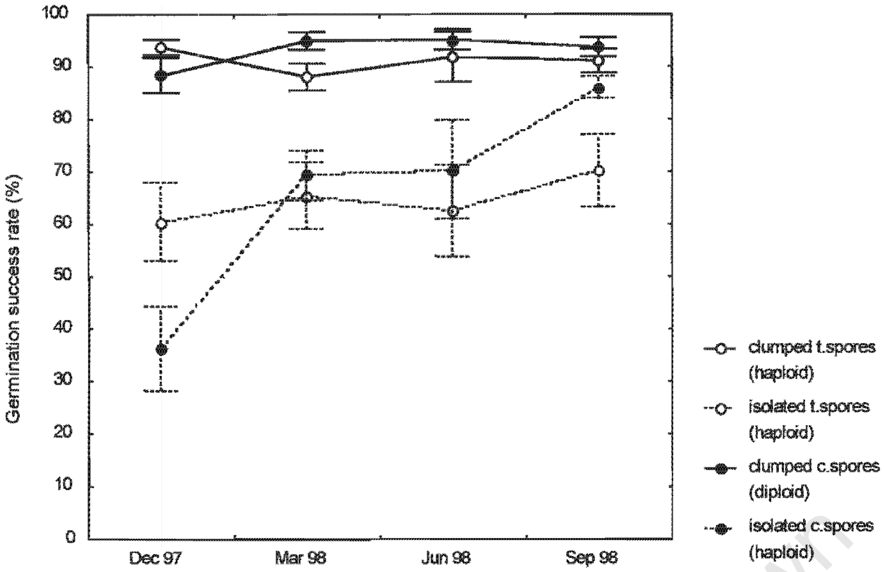

Fig. 4.10: Germination success rate of clumped and isolated tetraspores (t.spores) and carpospores (c.spores) of *Sarcothalia scutellata* from December 1997 to September 1998. Mean ± SE; n = 10.

Discussion

As fertile fronds of *Sarcothalia scutellata* are encountered throughout the year, this species could be collected at any time for culturing from spores. The optimal time for collection as indicated by the number of reproductive structures is summer.

Two other species within the Gigartinaceae that are abundant on the South African west coast in the mid- to low-intertidal zone, *Sarcothalia stiriata* and *Gigartina polycarpa*, were noted to occur on exposed rocks between the rock pools containing *Sarcothalia scutellata*. Table 4.3 shows a comparison between the numbers of reproductive structures produced by these species as well as another member of the Gigartinaceae, *Chondrus crispus* from the North Atlantic.

	Sarcothalia scutellata		***Gigartina polycarpa***		***Sarcothalia stiriata***		***Chondrus crispus***	
	T	C	T	C	T	C	T	C
			500.17	140.69	181.00	55.83		
Spring	34 ± 20.1	40 ± 23.6						
Summer	45 ± 38.5	48 ± 31.1					151 ± 116	23 ± 21
Autumn	40 ± 52.1	36 ± 28.8					244 ± 174	102 ± 59
Winter	30 ± 20.0	27 ± 18.4						

Table 4.3: Comparison of results for numbers of reproductive structures per gram of fresh mass obtained for four species within the family Gigartinaceae: *Sarcothalia scutellata* from this study; *Gigartina polycarpa* and *Sarcothalia stiriata* from Levitt (1998) and *Chondrus crispus* from Scrosati *et al.* (1994). T= tetrasporangial sori; C = cystocarps. Mean ± SD. Values for *G. Polycarpa* and *S. stiriata* are means of values obtained for summer and winter data.

Sarcothalia stiriata produced slightly more cystocarps and far more tetrasporangial sori per gram of fresh mass, whereas *Gigartina polycarpa* produced far more reproductive structures in both phases than *Sarcothalia scutellata*. No seasonal variation was found in the number of reproductive structures or in the percentage of the thallus that was fertile for either of these two species. The number of reproductive structures in *Sarcothalia scutellata* was also somewhat less but comparable to that found for *Chondrus crispus*, a species that has a number of similarities, including a superficial resemblance in gross morphology, occurrence in clumps, similar frond mass and size of the cystocarps (1 to 3 mm in diameter) (Scrosati *et al.* 1994). The latter species exhibited a contrasting seasonal pattern as fewer reproductive structures were produced in summer than in autumn. A study on a species from the Gelidiaceae, *Gelidium robustum* from California, found that 50 to 100 tetrasporangial sori per gram of fresh mass were produced in autumn/winter (Melo and Neushul 1993) which is comparable to that of *Sarcothalia scutellata*. Greatest numbers of reproductive structures also occurred in summer but the seasonal variation was greater as 300 to 400 sori per gram of fresh weight were then produced. The authors found that the greater numbers of fertile structures corresponded to periods of increased water temperatures. The decrease in winter was attributed to the loss of necrotic tetrasporangial sori after spores had been released and stormier seas occurred.

There is concordance in the seasonal patterns of frond mass (see Fig. 2.5 in Chapter 2) and number of reproductive structures produced for both phases. The greatest number of reproductive structures occurred in summer which was also the period of greatest frond mass whereas fewest fertile structures occurred in winter, the period of lowest frond mass. The increased numbers of reproductive structures would contribute to frond mass. However, as the fertile structures tended to occur only towards the tips of fronds it seems unlikely that the weight contributed was that significant.

No obvious relationship was found between the number of reproductive structures produced and the number of spores released. Although most cystocarps of *Sarcothalia scutellata* were produced in summer (December 1997) the fronds did not shed spores as readily as during the other seasons. There is the possibility that the lower incidence of spore release in summer was due to spores having been already released in the wild. Greatest numbers of carpospores were shed per cystocarp in spring (mean of 109000 carpospores). The number of tetraspores released per tetrasporangial sorus, like the number of sori per gram of fresh mass, did not show significant seasonal variation. Melo and Neushul (1993) found a lack of correspondence in these two factors in *Gelidium robustum* as the highest numbers of tetrasporangial sori occurred in spring whereas the greatest number of tetraspores were released per sorus in winter.

Far more tetraspores were released per sorus of *Sarcothalia scutellata* than were produced within sori of *Gigartina polycarpa* and *Sarcothalia stiriata* (Table 4.4). However, both these species (especially *Gigartina polycarpa*) produced far more tetrasporangial sori per gram of fresh mass (Table 4.3), which would compensate for the fewer spores per sorus. The total number of spores released per cystocarp of *Sarcothalia scutellata* was, however, less than that produced by cystocarps of *Gigartina polycarpa* and *Sarcothalia stiriata*. Both *Gigartina polycarpa* and *Sarcothalia stiriata* showed no seasonal variation in the number of tetraspores produced (Levitt 1998), as is the case in this study. However, as occurred for *Sarcothalia scutellata*, seasonal differences in number of carpospores per cystocarp also occurred for *Gigartina polycarpa* with more produced in summer, whereas *Sarcothalia stiriata* did not show seasonal differences. Results are also presented for the number of spores released from reproductive structures of *Chondracanthus canaliculatus* (Harvey) Guiry (previously *Gigartina canaliculata* Harvey) which released far fewer than that of *Sarcothalia scutellata.*

	Sarcothalia scutellata		***Gigartina polycarpa***		***Sarcothalia stiriata***		***Chondracanthus canaliculatus***	
	t.spores	c.spores	t.spores	c.spores	t.spores	c.spores	t.spores	c.spores
							1 500 to 3 000	3 000 to 6000
Spring	73 000	109 000						
Summer	71 000	6 000	688	1 429 000	6 962	159 100		
Autumn	48 000	38 000						
Winter	44 000	70 000	675	1 293 000	6 299	143 900		

Table 4.4: Comparison of results for numbers of spores released per tetrasporangial sorus or cystocarp obtained for four species within the family Gigartinaceae: *Sarcothalia scutellata* from this study; *Gigartina polycarpa* and *Sarcothalia stiriata* from Levitt (1998) and *Chondracanthus canaliculatus* from Pacheco-Ruiz *et al.* (1989). t.spoores = tetraspores; c.spores = carpospores.

The number of spores released was also far higher than that reported for a species within the Gelidiaceae, *Gelidium robustum*, which released about 450 to 750 spores per tetrasporangial sorus over three days in winter and 150 in spring/summer (Melo and Neushul 1993). Again, increased numbers of tetrasporangial sori in summer (300 too 400 sori per gram fresh mass) would compensate for lower spore output per sorus.

Differences in fertility and fecundity did not appear to contribute to gametophyte dominance in *Sarcothalia scutellata*. The number of tetrasporangial sori per gram of fresh mass was not significantly different to the number of cystocarps. Both phases tended to release large quantities of spores simultaneously with most spores released within the first 24 hours. The cystocarps released significantly more spores than did the tetrasporangial sori in spring which would encourage tetrasporophyte rather than gametophyte dominance. However, cystocarps showed a decreased inclination to release spores during summer. The significantly larger frond size of tetrasporophytes in spring and summer (see Fig. 2.5 of Chapter 2) may compensate for a lower fecundity.

A carpospore to tetraspore ratio of 0.25 might be expected to occur in species that have a G:T ratio close 2.8 as occurred for *Sarcothalia scutellata*. This is the G:T ratio obtained in demographic models of triennial and perennial thalli that produced four tetraspores per tetrasporophyte and one carpospore per male-female gametophyte pair (Scrosati and DeWreede 1999). However, the ratio of mean number of carpospores per cystocarp to tetraspores per tetrasporangial sorus did not show this ratio which ranged from 0.81 in autumn to 1.59 in winter. .

Other studies of gametophyte-dominated populations within the Gigartinaceae also reported greater production of carpospores compared to tetraspores which should favour the establishment of the diploid phase. Both *Sarcothalia stiriata* and *Gigartina polycarpa* produced far more carpospores than tetraspores per gram of thallus fresh weight and per m^2 of substratum (Levitt 1998). *Chondrus crispus* released more carpospores than tetraspores in all but two months with a general ratio of 4.7:1 (Bhattacharya 1985). *Chondracanthus canaliculatus* displayed a tendency for more carpospores than tetraspores to be released but differences were not significant (Pacheco-Ruiz *et al.* 1989). This latter study did not however mention phase dominance. Some other factor must therefore be involved that favours gametophyte development.

The large numbers of spores that were released simultaneously from tetrasporangial sori and cystocarps of *Sarcothalia scutellata* were loosely bound by mucilage. The presence of mucilage binding spores was also noted for *Chondrus crispus*, *Mastocarpus stellatus* (Stackhouse) Guiry (previously *Gigartina stellata* (Stackhouse) Batters) (Tveter and Mathieson 1976), *Chondracanthus canaliculatus* (Pacheco-Ruiz *et al.* 1989), *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham (previously *Gracilaria verrucosa* (Hudson) Papenfuss) (Jones 1957) and *Rhodymenia pertusa* (Postels *et* Ruprecht) J. Agardh (Boney 1978). The postulated advantages associated with aggregation of spores include increased sedimentation rate (Coon *et al.* 1971) and reduction of drift from the parental habitat (Pacheco-Ruiz *et al.* 1989). It is also possible that the mucilage promotes coalescence. The advantage of this may include physiological integration promoting survival of smaller fronds (Scrosati and DeWreede 1997) and the subsequent frond crowding allowing the seaweed to withstand environmental stresses (Scrosati and DeWreede 1998). Upright fronds originating from coalesced crusts have been noted to grow faster than those of non-coalesced crusts (Tveter and Mathieson 1976).

The final factor related to reproductive characteristics investigated in this study was that of germination. In both phases the majority of spores tended to settle in dense clumps. Such spores did not exhibit seasonal variation in germination rates for either phase. The two phases did not display differences in germination rates to each other either. High success rates were obtained for the clumped spores, ranging from 88 to 95%. These values are very high compared to that obtained for other species. Tetraspores of *Chondrus crispus* had germination rates of 15% which was higher than the carpospores at 9% (Scrosati *et al.* 1994). Little variation occurred in germination rates over the period studied (June to October). *Gelidium robustum* showed seasonal variation in tetraspore germination with less than 10% germinating in winter and 50 to 60% germinating in spring/summer (Melo and Neushul 1993). The highest germination rates coincided with the period of maximal spore output per plant. *Gelidium pristoides* had germination success rates of 5 to 15% for bispores and 15 to 37% for carpospores (Carter 1985). The clumped settling pattern of carpospores of the latter species was thought to have an influence on the greater success of carpospore germination. It was hypothesized that some germination stimulating compound may be present in greater quantities amongst the clumped spores.

Clumping also appeared to enhance germination within *Sarcothalia scutellata*. It is possible that the lower germination rates for isolated spores was due to a delay rather than an actual failure in germination. It would be interesting to note germination success rate on several consecutive days rather than on just the third day after spore release. It is possible that physical contact stimulates germination in some manner. This enhancement of germination in clumped spores again raises the topic of benefits of coalescence.

In conclusion there do not appear to be significant differences in reproductive capacity in terms of numbers of reproductive structures produced, numbers of spores released and germination rates, to account for gametophyte dominance in *Sarcothalia scutellata*. The production of larger tetrasporic fronds could mean the availability of more tetraspores that might have contributed to gametophyte dominance. For the purposes of mariculturing the species from spores fertile blades could be collected at any time of the year but the optimal period for the collection of cystocarpic fronds would be spring when most spores were released, whereas tetrasporic fronds showed similar fertility and fecundity in all seasons.

Chapter 5

Seasonal fertility, fecundity and germination of *Grateloupia filicina* (Lamouroux) C. Agardh (Halymeniaceae, Rhodophyta) from the west coast of South Africa

Introduction

Reproductive characteristics were investigated to determine if seasonal variation in fertility (number of reproductive structures produced), fecundity (number of spores released) and germination occurs in *Grateloupia filicina* (Lamouroux) C. Agardh. Life-history phases were compared to each other to determine if differences in reproductive characteristics could be contributing to the high level of tetrasporophyte dominance within the population.

Determining the number of fertile structures was more complicated for this species, as they required microscopic examination. Only mature carposporophytes can be viewed as dark spots by the naked eye. The tetrasporangia were not grouped in sori. A very different method was thus required to obtain an idea of fertility to those used for *Sarcothalia scutellata*.

The reproductive structures of the tetrasporophytes consist of cruciately divided tetrasporangia, about 15 by 35 µm, that are scattered throughout the cortex (Fig. 5.1) (Irvine 1983; Womersley 1994; Stegenga *et al.* 1997). It was noted that the tetrasporangia were released from the cortex in their entirety. Sometime after release the four sections moved apart and rapidly formed spherical spores. The latter process occurred within seconds. The carposporophytes consist of dense globose clusters, about 300 µm in diameter, that are found scattered throughout the thallus beneath the cortex (Fig. 5.2).

The number of spores released also required a different method of investigation due to the dispersed arrangement of the tetrasporangia and the difficulty of isolating single carposporophytes. Comparisons of seasonal spore release and between phases were thus achieved by comparing spores released from similar sized thallus sections. Methods to encourage spore release in this species have previously been investigated (Vetter 1996). Desiccation, brief immersion in fresh water and temperature stress (hot and cold) all resulted in a high percentage of spore release. However, this was not appreciably greater than that for non-treated thalli.

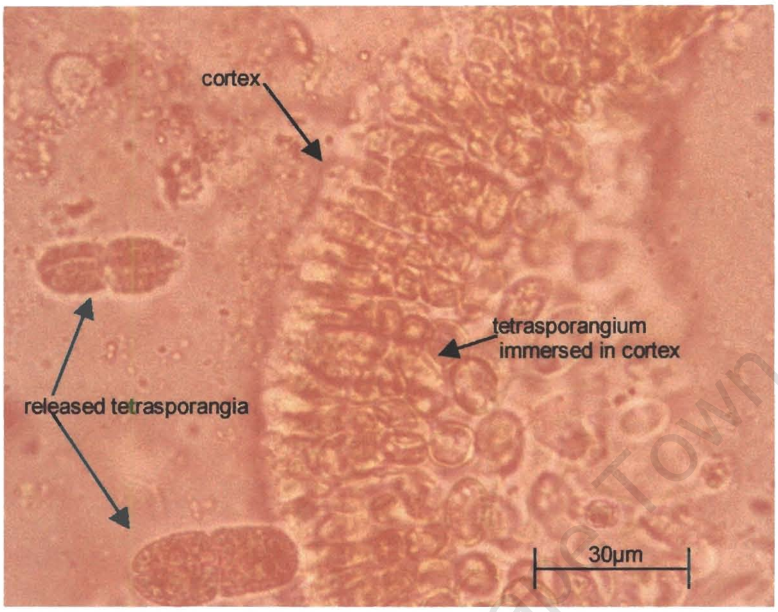

Fig. 5.1: Cross section through tetrasporic frond of *Grateloupia filicina* showing immersed and released tetrasporangia

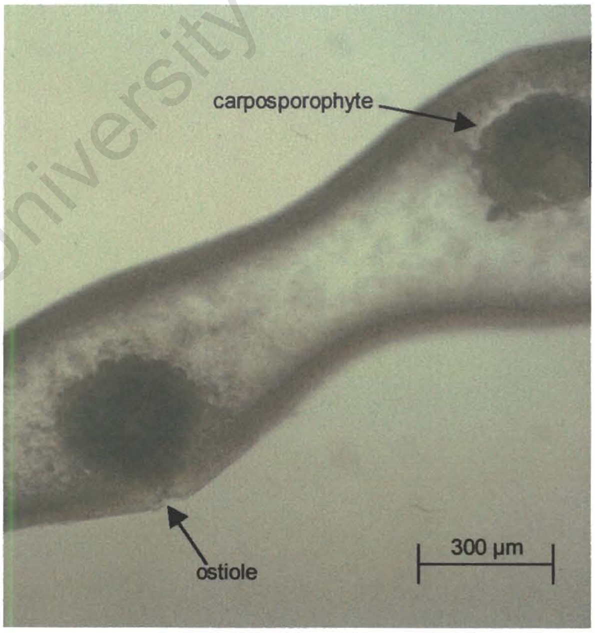

Fig. 5.2: Cross section through carposporic frond of *Grateloupia filicina* showing carposporophytes.

The final factor related to reproductive characteristics that was investigated was that of spore germination. Spore germination in *Grateloupia filicina* resembles either the *Gelidium* or *Nemalion* type of spore germination mentioned in Guiry (1990) where spore contents are described as passing into a protuberance. Algal spores germinate rapidly under a wide range of environmental conditions possibly due to either a lack of structures to promote long term survival or the need to ensure firm attachment (Dring 1982). Germination of spores in culture was investigated to determine if there were differences in success rate between different seasons, phases and clumped and isolated settling positions. In addition, an experiment was conducted to see if temperature had any effect on germination. Previous studies on a variety of algal species suggest that temperature does not have an effect on percentage germination provided temperatures are below lethal limits for the species. For example, there was little difference in germination rates for laminarian zoospores and fucoid zygotes at temperatures from 2-3°C up to the lethal limits for these plants at 20 to 25°C (Dring 1982). Thereafter germination success rates decreased. Experiments on temperate red algal species (two species of *Gelidium* from Chile), found that temperature, photoperiod and irradiance levels did not have significant effects on germination success rate (Correa *et al.* 1985).

Method

Number of reproductive structures

Ten tetrasporic and ten carposporic fronds were randomly selected from each of the four different collections (December 1997; March 1998; June 1998 and September 1998). No seawater was placed within the plastic bags in which fronds were transported to the laboratory to minimize spore release in transit.

The tetrasporangia were noted to occur largely in small, ultimate, terete branchlets, which turned an olive colour when fertile as opposed to the reddish brown of the rest of the thallus. To determine the density of tetrasporangia, three of these branchlets were removed from each frond and the average number of tetrasporangia that occurred within an area of 250 µm × 250 µm on each branchlet was counted.

The total number of fertile branchlets per frond was calculated by measuring the length of the thallus that contained fertile branchlets and then counting the number of fertile branchlets that occurred along three different 30mm lengths of the frond. The average of these three numbers was then used to estimate the total number of fertile branchlets per frond.

To determine the number of tetrasporangia per gram of fresh weight, the number of tetrasporangia per 250 µm × 250 µm was first utilized to determine the number of tetrasporangia occurring in the entire branchlet by measuring the area of the branchlet that was fertile. An average of three measurements was obtained. The number of fertile branchlets (as measured above) was then used to determine the total number of tetrasporangia per frond. The fresh mass of the frond was then measured to determine the number of tetrasporangia per gram of fresh mass.

The fertile areas of the carposporic fronds were not concentrated in the branchlets but were scattered over the thallus. Two methods were used to obtain an idea of frond fertility, firstly, the percentage of the frond that was reproductive and, secondly, the density of carposporophytes. In the first method, the fertile areas were separated from infertile areas and the weights of each were measured. In the second method, the number of carposporophytes that occurred within an approximately 2mm X 2mm section of thallus was counted using 40X magnification. This was performed three times per frond for each of the ten fronds.

Number of spores released

The pattern and number of spores released was determined on five different occasions (October 1997, December 1997, March 1998, June 1998 and September 1998) over five consecutive days. Five fertile fronds of each phase were stored in plastic bags overnight in a dark room at 0°C. Such an environment allowed the fronds to be maintained overnight in good condition as time constraints prevented the immediate measuring of fertility. A fertile section of approximately 4mm^2 in area was cut out of each of the five tetrasporic and five carposporic fronds. The exact area of each section was measured using the microscope. The experiment was conducted in two plastic culture dishes, each of which consisted of 25 square-shaped chambers of 6cm^3 volume (the same type of dishes as diagrammed in Fig.4.4 of Chapter 4). Each thallus section was placed in a chamber containing 4ml of one-third strength Provasoli Enriched Seawater (Provasoli 1968). Diatom contamination was inhibited by adding 1ml.l^{-1} of saturated GeO_2 (Markham and Hagmeier 1982). The dishes were placed at 15°C, under cool white fluorescent lamps at an irradiance of 60µ mol photons m^{-2} s^{-1} and a day:night cycle of 16:8 hours. The fertile thallus sections were transferred daily to new chambers with fresh media for five consecutive days. The number of spores released in each chamber was counted using an inverted compound microscope. A rough approximation of the mean number of spores released per fertile frond was obtained using the mean number of spores released per fertile section, which had an average mass of 0.002g, to calculate the mean number of spores released per mean fresh frond mass each season.

Germination Success Rate

The effect of season, phase and settling position on germination was examined. The experiment was performed in October 1997, December 1997, March 1998, June 1998 and September 1998. On each occasion, twenty-five fertile thallus sections of approximately 6mm^2 from each phase were rinsed for 20 to 30 seconds in 0.5% Povidone iodine in seawater (Haritonidis 1992) and placed in 4ml of culture medium (as described above) in separate culture chambers. The culture dishes were placed in a temperature-controlled room at 15°C, irradiance of 60µ mol photons m^{-2} s^{-1} and a day: night cycle of 16:8 hours. The fertile thallus sections were removed after 24 hours. The percentage of spores that had germinated in both the clumped and isolated positions on the third day after the removal of the parent thalli was calculated.

An additional experiment was performed in October 1997 to see if temperature had an effect on germination. As well as the set of culture dishes that were placed at 15°C, an extra set was prepared in the same way as described above, but were placed in a temperature-controlled room set to 20°C.

Results

Number of reproductive structures

Grateloupia filicina produced fertile material throughout the year. The density of tetrasporangia per 250 µm × 250 µm sections of thallus increased from December 1997 to September 1998 (Fig. 5.3). Kruskal-Wallis ANOVA by ranks indicated that tetrasporangial density was significantly different for each month with the exception of June and September 1998 (Table 5.1).

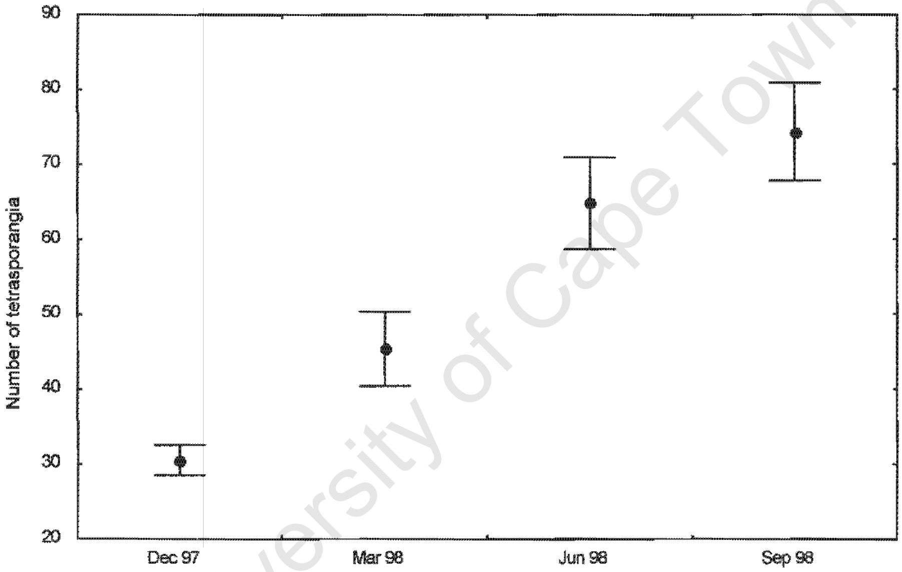

Fig. 5.3: Number of tetrasporangia occurring within a 250µm × 250µm area of thallus of *Grateloupia filicina*. Mean ± SE; n = 10.

Date	Mean	Dec 97	Mar 98	June 98	Sep 98
Dec 97	30.5		0.034*	0.001***	0.000***
Mar 98	45.4	0.034*		0.034*	0.003***
Jun 98	64.8	0.001***	0.028*		0.496
Sep 98	74.4	0.000***	0.003***	0.496	

Table 5.1: Significant differences in density of tetrasporangia of *Grateloupia filicina*. (Kruskal-Wallis ANOVA by ranks). * $p < 0.05$; ** $p < 0.01$; ***$p < 0.005$.

The fronds however, did not necessarily produce more tetrasporangia at each successive period because when the number of fertile branchlets per frond was taken into account a different pattern emerged. The greatest numbers of fertile branchlets were produced in September 1998 and the lowest in June 1998 (Fig. 5.4). Significant differences are indicated in Table 5.2.

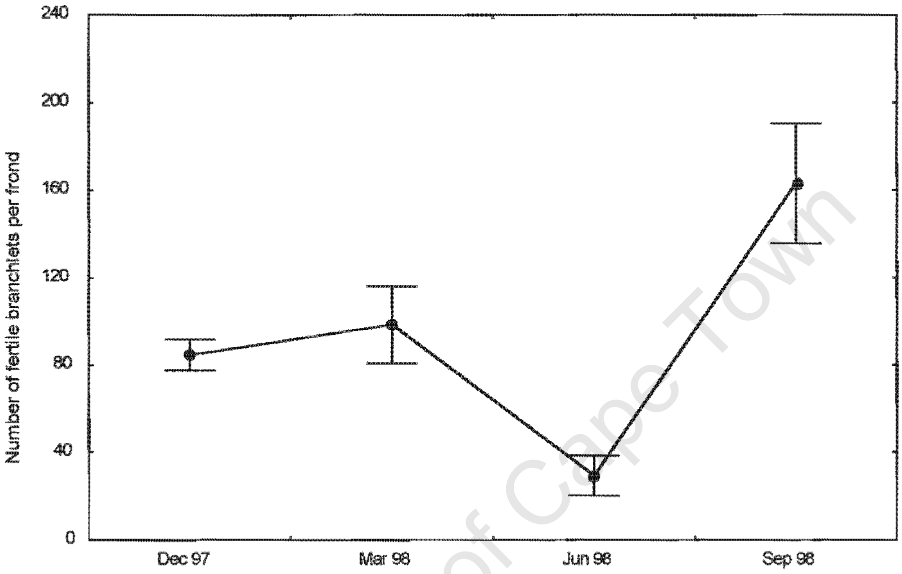

Fig. 5.4: Number of fertile branchlets per frond of *Grateloupia filicina* in December 1997, March 1998, June 1998 and September 1998. Mean ± SE; n = 10.

Date	Mean	Dec 97	Mar 98	Jun 98	Sep 98
Dec 97	84.9		0.496	0.002***	0.034*
Mar 98	98.6	0.496		0.003***	0.096
Jun 98	29.5	0.002***	0.003***		0.001***
Sep 98	163.1	0.034*	0.096	0.001***	

Table 5.2: Significant differences in number of fertile branchlets per frond of *Grateloupia filicina*. (Kruskal-Wallis ANOVA by ranks). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

The mean number of tetrasporangia produced per gram of seaweed ranged from 200 000 in June 1998 to 1 600 000 in September 1998 (Fig 5.5). The seasonal pattern is similar to that of the number of fertile branchlets per frond.

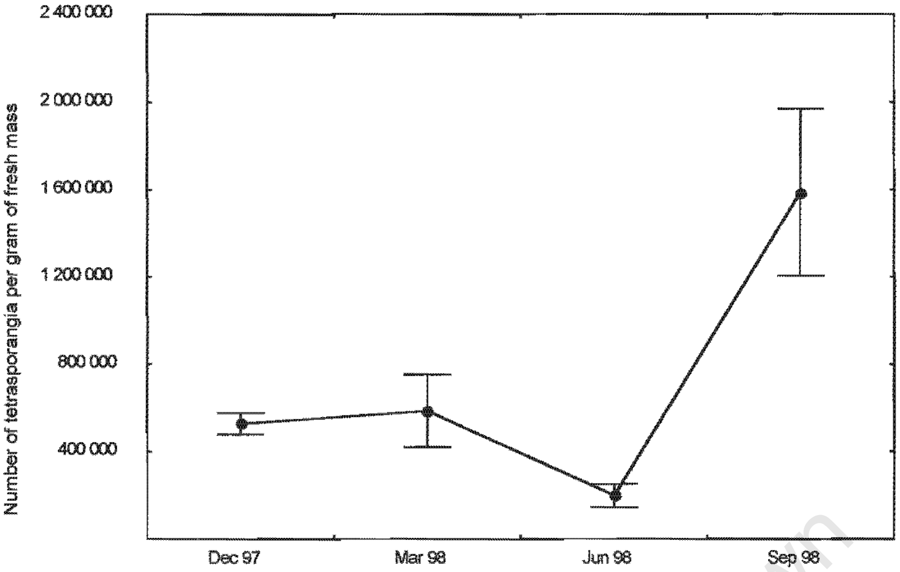

Fig. 5.5: Number of tetrasporangia per gram of fresh mass of *Grateloupia filicina* in December 1998, March 1998, June 1998 and September 1998. Mean ± SE; n = 10.

The percentage of the carposporic fronds that was reproductive ranged from a mean of 42% in March 1998 to 75% in September 1998 (Fig. 5.6). Insufficient carposporic fronds in the December 1997 collection (only 3 fronds) prevented an equal sample size for each season. Kruskal-Wallis ANOVA by ranks revealed no significant differences between seasons.

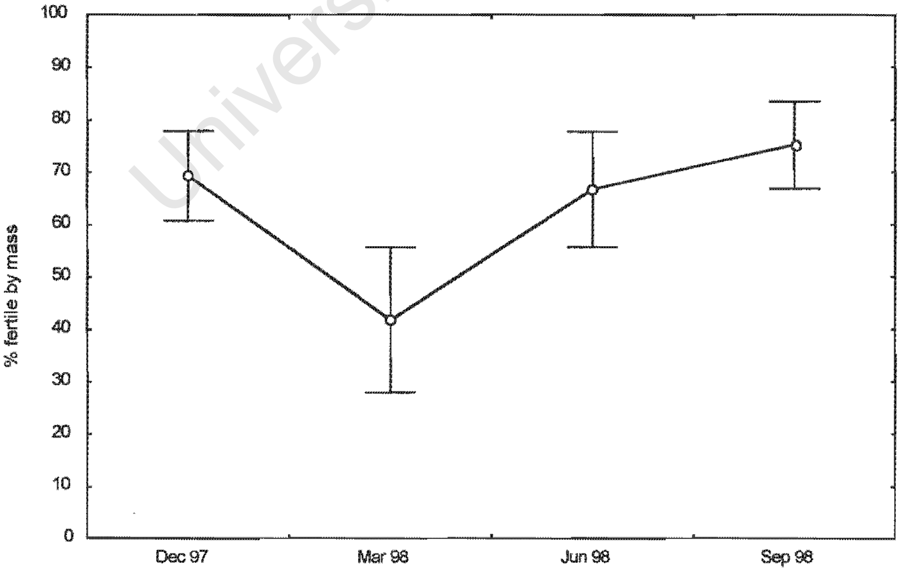

Fig. 5.6: Percentage of carposporic frond mass that was fertile in December 1998, March 1998, June 1998 and September 1998. Mean ± SE; n = 3 for Dec 97 and n = 10 for Mar, Jun and Sep 98.

The number of carposporophytes per 2mm × 2mm showed no significant differences between seasons (Fig.5.7).

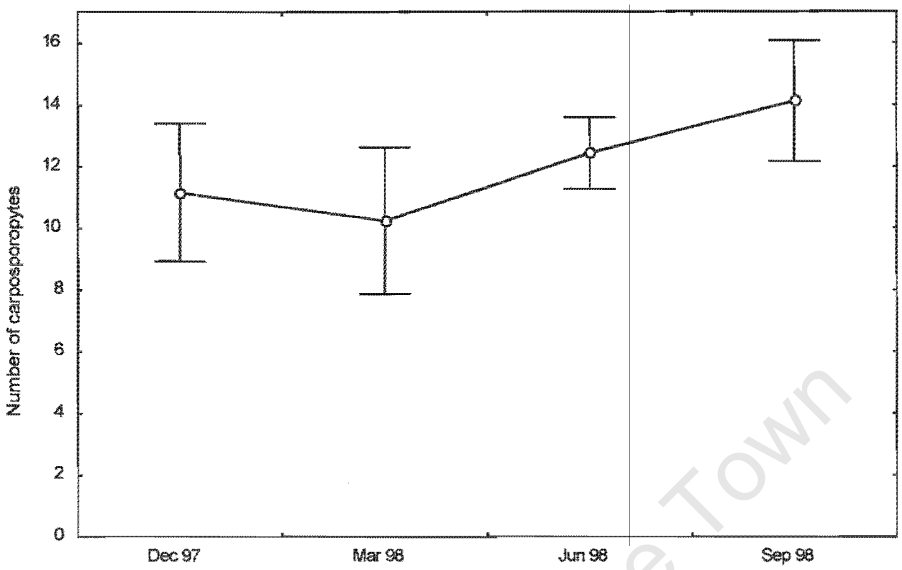

Fig. 5.7: Number of carposporophytes per 2mm × 2mm section of thallus of *Grateloupia filicina*. Mean ± SE; n = 3 for Dec 97 and n = 10 for Mar, Jun and Sep 98.

Number of spores released

The 4mm^2 fertile sections generally released spores in the thousands on the first day and continued to release spores over the subsequent four days but in diminishing numbers (Fig 5.8). Figure 5.8 depicts the combined data for October 1997, December 1997, March 1998, June 1998 and September 1998. No data are available for the number of tetraspores released in December 1997 and none of the five parent thalli released any carpospores in March 1998. The carposporic sections generally released more spores than the tetrasporic material. The spores were not bound by as much mucilage as those of *Sarcothalia scutellata*.

The total number of spores released per 4mm^2 areas of parent thallus over a period of five days for each date of collection is indicated in Figure 5.9. The total number of tetraspores released did not show significant differences with season and ranged from a mean of 1965 in March 1998 to 5225 in September 1998. All five of the carposporic parent thalli failed to release carpospores in March 1998. Otherwise, the mean number of carpospores released ranged from 3228 in December 1997 to 11591 in October 1997. Greatest numbers of carpospores were thus released in spring. Significant differences in numbers of carpospores released are indicated in Table 5.3. Significantly more carpospores than tetraspores were released in October 1997 (p = 0.009) only.

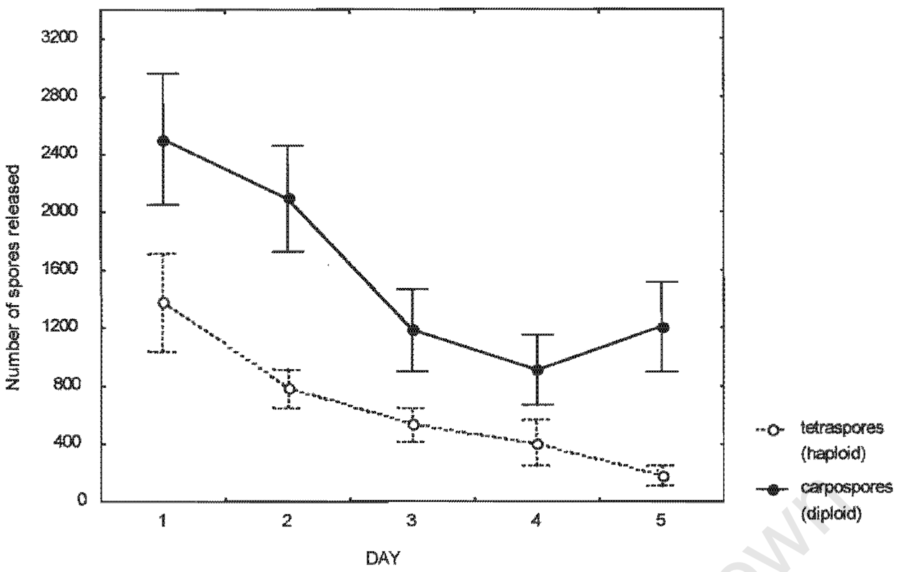

Fig. 5.8: Number of spores released from 4mm² sections of tetrasporic and carposporic fronds of *Grateloupia filicina* over five consecutive days. Data collected on Oct 97, Dec 97, Mar 98, Jun 98 and Sep 98 is combined. Mean ± SE; n = 20. (no data available for tetraspores of Dec 97 or carpospores of Mar 98).

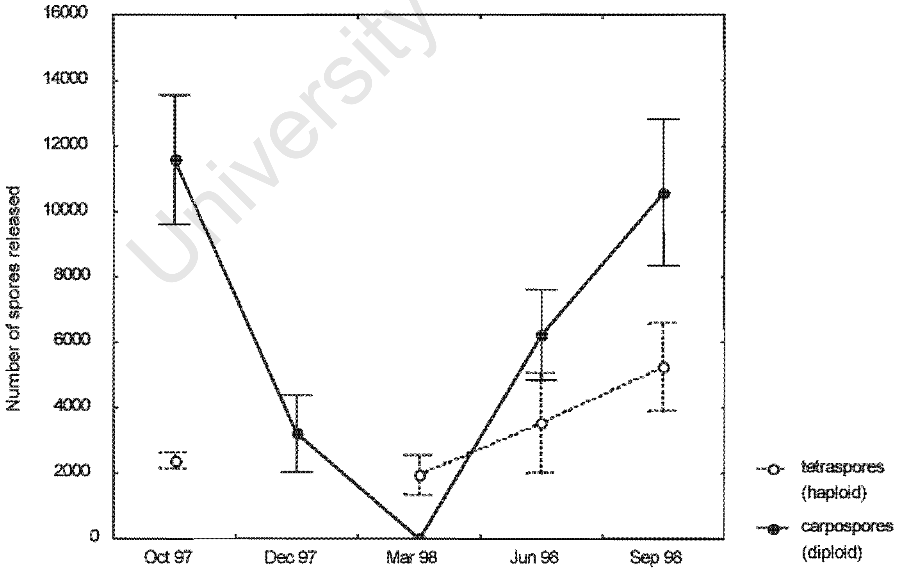

Fig. 5.9: Total number of spores released from 4mm² fertile sections of tetrasporic and carposporic fronds of *Grateloupia filicina* in October 1997, December 1997, March 1998, June 1998 and September 1998. Mean ± SE; n = 5. (No data for tetraspores of Dec 97).

Date	mean	Oct 97	Dec 98	Mar 98	Jun 98	Sep 98
Oct 97	11591		0.016*	0.005**	0.117	0.754
Dec 97	3228	0.016*		0.005**	0.076	0.047*
Mar 98	0	0.005**	0.005**		0.005**	0.005**
Jun 98	6228	0.117	0.076	0.005**		0.175
Sep 98	10592	0.754	0.047*	0.005**	0.175	

Table 5.3: Significant differences in number of carpospores released from $4mm^2$ fertile sections of *Grateloupia filicina*. (Kruskal-Wallis ANOVA by ranks). * $p < 0.05$; ** $p < 0.01$; ***$p < 0.005$.

An approximation of the mean number of spores released per fertile frond was calculated by using the mean number of spores released per fertile section to estimate the mean number of spores released per mean fresh frond mass (Table 5.4).

Month	Tetraspores	Carpospores
Oct 97	5 547 000	27 239 000
Dec 97	ND	12 621 000
Mar 98	5 364 000	ND
Jun 98	11 433 000	12 020 000
Sep 98	17 661 000	29 234 000

Table 5.4: Mean number of spores released per fertile frond of *Grateloupia filicina* from October 1997 to September 1998.

Germination Success Rate

Germination of spores of *Grateloupia filicina* was easily observed as the spore contents were extruded, leaving an empty capsule. The extruded contents formed either a circular crust or a tube-like extension (Fig. 5.10). The latter structure appeared to form a circular crust once contact with the substratum was achieved. The carpospores settled in dense clumps as well as in scattered positions. The tetraspores, which are released from tetrasporangia that are dispersed throughout the parent thallus, did not settle in clumps as dense as those of the carpospores. However, in the area of the chamber directly beneath the parent tetrasporic thallus, there were numerous tetraspores, many of which were in physical contact with each other. These were categorized as occurring in the clumped position.

Fig. 5.10: Germinating carposporelings (diploid phase) of *Grateloupia filicina*. Tetrasporelings are of similar appearance but originating from spores of slightly lower mean diameter.

The time of year in which the parent material was collected and whether the spores settled in a clumped or isolated position caused significant differences in germination rate for both phases (Fig. 5.11; Table 5.5). Germination success rates were highest for both tetraspores and carpospores in October 1997 and decreased with each subsequent collection. There are no data for tetraspores in December 1997 and the fertile carposporic thalli failed to release spores in March 1998. The clumped tetraspores had similar germination success rates in October 1997 and March 1998 that differed significantly from those of June and September 1998. The isolated tetraspores had significantly different germination success rates in each month with the exception of June and September 1998. The clumped carpospores had significantly different germination rates on each date. Germination rates of isolated carpospores in October 1997 differed from those of June and September 1998, whereas those of December 1997 differed only from September 1998.

Comparisons between clumped tetraspores and clumped carpospores on each sampling date revealed no significant differences in germination success rate between the two phases. No significant differences occurred either between isolated tetraspores and carpospores on each date. However, a comparison between clumped and isolated spores revealed significant differences for both phases and on each sampling date ($p < 0.0001$ for all comparisons except between clumped and isolated tetraspores on Oct 97 where $p = 0.001$).

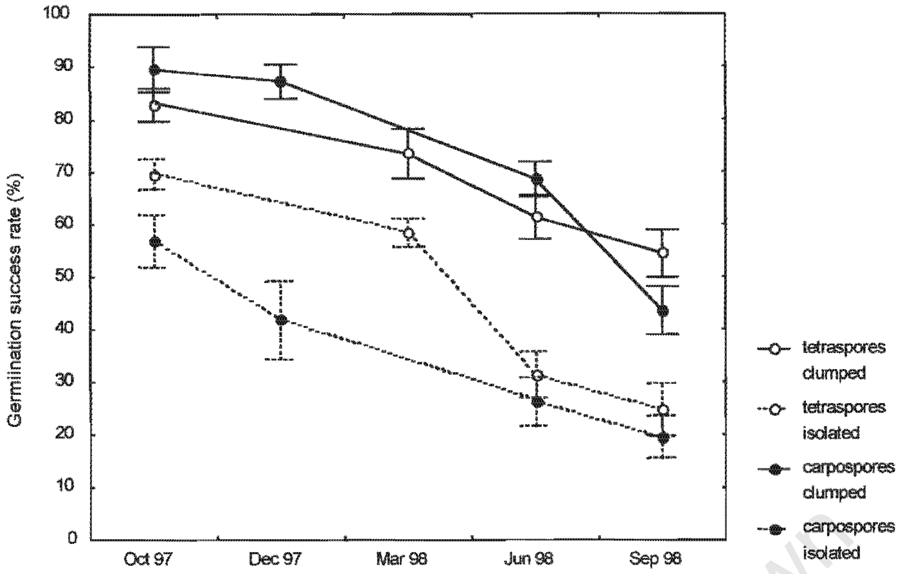

Fig. 5.11: Germination success rate of carpospores and tetraspores of *Grateloupia filicina* in October 1997, December 1997, March 1998, June 1998 and September 1998. Values were obtained for spores that settled in clumped and isolated positions. No data available for tetraspores in Dec 1997 and carposporic sections did not release carpospores in Mar 98. Mean ± SE;

	Clumped							
	Tetraspores				Carpospores			
	Oct 97	Mar 98	Jun 98	Sep 98	Oct 97	Dec 98	Jun 98	Sep 98
Oct 97		0.074	0.000***	0.000***		0.007**	0.000***	0.000***
Dec 97	ND	ND	ND	ND	0.007**		0.001***	0.000***
Mar 98	0.074		0.006**	0.002***	ND	ND	ND	ND
Jun 98	0.000***	0.006**		0.222	0.000***	0.001***		0.000***
Sep 98	0.000***	0.002***	0.222		0.000***	0.000***	0.000***	

	Isolated							
	Tetraspores				Carpospores			
	Oct 97	Mar 98	Jun 98	Sep 98	Oct 97	Dec 98	Jun 98	Sep 98
Oct 97		0.003***	0.000***	0.000***		0.163	0.000***	0.000***
Dec 97	ND	ND	ND	ND	0.163		0.135	0.020*
Mar 98	0.003***		0.000***	0.000***	ND	ND	ND	ND
Jun 98	0.000***	0.000***		0.299	0.000***	0.135		0.217
Sep 98	0.000***	0.000***	0.299		0.000***	0.020*	0.217	

Table 5.5: Significant differences in germination success rates of tetraspores and carpospores of *Grateloupia filicina* as indicated by Kruskal-Wallis ANOVA by ranks. * $p < 0.05$; ** $p < 0.01$; ***$p < 0.005$. ND = no data available.

Germination at two different temperatures indicated that the success rate was greater at 15°C than at 20°C for both the clumped and isolated settling positions (Fig. 5.12). The germination rate of the clumped tetraspores at 15°C was significantly greater than that at 20°C ($p = 0.025$) as was that of the clumped carpospores ($p = 0.032$). Germination of isolated tetraspores and carpospores also showed significant differences between the two temperatures ($p = 0.002$ and $p = 0.001$ respectively).

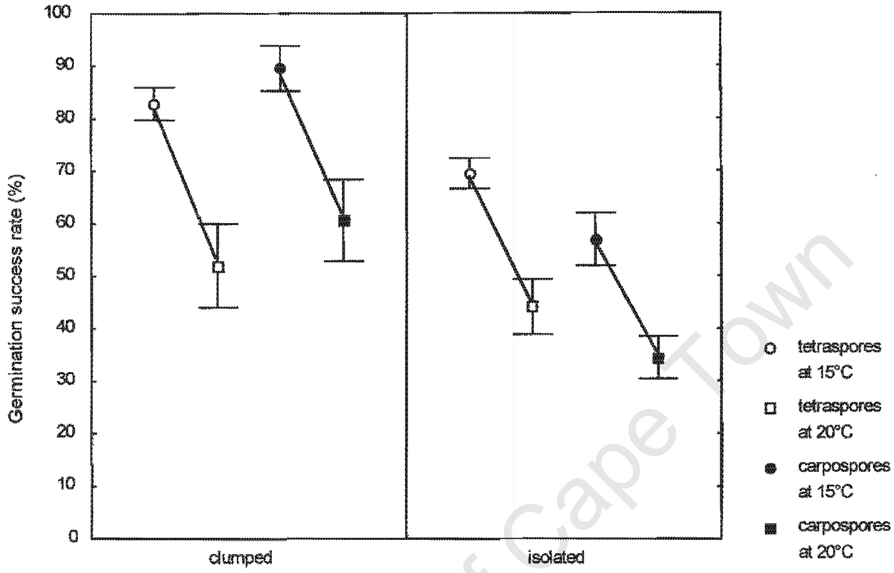

Fig. 5.12: Germination success rate of spores of *Grateloupia filicina* at 15°C and 20°C in clumped and isolated positions. Parent thalli collected in October 1997. Mean ± SE; n = 25.

Discussion

Grateloupia filicina can be collected from natural populations in any season for culturing from spores as fertile fronds occur throughout the year. The tetrasporic fronds exhibited greatest fertility in spring in terms of density of tetrasporangia on the parent thallus, number of fertile branchlets produced and number of tetrasporangia per gram of fresh mass. The carposporic fronds did not exhibit any seasonality in either the percentage of the thallus that was fertile or the density of carposporophytes.

Other factors beside the production of reproductive structures need to be examined to get a full picture of reproductive phenology. For example, the production of reproductive structures and spore release may be responding to different environmental or physiological cues (Melo and Neushul 1993). In *Grateloupia filicina* the tetrasporic fronds, which showed some seasonality in the density and number of tetrasporangia, exhibited little seasonality in numbers of spores released per fertile area. The carposporic fronds on the other hand, which showed no seasonality in production of carposporophytes, released far more spores in spring. Other studies mention seasonal variation in spore release. Bhattacharya (1985) found greatest number of spores were released in autumn for both phases of *Chondrus crispus*, which was correlated with the number of fertile structures produced. Melo and Neushul (1993) found greatest number of spores of *Gelidium robustum* (Gardner) Hollenberg *et* Abbott to be released in winter but this did not correspond to highest soral production.

It has been noted that intertidal species time spore discharge to periods of immersion (Kain and Norton 1990). *Grateloupia filicina* usually is permanently immersed, even during spring low tides, as it tends to occur in permanent rock pools. As spore discharge was always measured from fronds removed at the time of the spring low it is uncertain whether spore discharge is related to tidal movements.

Desiccation, temperature, irradiance, photoperiod, and salinity were all found to affect spore shedding of *Gelidium pusillum* (Stackhouse) Le Jolis, *Pterocladia heteroplatos* (Boergesen) Umamaheswara Rao *et* Kaliaperumal and *Gelidiopsis variabilis* (Greville) Schmitz from the low intertidal of tropical waters in India (Umamaheswara Rao and Kaliaperumal 1983). Maximum spore output occurred when immersed for all three species. A combination of low irradiance and long day length also produced maximal spore output. Optimal salinity and temperature appeared to be related to that encountered in the environment. Minimal spore release occurred at 10 to 15°C with an increase at 20°C and optimal release occurring around 25 to 30°C. Gierdien (1992) also found that temperature influenced the number of spores released in *Gigartina pistillata* (Gmellin) Stackhouse from False Bay in South Africa. More spores were released at 18°C than at 12°C but spore release was completely inhibited at 25°C. Maximum output thus appeared to occur at sea water temperatures most commonly encountered in False Bay.

In this study, the pattern of spore release in *Grateloupia filicina* was determined per 4mm^2 section of parent thallus rather than per reproductive structure as the tetrasporangia did not occur in localized sori and the carposporophytes were too small to isolate single structures. It was thus difficult to tell whether the carpospores originated from a single carposporophyte or not. Over a period of five days, both phases released spores numbered in the thousands from these fertile sections. More carpospores were released than tetraspores, which might be a factor contributing to tetrasporophyte dominance. If frond size is taken into account the number of spores released per frond still indicated that more carpospores than tetraspores are released. As discussed in Chapter 4, a number of species within the Gigartinaceae also produced more carpospores than tetraspores (Bhattacharya 1985; Pacheco-Ruiz *et al.* 1989; Levitt 1998). These species however, in contrast to *Grateloupia filicina*, tended to be dominated by the gametophyte phase.

Despite year round availability of fertile material and spore release, the spores may differ in their ability to germinate. The germination rate of spores appears to be affected by the environment from which the parent thalli were removed as spores grown from fronds collected in different seasons displayed differences in germination rates when grown in identical laboratory conditions. A similar finding occurred for *Gelidium robustum* (Melo and Neushul 1993). Spores of *Grateloupia filicina* had a progressively lower germination success rate from October 1997 to September 1998. The lower germination rates in September 1998 were surprising, as this tended to be the period of highest fertility otherwise. As germination rates were high in October in the previous year, there is no obvious seasonal pattern. The parent fronds may have been subjected to some unpredictable environmental factor that affected spore germination.

The temperature at which the spores were germinated in the laboratory also had an effect on germination success rate. Spores that were placed at 20°C had a lower germination rate than those at 15°C. This may indicate that 20°C is approaching the lethal limits for this species as germination is generally not affected by temperature differences unless the lethal limit is approached (Dring 1982). Investigation of growth of sporelings at different temperatures indicated that death occurred at 24°C (see Chapter 6). However, *Grateloupia filicina* has a wide distribution in warm temperate seas (Stegenga *et al.* 1997) and is also recorded across the tropical Indian Ocean from southern Africa to Australia, including India and Indonesia (Silva *et al.* 1996). The South African species thus appears to be adapted to cooler water. More extensive taxonomical studies may be required to determine whether this species is actually the same as those occurring elsewhere.

The third factor that had an effect on germination rates was the position in which the spores settled, namely, whether they occurred in clumped or isolated positions. The clumped spores had significantly higher germination success rates. However, both the clumped and isolated spores had high germination rates compared to other species in which this has been studied, with the exception of *Sarcothalia scutellata* whose densely clumped spores exhibited consistently high germination rates (see Chapter 4). The germination success rate of the clumped spores of *Grateloupia filicina* ranged from 44 to 90% and isolated spores ranged from 19 to 70%. (Examples of germination rates for other species can be found in the discussion of Chapter 4).

There was no difference in germination success rate between tetraspores and carpospores. However, as the carpospores were released in clumps whereas the tetraspores were released from dispersed tetrasporangia, the latter would have more spores in isolated positions, so ultimately there is a difference between the phases. This factor might contribute to tetrasporophyte dominance. Carpospores of *Gelidium pristoides* (Turner) Kützing (Carter 1985) also tended to have a more clumped settlement pattern than bispores. The germination success of the carpospores was 29.7 ± 8.48 % and was significantly greater than that of the bispores, which displayed rates of 10.9 ± 3.5 %. Carter (1985) suggested that a germination-stimulating compound may exist that only reaches sufficiently high levels under the more crowded conditions. It is also possible that physical contact may stimulate germination in some way. A comparison with spores that have not settled and thus received no physical contact at all would be interesting.

In conclusion, this study reveals differences in fertility between the two phases that may have allowed the tetrasporic phase to become dominant. More carpospores than tetraspores were released per fertile area. Clumped spores had higher germination success rates than isolated spores and more carpospores than tetraspores settled in clumps.

The practical implication of the findings for mariculture is that although fertile fronds may be collected in any season, the best time to collect tetrasporic fronds appears to be in spring. However, germination in September 1998 was less than that of other months. Carposporic fronds could be collected at any time except autumn, when spore release appears to be less effective. A larger sample size needs to be investigated before it can be concluded that no spore release occurs at this time of year. In culturing, the spores should be germinated at 15 rather than 20°C.

Chapter 6

Temperature and Light Responses of *Sarcothalia scutellata* (Hering) Leister and *Grateloupia filicina* (Lamouroux) C. Agardh (Rhodophyta) in culture

Introduction

The region in which this study was conducted falls within the cool temperate Benguela marine region as defined by Bolton and Anderson (1997). The minimum and maximum mean monthly temperatures found for this region were 11.5°C and 14°C respectively with an annual mean of 12 to 13°C (Bolton 1986). Cold temperate regions are defined as those areas that have a mean monthly temperature in the warmer months greater than 10°C and in the cooler months less than 10°C. Warm temperate regions are defined as having mean monthly temperatures over 15°C in the warmer months and less than 20°C in the colder months. The temperatures of the Benguela region is thus intermediate between these two definitions leading to the above appellation of cool temperate.

Sarcothalia scutellata, a southern African endemic (Stegenga *et al.* 1997), occurs largely within the cool temperate Benguela marine region. It extends south from Namibia and also occurs within the western overlap region that stretches from Cape Point to Cape Agulhas. This region separates the cool temperate Benguela marine province of the west coast and the warm temperate Agulhas marine province on the south coast (Bolton and Anderson 1997). The latter is characterized by a mean annual temperature of 15 to 16°C and a range from 13 to 20°C (Bolton 1986).

Grateloupia filicina also occurs along the Cape west coast and western overlap region. However, this species also extends into the warm temperate region as far as the Kowie area (Stegenga *et al.* 1997) near Port Alfred. This region has an annual mean of 17.2 to 18.2°C and a minimum monthly mean of 13.7°C and maximum monthly mean of 21.2°C (Bolton 1986). It is a cosmopolitan species occurring in most warmer seas including southern England, the Mediterranean, Africa, the Indo-Pacific, Western Australia, Tasmania and the Americas from North Carolina to Brazil including the Caribbean (Irvine 1983; Womersley 1994; Silva *et al.* 1996). It has also been described as a pantropical species (Lüning 1990). Silva *et al.* (1996) described its Indian ocean distribution, which included India, Indonesia, Kenya, Mauritius, Seychelles, Somalia, South Africa, Sri Lanka and Yemen. However, the South African species has an eastern limit at the Kowie area (Stegenga *et al.* 1997). Failure to extend into the tropical waters of the South African east coast suggests that the tropical and temperate species may differ. The nature of ocean currents affecting the coast suggests temperate and polar regions of the South Pacific as the origin of west coast species (Bolton and Levitt 1987). Thus further taxonomic revision is indicated. The type-specimen is from the Adriatic sea at Trieste in Italy. Minimum seawater temperatures here are 7 or 10°C and rarely surpass 22°C (Lüning 1990). Optimal temperatures for growth for cold temperate species range between 10 to 15°C, for warm temperate species between 10 to 20°C and for tropical species between 15 to 30°C (Lüning 1990).

This study was conducted on the west coast, as both species were more abundant in intertidal rock pools on the Atlantic compared to the False Bay side of the Cape Peninsula. *Sarcothalia scutellata* was more abundant in mid- to low-intertidal rock pools whereas *Grateloupia filicina* was more abundant in high-intertidal rock pools although there was some overlap in their vertical distribution. Temperatures in March within rock pools at different intertidal levels on the west coast were monitored by Huggett and Griffiths (1986). Maximum pool temperature was related to height above LWS (Low Water of Spring tide) and indicated that the highest rock pools at 2.2m above LWS had maximum temperatures of 30°C. The latter study found that the limit of vertical distribution for *Grateloupia filicina* was in pools at 1.0m above LWS which experienced maximum temperatures of 22 to 28°C. *Sarcothalia scutellata* was recorded at about the LWS level at which maximum temperatures were around 14 to 17°C.

An idea of the light intensity experienced in the natural environment can be obtained from a study by Anderson and Bolton (1985). Surface irradiance at midday in summer was 2300 μ mol photons m^{-2} s^{-1} and the maximum irradiance on a cloudy day was 1400 to 1600 μ mol photons m^{-2} s^{-1}. Thus algae inhabiting the intertidal are exposed to high irradiances and need to be adapted as "sun plants" (Lüning 1990). Examples of light saturation levels for growth of vegetative thalli ranged from 80 to 400 μ mol photons m^{-2} s^{-1} for various intertidal red algae (Levitt and Bolton 1990; Engledow and Bolton 1992; Levitt 1993; Zinoun *et al.* 1993). The optimal irradiance for the growth of sporelings would probably be less than that for larger plants as they would need to be acclimated to the lower light levels beneath the canopy of the larger plants. Light saturation for growth of sporelings of intertidal species occurred at quite low irradiances that ranged from 50 to 75 μ mol photons m^{-2} s^{-1} for *Mazzaella laminarioides* (Bory) Fredericq (formerly *Iridaea laminarioides* Bory) (Hannach and Santelices 1985), species of *Gelidium* (Lüning 1990), *Sarcothalia scutellata* and *Gigartina polycarpa* (Kützing) Setchell *et* Gardner (Levitt 1998).

The aims of this study were to determine optimum conditions for growth of sporelings in culture in terms of temperature and irradiance. This would provide guidelines for mariculturing the species. How well the optimal water temperatures for growth correlate with geographic distribution could then be determined. Ecological implications of differences in the growth response between the two species and between phases within species were explored.

Method

Three fertile fronds of each phase of each species were collected from Olifantsbos (lat. 34° 16' S, long. 18°23' E) on the west coast of the Cape Peninsula. The phases were stored separately overnight in sealed plastic bags in the dark at 0°C. Fertile sections were cut out of the fronds. To decrease epiphytes the sections were cleaned with paper toweling and rinsed in 0.5% Povidone iodine in seawater (Haritonidis 1992) for 2 minutes. The sections were also placed in fresh water for 1 minute to encourage spore release. Finally, the sections were washed in sterile seawater.

The fertile sections were placed upon coverslips in crystallizing dishes containing 175ml of one-third strength ($6ml.l^{-1}$) Provasoli Enriched Seawater (Provasoli 1968). A saturated solution of GeO_2 ($1ml.l^{-1}$) was added to the media to inhibit diatom growth. The concentration was based on that recommended by Markham and Hagmeier (1982). Three thallus sections, one from each parent frond, were placed in each crystallizing dish. The dishes were initially kept overnight at 15°C and at a light intensity of 60 µ mol photons m^{-2} s^{-1}. The parent thalli were then discarded and the diameters of 30 spores of each phase were measured.

The temperature experiment was first performed on *Grateloupia filicina* in water baths at a wide range of temperatures: 6°C, 12°C, 18°C and 24°C. *Sarcothalia scutellata* was then grown at a narrower range of 12°C, 15°C, 18°C and 21°C. Spores of this species had been noted previously to die at 24°C so this temperature was not included. Three crystallizing dishes were placed at each temperature at a light intensity of 80 µ mol photons m^{-2} s^{-1}.

Water temperatures were measured in the five high-intertidal rock pools containing *Grateloupia filicina* and the five mid- to low-intertidal rock pools containing *Sarcothalia scutellata*. Temperature measurements were taken on seven occasions between July 1997 and September 1998 during the period of the spring low tide.

The light experiment was conducted in waterbaths set to 18°C. Sporeling growth was measured at six irradiances: 10, 30, 60, 90, 120 and 200 μ mol photons $m^{-2} s^{-1}$. Three dishes were placed at each light intensity.

The experiments were conducted at a light: dark cycle of 16:8 hours and over a period of three weeks. The growth medium was changed weekly and the position of the dishes rotated daily. The diameters of ten crusts per coverslip were measured weekly in the temperature experiment resulting in a sample size of 90 diameters per temperature. As certain of the parent thalli did not release sufficient spores in the light response experiment, only ten crusts per dish were measured resulting in a sample size of 30 diameters per irradiance level. As the crusts began producing uprights by the end of the third week, further measurement was not meaningful.

Results

Both species possessed spores of a uniform spherical shape that were indistinguishable between the phases. Carpospores of *Grateloupia filicina* had a mean diameter of 16 ± 3.0 μm. The tetraspores were slightly smaller with a mean diameter of 14 ± 2.9 μm. The mean carpospore diameter of *Sarcothalia scutellata* was 16 ± 1.4 μm, a similar size to the tetraspores which had a mean diameter of 15 ± 0.4 μm.

Temperature Responses

At 6°C there was a slight increase in the size of the crust diameters of sporelings of *Grateloupia filicina* compared to that of the ungerminated spores but very little growth occurred (Fig 6.1). The sporelings had a healthy appearance at this temperature despite little growth. Mean crust diameter after 21 days was 24 ± 3.8 μm for the diploid phase and 26 ± 4.3 μm for the haploid phase. The haploid sporelings, despite originating from spores with smaller diameters, had achieved significantly greater crust diameters than the diploid carposporelings ($p = 0.003$).

Significantly greater growth occurred at 12°C than at 6°C for both the diploid ($p < 0.0001$) and the haploid ($p < 0.0001$) phases. Mean diploid crust diameter was 68 ± 24.0 μm and mean haploid crust diameter was 74 ± 30.4 μm after 21 days. At this temperature there was no significant difference in crust diameter between the two phases ($p = 0.192$).

Greatest growth occurred at 18°C and was significantly greater than that at 12°C for both phases ($p < 0.0001$). Mean diploid crust diameter was 156 ± 83.0 μm and mean haploid crust diameter was 261 ± 104.1 μm after 21 days. The haploid crusts were significantly larger than the diploid crusts ($p < 0.0001$).

Sporelings survived for about a week at 24°C. Greater numbers of diploid crusts were still viable by the end of the first week compared to haploid crusts, but neither survived the second week at this temperature.

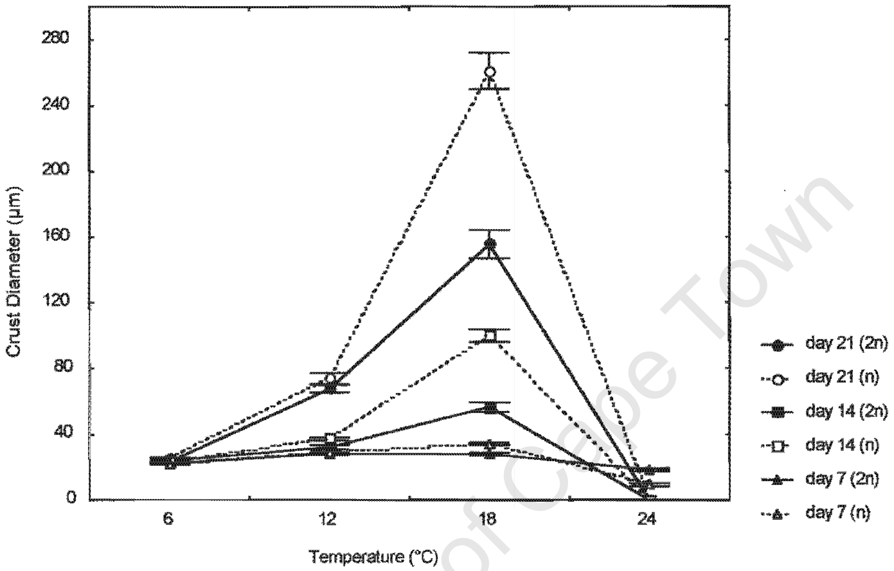

Fig. 6.1: Crust diameters (µm) of sporelings of *Grateloupia filicina* after growth for 7, 14 and 21 days at four different temperatures. 2n = diploid crusts; n = haploid crusts. Mean ± SE; n = 90.

Sporelings of *Sarcothalia scutellata* exhibited slow growth at 12°C (Fig. 6.2). Mean diameter after 21 days for diploid crusts was 60 ± 35.5 μm and for haploid crusts was 57 ± 30.7 μm. There was no difference in size between the two phases at this temperature ($p = 0.658$).

Growth was much enhanced at the three higher temperatures compared to that at 12°C. Significantly greater growth occurred at 15°C for both phases ($p < 0.0001$). Diploid crusts reached mean diameters of 225 ± 65.1 μm and haploid crusts 212 ± 65.9 μm. The two phases did not differ in size at this temperature ($p = 0.134$).

Growth did not show as much of an increase at the higher levels compared to that between 12°C and 15°C. However, the crust diameters were still significantly larger at 18°C than at 15°C ($p < 0.0001$ for the diploid phase and $p = 0.001$ for the haploid phase). Mean diploid crust diameter was 271 ± 60.8 μm and mean haploid crust diameter was 244 ± 60.5 μm. Diploid crusts were significantly larger than the haploid crusts ($p = 0.001$).

The diploid, but not the haploid phase, had significantly higher growth at 21°C ($p < 0.0001$ and $p = 0.216$ respectively). Diploid crusts had a mean diameter of 324 µm ± 70.2 and haploid crusts 253 ± 62.8 µm. Diploid crust diameters were significantly larger than the haploid crust diameters at this temperature ($p < 0.0001$).

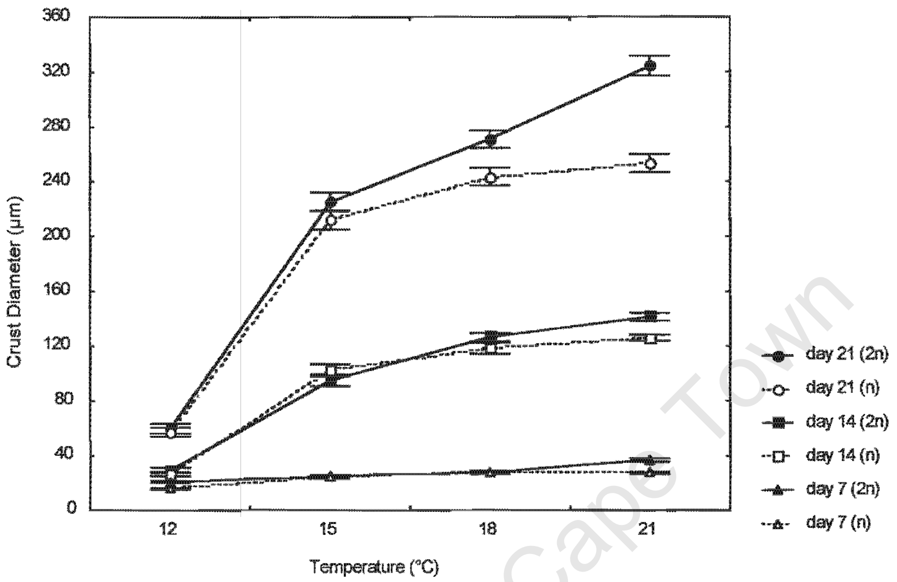

Fig. 6.2: Crust diameters (µm) of sporelings of *Sarcothalia scutellata* after growth for 7, 14 and 21 days at 4 different temperatures. 2n = diploid; n = haploid. Mean ± SE; n = 90.

Temperatures occurring in the rock pools when measured during the spring low tides are indicated in Table 6.1.

	Grateloupia filicina	***Sarcothalia scutellata***
July 97	12 ± 0.5	12 ± 0.0
August 97	14 ± 1.1	14 ± 0.4
October 97	14 ± 0.4	16 ± 0.6
December 97	21 ± 0.3	17 ± 0.7
March 98	18 ± 1.3	15 ± 0.4
June 98	15 ± 0.2	15 ± 0.6
September 98	16 ± 1.2	14 ± 0.0

Table 6.1: Mean water temperature (°C) of seawater in rock pools in which the two species occurred during the spring low tide. n=5.

The temperature in the high-intertidal rock pools in which *Grateloupia filicina* occurred ranged from 12°C (July 1997) to 21°C (December 1997) during the spring low tides. In the mid- to low-intertidal rock pools containing *Sarcothalia scutellata* it ranged form 12°C (July 1997) to 17°C (December 1997). As these measurements were taken only when seaweed was being collected about every second month, they do not reflect the full range of temperatures that occurred in the rock pools.

Light responses

Growth of sporelings of *Grateloupia filicina* at six light intensities showed that crust diameters were far smaller at 10 μ mol photons m^{-2} s^{-1} than at the other light intensities tested (Fig 6.3). Saturation appeared to occur around 30 μ mol photons m^{-2} s^{-1} where the diploid and haploid crusts attained mean diameters of 168 ± 58.6 μm and 198 ± 48.7 μm respectively. However, the largest crust diameters for both phases occurred at the highest light intensity of 200 μ mol photons m^{-2} s^{-1}. Mean crust diameter was significantly larger at this irradiance than at all lower irradiances for the diploid crusts whereas it was significantly larger than at all lower irradiances except for 60 μ mol photons m^{-2} s^{-1} for the haploid phase (Table 6.2(A)). The mean diameter of the crusts after 21 days at this irradiance was 283 ± 91.8 μm for diploid crusts and 241± 81.1 μm for haploid crusts. The haploid crusts had significantly larger diameters than the diploid crusts at 30 μ mol photons m^{-2} s^{-1} (p = 0.043) and 90 μ mol photons m^{-2} s^{-1} (p = 0.004).

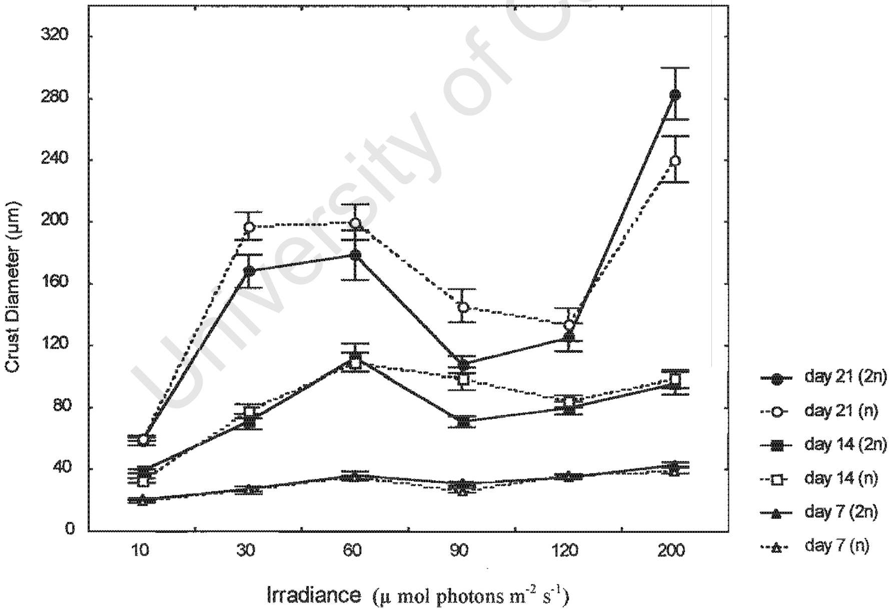

Fig. 6.3: Crust diameters (μm) of sporelings of *Grateloupia filicina* after growth for 7, 14 and 21 days at six light intensities. 2n = diploid; n = haploid. Mean ± SE; n = 30.

Sarcothalia scutellata showed a similar light response with growth at 10 μ mol photons m^{-2} s^{-1} much reduced compared to that at the five higher light intensities (Fig 6.4). Saturation occurred around 30 to 60 μ mol photons m^{-2} s^{-1} but significantly larger mean diameters were again attained at the highest light intensity of 200 μ mol photons m^{-2} s^{-1}(Table 6.2 (B)). Mean crust diameter at

30 μmol photons m^{-2} s^{-1} was 161 ± 30.2 μm for diploid crusts and 167 ± 36.0 μm for haploid crusts. Mean diameter at 200 μ mol photons m^{-2} s^{-1} was 263 ± 45.4 μm and 259 ± 61.5 μm for diploid and haploid crusts respectively. Significant differences in crust diameter between the diploid and the haploid phases occurred at 10 μ mol photons m^{-2} s^{-1} ($p = 0.037$) and 60 μ mol photons m^{-2} s^{-1} ($p < 0.0001$).

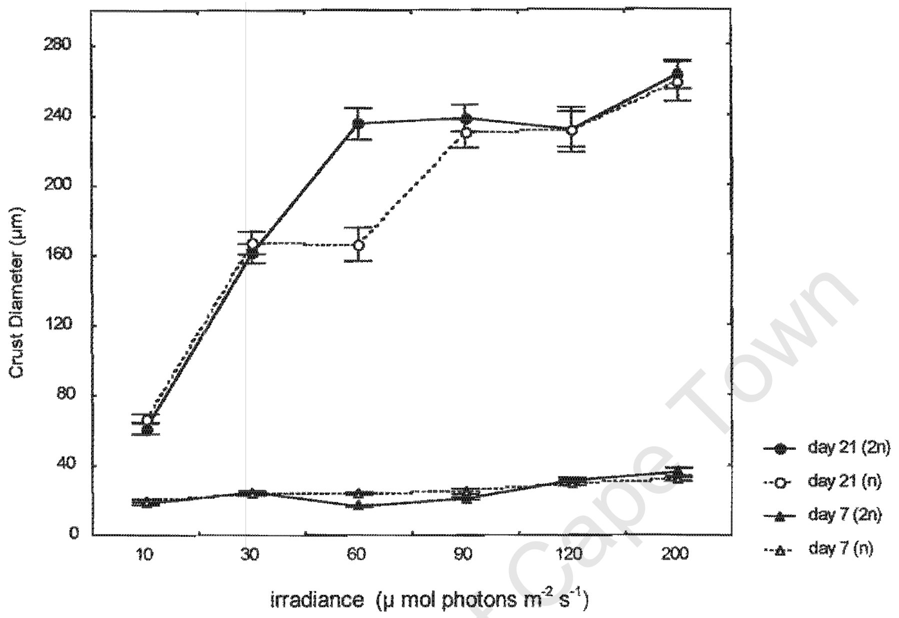

Fig. 6.4: Crust diameters (μm) of sporelings of *Sarcothalia scutellata* after growth for 7 and 21 days at six light intensities. 2n = diploid; n = haploid, Mean ± SE; n = 30.

(A)	***Grateloupia filicina***											
	Diploid						**Haploid**					
	10	**30**	**60**	**90**	**120**	**200**	**10**	**30**	**60**	**90**	**120**	**200**
10		***	***	***	***	***		***	***	***	***	***
30	0.000		ns	***	***	***	0.000		ns	***	***	*
60	0.000	0.959		***	*	***	0.000	0.830		***	***	ns
90	0.000	0.000	0.003		ns	***	0.000	0.000	0.001		ns	***
120	0.000	0.002	0.020	0.300		***	0.000	0.000	0.000	0.245		***
200	0.000	0.000	0.000	0.000	0.000		0.000	0.043	0.053	0.000	0.000	

(B)	***Sarcothalia scutellata***											
	Diploid						**Haploid**					
	10	**30**	**60**	**90**	**120**	**200**	**10**	**30**	**60**	**90**	**120**	**200**
10		***	***	***	***	***		***	***	***	***	***
30	0.000		***	***	***	***	0.000		ns	***	***	***
60	0.000	0.000		ns	ns	*	0.000	0.623		***	***	***
90	0.000	0.000	0.695		ns	*	0.000	0.000	0.000		ns	*
120	0.000	0.000	0.853	0.983		*	0.000	0.001	0.000	0.845		ns
200	0.000	0.000	0.029	0.027	0.033		0.000	0.000	0.000	0.021	0.174	

Table 6.2: Significant differences in crust diameter at different light intensities for *Grateloupia filicina* (A) and *Sarcothalia scutellata* (B). (Kruskal-Wallis ANOVA by ranks). ns = not significant; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$.

Discussion

Temperature responses

Despite *Grateloupia filicina* occurring in the warmer higher intertidal rock pools and having a distribution that extends into the warmer Agulhas province it did not exhibit higher growth optima than *Sarcothalia scutellata*. The optimal temperature for growth from spores for these two species was around 18°C (to 21°C). Growth occurred at all temperatures tested except for 24°C, which neither species could tolerate.

The larger spore diameters possessed by the carpospores of *Grateloupia filicina* did not confer a competitive advantage over the smaller tetraspores. This factor did not therefore contribute to the dominance of the diploid phase that occurs in the natural environment. The haploid crusts had faster growth rates, particularly at the optimal temperature of 18°C. The haploid phase, however, succumbed more rapidly to the stressful temperature of 24°C, as can be seen by the larger mean crust diameter for diploid crusts at this temperature by the end of the first week.

It is possible that the haploid phase is capable of faster growth if stressful conditions are not encountered. Thus in optimal conditions the haploid phase might predominate. The highest temperature recorded in the high-intertidal rock pools was 21°C, which occurred in three of the rock pools during December 1997. It is likely that higher temperatures are encountered on hot sunny summer days. The maximum temperatures recorded in March for pools at 1.0m above LWS, which was the vertical limit recorded for *Grateloupia filicina*, was 22 to 28°C (Huggett and Griffiths 1986). The periodic subjection to temperatures approaching 24°C in the higher rock pools may have contributed to diploid dominance. The existence of this species in high-intertidal rock pools that experience temperatures of 24°C and possibly higher, whereas sporelings died at 24°C in culture, is probably due to the former experiencing high temperatures periodically at low tide, whereas the sporelings were permanently subjected to the high temperature. The species may also experience other physical stresses such as increased salinity in these high rock pools. The species may be displaced from the less physically stressed conditions of the lower rock pools by other species that are superior competitors.

However, in *Sarcothalia scutellata*, the haploid phase did not grow faster than the diploid phase under optimal conditions. The two phases showed similar growth at the lower temperatures of 12 and 15°C, which are the temperatures more commonly encountered in the mid- to low-intertidal rock pools of the west coast. The diploid phase had significantly greater growth at the higher temperatures of 18°C and 21°C. The highest temperatures recorded in the mid- to low-intertidal rock pools were in December 1997 when most of the pools had a temperature of 17°C and one pool had a temperature of 18°C. The vertical limit recorded for *Sarcothalia scutellata* by Huggett and Griffiths (1986) was in the region of the LWS, which did not experience maximum temperatures much above 14°C. However, in this study the latter species did occur above LWS in the region of *Gigartina polycarpa*. The study by Huggett and Griffiths (1986) recorded this species in pools with a maximum height of about 0.4m above LWS, which were subjected to a maximum temperature of 20°C. These lower rock pools do not experience as much of a temperature fluctuation as they are seldom isolated from the moderating influence of the ocean for any length of time. The species was noted to occur in higher pools and it would be interesting to note the phase dominance there. Difficulties may be experienced, however, as casual observation indicated that they were less fertile which would hinder phase recognition.

The idea of the diploid phase being more resistant is not borne out by *Iridaea laminarioides* Bory from Central Chile. Gametophytes showed greater desiccation tolerance, optimum growth at higher temperatures and molluscan grazers showed a preference for the tetrasporophytes (Luxoro and Santelices 1989).

The temperature responses indicated that the two species investigated in this study are capable of growth within a broad temperature range, but with optimal growth occurring at warmer temperatures such as those found on the south coast and thus they display characteristics of warm temperate species. The minimum monthly mean of the south coast is 13.7°C and the maximum monthly mean is 21.2°C with an annual mean of 17.2 to 18.2 °C (Bolton 1986). It is thus surprising that *Sarcothalia scutellata* does not extend to the East of Cape Agulhas. However, it is possible that sporelings have wider tolerances than adults do. Higher temperature optima for sporelings than the species distribution suggests have also been described for *Suhria vittata* (Lamouroux) J. Agardh (Anderson and Bolton 1985).

The two species investigated in this study were noted to be abundant in pools on the west coast but had a lower occurrence on the False Bay side of the Peninsula. This could be due to increased competition with other species on the warmer coast or the periodic occurrence of lethal temperatures in the intertidal zone. The latter seems unlikely in the case of the lower rock pools. Temperatures experienced in the Agulhas zone may limit reproduction, which often has a narrower range than vegetative growth. It was noted that the germination of spores of *Grateloupia filicina* decreased at 20°C (see Chapter 5).

The agarophyte *Suhria vittata* has a similar distribution to that of *Sarcothalia scutellata* and sporelings grown in culture showed a similar growth optimum at 15 to 20°C (Anderson and Bolton 1985). *Suhria vittata* has been recorded further north in Namibia at Möwe Bay to as far east as Cape Agulhas and was also more abundant on the west coast compared to False Bay (Anderson and Bolton 1985). The intertidal species *Gigartina polycarpa* is abundant at Lüderitz in Namibia, along the Cape west coast and along the south coast as far as Port Alfred (Stegenga *et al.* 1997). Sporelings of this species also showed optimal growth at 15 to 20°C (Levitt 1998). The latter species also died at 25°C and *Suhria vittata* showed a decrease in growth at 22.5°C. These two species have been considered as warm temperate species (Bolton and Levitt 1985; Levitt 1998). The temperature responses are also similar to that of *Gigartina pistillata* (Gmelin) Stackhouse from False Bay, which had optimal growth for sporelings at 18°C, and no growth at 25°C (Gierdien 1992). However, this warm temperate species does not occur on the west coast proper but is distributed from Smitswinkel Bay in False Bay to the Kowie area. Growth of these species, including the two in this study, at low temperatures also makes them suitable for the cooler west coast conditions. Growth of *Grateloupia filicina* and *Sarcothalia scutellata* occurred at 12°C and sporelings of the former species appeared healthy at 6°C, although little growth occurred.

Sarcothalia stiriata (Turner) Leister has a similar distribution to *Gigartina polycarpa* along the west coast but only extends as far as Cape Agulhas on the south coast. This species was more sensitive to higher temperatures as death occurred at 20°C and optimal growth occurred at 15°C (Levitt 1998). This species was considered more of a cool temperate species. *Sarcothalia scutellata* has a similar distribution pattern to *Sarcothalia stiriata* but temperature tolerances of the sporelings were those of a warm temperate species.

If all factors but temperature are ignored, neither phase of *Sarcothalia scutellata* would be expected to dominate the mid- to low-intertidal rock pools as no difference in growth rate occurred at 12 to 15°C. Gametophyte dominance must therefore result from some other factor. *Gigartina polycarpa* and *Sarcothalia stiriata* were also found to be gametophyte dominated but no difference in growth rate between the two phases was discovered (Levitt 1998). In the high intertidal rock pools that contained *Grateloupia filicina*, temperatures measured did not exceed 21°C, but temperatures recorded by Huggett and Griffiths (1986) indicated that these rock pools experience temperatures as high as 28°C. Periodic subjection to high temperatures during low tides may have been important in the initial establishment of the species from spores if the tetrasporelings succumbed more rapidly than the carposporelings to the high temperatures as appeared to happen at 24°C in culture. Thus, diploid dominance of this species may be due to periodic stressful temperatures or some other physical factor such as high salinity. *Gelidium pristoides* from Port Alfred in the Eastern Cape, which was also dominated by the diploid phase, showed no difference in growth between the two phases (Carter 1985). However, this species occurs in the mid- to low-intertidal (Bolton and Anderson 1997; Stegenga *et al.* 1997) rather than in high rock pools.

Light Responses

Light saturation for both species and phases occurs around 30 to 60 µ mol photons m^{-2} s^{-1}. The experiment was conducted at the optimal growth temperature of 18°C and the haploid phase of *Grateloupia filicina* again showed a higher growth rate compared to the diploid phase except at the highest light intensity of 200 µ mol photons m^{-2} s^{-1}. The reason for faster haploid growth is not known.

Saturation at around 30 to 60 µ mol photons m^{-2} s^{-1} is similar to that found for sporelings of red seaweeds of other species. *Sarcothalia stiriata* and *Gigartina polycarpa* showed a large increase in growth at 30 µ mol photons m^{-2} s^{-1} and saturation around 50 µ mol photons m^{-2} s^{-1} (Levitt 1998). *Suhria vittata*, a species that is epiphytic in the subtidal region of the South African west coast might be expected to show lower saturation levels than the intertidal species but it too showed saturation around 40 µ mol photons m^{-2} s^{-1} (Anderson and Bolton 1985). Sporelings of species of *Gelidium* showed growth saturation at 50 to 75 µ mol photons m^{-2} s^{-1} (Lüning 1990). Sporelings of the Chilean intertidal species *Mazzaella laminarioides* also showed saturation at 60 µ mol photons m^{-2} s^{-1} (Hannach and Santelices 1985). The gametophytes had significantly larger diameters under optimal conditions (15°C and at 35 and 60 µ mol photons m^{-2} s^{-1}). Sporophytes of another Chilean intertidal species, *Iridaea ciliata* Kützing, tended to grow faster than the gametophytes except at optimal conditions (also at 15°C and at 35 and 60 µ mol photons m^{-2} s^{-1}.) where there was no significant difference (Hannach and Santelices 1985).

The irradiance at which sporelings become light saturated differs markedly from that of mature thalli. For example, vegetative pieces of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham (previously *Gracilaria verrucosa* (Hudson) Papenfuss) became light saturated at 80 µ mol photons m^{-2} s^{-1} (Engledow and Bolton 1992). Mature thalli of *Gigartina polycarpa* from sublittoral populations saturated at 200 µ mol photons m^{-2} s^{-1} (Levitt and Bolton 1990) and from eulittoral populations at 400 µ mol photons m^{-2} s^{-1} (Levitt 1993). Thallus pieces of *Gigartina teedii* (Roth) Lamouroux from Brittany became light saturated at 500 µ mol photons m^{-2} s^{-1} (Zinoun *et al.* 1993). This difference in saturation levels between sporelings and mature thalli is probably due to the dense shading that occurs in the seaweed understory.

In conclusion, the recommended temperatures and irradiance at which sporelings of *Grateloupia filicina* and *Sarcothalia scutellata* should be cultured is at 18°C and above 30 to 60 µ mol photons $m^{-2} s^{-1}$. Investigations are still required to determine how the species will fare at irradiances above 200 µ mol photons $m^{-2} s^{-1}$. Diploid dominance of *Grateloupia filicina* may be influenced by the periodic occurrence of high temperatures in rock pools. The temperatures of the rock pools in which *Sarcothalia scutellata* occurred would not cause difference in growth rate between the two phases so this factor would not contribute to haploid dominance. The species tended to be most abundant in areas that were colder than the temperature optima for growth of sporelings, thus sporelings probably have higher temperature optima than the species distribution suggests.

Chapter 7

General discussion

Carrageenan content

Of the two species investigated in this study, only *Sarcothalia scutellata* appears to have potential for use in the carrageenan industry. However, the λ-carrageenan of the tetrasporophytes was only 14 to 24% of the dry mass whereas the κ-carrageenan formed 33 to 58% of the dry mass. The former thus falls below the level of 30 to 70% usually required in exploited species. *Grateloupia filicina* from intertidal pools on the west coast of South Africa does not have potential for use in the carrageenan industry but it may still be useful in the food industry. In-depth market research is required however to determine the economic viability of such an enterprise. As populations of *Grateloupia filicina* in other parts of the world possessed exploitable quantities of carrageenan, systematic research may be required to identify whether these are in fact the same species. The species that occurs in the western Cape does not extend to the east coast as the species has temperature tolerances below that of the tropics. Silva *et al.* (1996) however, includes the Kenyan coast as part of the Indian ocean distribution for this species. This further indicates the possibility of the existence of more than one species.

Phase composition

The difference in abundance of isomorphic phases is a puzzling phenomenon. As the different phases may possess different carrageenans, this has commercial implications for those species that are used in the carrageenan industry.

It is more common for the diploid phase to be dominant as is the case for *Grateloupia filicina*, which had a particularly pronounced difference in phase representation. The Gigartinaceae are a notable exception to diploid dominance as the gametophytic phase often dominates (Bhattacharya 1985; Hannach and Santelices 1985; May 1986; Braga 1990; Chopin and Floc'h 1992; Scrosati *et al.* 1994; Piriz 1996; Westermeier and Sigel 1997; Scrosati 1998a). *Sarcothalia scutellata*, a member of the Gigartinaceae, conformed to this pattern.

Dominance of one phase may be expected during specific environmental conditions in species with heteromorphic alternation of generations. For example, the different growth forms of kelps and *Porphyra* are adapted to different environments (Lobban and Harrison 1994). Many species that have heteromorphic life histories use photoperiod as a cue to switch to the alternate phase that is better adapted for the next season (Lobban and Harrison 1994). For example, the heteromorphic red alga *Acrosymphyton purpuriferum* from the Mediterranean Sea has erect fleshy gametophytes present only in spring and summer whereas crustose tetrasporophytes occur in short days (Cortel-Breeman and ten Hoopen 1978 cited in Lüning 1990). In terms of the functional - form theory, if natural selection acts on the phenotype and if the macromorphological attributes of an alga adapt it to the environment, algae with similar morphologies should not have ecological differences (Littler and Littler 1980). There may however be other differences, such as metabolic and physiological differences between the phases.

There is a general trend of increasingly complex organisms reducing the size of the haploid phase until a free-living multicellular haploid phase is omitted altogether. Haploidy is thought to be less favored as all recessive alleles are exposed to selection. Haploid members would only be favoured in environments that selected for recessive homozygosity. Heterozygosity can only occur in diploid plants and is thought to make individuals more fit (Begon *et al.* 1990). There is no explanation yet as to why the haploid phase of the Gigartinaceae often predominates. However, determination of phase dominance can be problematic, for example, if one phase produces more fronds than the other but does not differ in the number of distinct plants produced, the sampling of fronds would indicate phase dominance.

Hannach and Santelices (1985) state that in a stressful environment a species is more likely to show dissimilar representation of phases than those inhabiting more stable environments due to one of the phases being more adaptable to the stresses encountered. Laura Lazo *et al.* (1989) suggest the opposite in stating that environments subjected to disturbances would have a more equal representation of phases whereas dominance would occur in stable environments. According to this theory, the two phases are not mutually dependent upon one another, as are the two sexes of dioecious species. Competition for resources occurs and the competitive exclusion principle applies. In an unstable environment, such as turbulence acting on a soft, friable substratum, as occurred in the area in which they studied on Prince Edward Island, Canada, new space is continually made available and either phase has the opportunity to reestablish itself. This results in a similar abundance of each phase, as occurred for *Chondrus crispus* in this environment, as opposed to the more stable rocky substratum of Nova Scotia where gametophytic dominance of this species had been reported (Bhattacharya 1985; Scrosati *et al.* 1994). The stable environment allows the superior competitor to become established at the expense of the alternate phase.

The two seemingly conflicting ideas are not mutually exclusive if one distinguishes between a stressed and a disturbed environment. In the former, harsh environmental stresses enable the superior competitor to dominate, whereas in the latter, the stresses are of such a nature as to remove entire thalli and clear patches of the substratum. The former would thus be characterized by a substratum that is completely colonized, whereas the latter would be identified by open spaces between plants. It would be interesting to determine if a correlation exists between the amount of space available for colonization and phase dominance.

Is any pattern evident in the literature between the nature of the substratum, the existence of environmental stresses or disturbance and phase dominance? The substratum on which *Chondrus crispus* in Nova Scotia grew in the study by Bhattacharya (1985) was described as having little new space for colonization as well as being hard and stable and this was highly dominated by the gametophyte generation. Most recruitment into the stand was via regrowth of fronds from existing holdfasts. In previous unpublished data he found that in areas that were artificially denuded there was a higher occurrence of the diploid stage, particularly at the upper edge of the intertidal zone.

A gametophyte-dominated population of *Gigartina teedii* was described as growing on a rocky substratum in the intertidal zone in which there was little colonizing space as various turf forming seaweeds covered the substratum (Braga 1990). Vegetative reproduction appeared to maintain this dominance.

In the study of *Mazzaella cornucopiae* growing on a rocky substratum in the intertidal zone (Scrosati 1998a), gametophyte dominance again corresponded with a small amount of space available for colonization and vegetative reproduction. This species was described as conforming to a K -strategy, as holdfasts were perennial, reproduction delayed and iteroparous. The effect of wave exposure was also investigated but no significant effects were discovered. However, it was mentioned that there was greater colonizing space at lower elevations and although still gametophyte dominated, there was a greater occurrence of tetrasporophytes.

The above three examples all have a combination of little colonizing space, phase dominance and vegetative reproduction. Once dominance is established it is possible that regeneration from previous tissue might become more important than spore production.

A worldwide study of *Gracilaria gracilis* (Kain and Destombe 1995) found the full range of possibilities occurring among populations from all gametophytes, gametophyte dominated, equal ratios, sporophyte dominated to all sporophytes. The most common, however, was equal ratios and tetrasporophyte dominance. Local conditions rather than geographical location were more important as different populations in nearby localities were found to exhibit opposite extremes. Extreme differences in habitat were mentioned but it is not clear in what manner they differed. They do mention that substratum is important and suggest that in areas with soft substrata vegetative reproduction is more likely than development from spores and a single phase would thus be favoured whereas hard substrata are more suitable for spore settlement, and thus the completion of the life cycle resulting in a mixture of phases.

In a study in Brittany (Chopin and Floc'h 1992), the midlittoral was described as more unstable than the infralittoral zone. Although gametophytic dominance of *Chondrus crispus* occurred in both zones it was less marked in the more stable infralittoral zone and they concluded that the less stable environment promoted dominance. However, space available for colonization was not mentioned. Hannach and Santelices (1985) also stated that the lower intertidal was more protected and subjected to less drastic fluctuations than the high-intertidal. The latter zone was highly dominated by the cystocarpic phase of *Mazzaella laminarioides* (previously *Iridaea laminarioides*) whereas a more equitable representation of phases occurred in the low-intertidal. *Iridaea ciliata* on the other hand had similar representation of phases in the mid-intertidal (the species did not occur in the high-intertidal) and increased gametophytic dominance in the low-intertidal but this was not as marked as *Mazzaella laminarioides* in the high-intertidal. Longer exposure to stress in the high-intertidal was thought to promote a greater divergence in phase representation than that of the more stable low-intertidal. The amount of colonizing space was not mentioned. This contradicts the idea that an unstable environment promotes equal representation, but it is possible that such environments are stressed rather than unstable. A measurement of colonizing space may be a more important indicator. It is also unclear as to what is meant by "more unstable environment" or how it is compared between areas. However, growth was greater in the infralittoral compared to the midlittoral form of *Chondrus crispus*, which also implies a less stressed environment (Chopin and Floc'h 1992).

But what attributes does the dominant phase possess that enables it to outcompete the other phase?

One possibility is that the dominant phase has the capacity for faster growth in a certain set of environmental conditions. In comparing growth rate between the phases of *Sarcothalia scutellata*, the tetrasporophytes were found to have significantly higher growth rates in culture at 18 and

21°C, but showed little difference at 12 and 15°C (See Chapter 6). The temperatures encountered during spring low tides in the rock pools in which this species occurred were 12 to 16°C in winter and 14 to 18°C in summer. These rock pools are mostly covered by the sea so would mostly experience prevailing sea temperatures that range from about 11 to 17°C. Gametophyte dominance must therefore be attributed to some other factor in this species. *Grateloupia filicina* showed faster growth of the haploid phase at 18°C and at the light intensities of 30 to 120 μ mol photons m^{-1} s^{-1}. However, the diploid phase survived longer at 24°C and crusts were larger, although not significantly, at 200 μ mol photons m^{-1} s^{-1}. More investigation is required to determine if the diploid phase is more resistant to higher temperatures and irradiances and thus contributing to diploid dominance in this species.

Hannach and Santelices (1985) grew *Mazzaella laminarioides* and *Iridaea ciliata* from spores under a range of temperature and light conditions and found differences in growth rates. There was a tendency for the gametophytic phase to have superior or equal growth at optimal conditions of temperature and light (15°C and 35 to 60 μ mol photons m^{-2} s^{-1}) but for the sporophytic phase to have higher growth rates otherwise. Gametophytes of *Mazzaella laminarioides* appeared more tolerant of desiccation and higher temperatures (Luxoro and Santelices 1989).

Bhattacharya (1985) found differences in the growth rates in an intertidal population of *Chondrus crispus* with the haploid phase having faster growth than the diploid phase at all times except summer. In an intertidal population of *Gigartina polycarpa*, tetrasporophytes showed greater growth rates than female gametophytes, whereas no significant differences occurred between the phases of *Sarcothalia stiriata* (Levitt 1998). The growth rate was measured by determining the length of the fronds of tagged individuals over the course of a year. However, growth of these two species from spores showed no differences between the phases for either species when grown at a range of temperature and light intensities (Levitt 1998).

The phases may differ in survival strategy. One phase may increase survival by greater allocation of resources to mechanical structures to withstand physical stresses or to chemical means to withstand predation. In an analysis of frond survivorship the diploid phase of *Chondrus crispus* either equalled or was superior to that of the haploid phase whereas the latter exhibited a higher recruitment rate (Bhattacharya 1985). Dyck and DeWreede (1995) describe tetrasporophytes of *Mazzaella splendens* as having lower growth but higher survival rates whereas gametophytes showed much greater density fluctuations and appeared to exploit favourable conditions to offset greater loss in unfavourable times. The survival rate of juvenile plants of the diploid phase of *Gracilaria gracilis* in France was twice that of the haploid phase (Destombe *et al.* 1989). A major difference in size between phases was reported for subtidal *Gigartina skottsbergii* with tetrasporophytes being much smaller, but the idea that tetrasporophytes would be more resistant is not supported as the spore shedding process resulted in considerably more damage to tetrasporophytes than gametophytes (Piriz 1996). However, in *Gracilaria tikvahiae* the tetrasporophytes do not degenerate after reproducing in summer, as do the gametophytes. In unattached populations in environments that prevent the formation of holdfasts, such as sandy substrata, the diploid phase would have the advantage (Kain and Destombe 1995). Tetrasporic fronds of *Mazzaella splendens* were more resistant to hydrodynamic forces than gametophytic fronds (Shaughnessy *et al.* 1996). The former thus predominated in wave exposed sites whereas the latter predominated in more sheltered areas.

The influence of other organisms may have an effect on phase distribution. For example, a limpet was found to show a preference for the gametophytic phase of *Mazzaella laminarioides* (Hannach and Santelices 1985) which, although this does not account for gametophytic dominance, does support the idea that tetrasporophytes may be more resistant. However, preferential infection of sporophytes of *Chondrus crispus* and *Iridaea cordata* by green endophytes has been encountered (Correa and McLachlan 1991). Molluscan grazers showed a preference for tetrasporophytes of *Mazzaella laminarioides* (Luxoro and Santelices 1989). The tetrasporophyte preferences may be due to a carrageenan preference rather than superior resistance in the gametophytes. It has also been noted that amphipods that showed a preference for mature cystocarpic tissues of *Mazzaella laminarioides* appeared to facilitate the release and dispersal of carpospores (Buschmann and Santelices 1987).

The two phases may differ in reproductive capacity. Carposporic plants of *Grateloupia filicina* tended to release more spores per thallus area than tetrasporic plants. A higher proportion of carpospores compared to tetraspores settled in clumped positions, which tended to have a higher germination success rate than isolated spores. This may have contributed to the tetrasporic phase originally becoming more abundant.

The enhanced germination of clumped spores may be related to benefits conferred by sporeling coalescence. A number of species within the Rhodophyta have spores that form basal crusts that are capable of coalescence (Santelices *et al.* 1996; Maggs and Cheney 1990). Intraspecific cooperation that results from such coalescence may have advantages, such as protection from bleaching, that outweigh the disadvantages of crowding (Scrosati 1998a). Maggs and Cheney (1990) suggested possible advantages such as: a larger basal crust forming a more efficient holdfast; metabolic interactions between coalesced sporelings that may promote growth; greater resistance to grazing and maintenance of genetic diversity. They suggested that the sticky mucilaginous plug in which spores are released might promote sporeling coalescence. Transgressions of self-thinning laws by certain species may result from physical integration of fronds produced from coalesced sporelings promoting cooperation rather than competition (Martínez and Santelices 1992).

The number and seasonal pattern of fertile structures produced was similar for both phases of *Sarcothalia scutellata.* The number of spores released by each phase was similar in autumn and winter, whereas more carpospores were released in spring, but fewer carposporophytes released spores in summer. Greater frond size of tetrasporic fronds in spring and summer may mean that ultimately more tetraspores are released. The number of spores released per frond each season can be approximated using mean number of spores per fertile structure, mean number of fertile structures per frond and mean frond masses (see Table 4.2 of Chapter 4). The mean number of spores per tetrasporic frond ranged from a minimum of 1.8×10^6 in June 1998 to a maximum of 1.4×10^7 in December 1997. The mean number of spores per cystocarpic frond ranged from a minimum of 8.8×10^5 in December 1997 to a maximum of 8.2×10^6 in September 1998. Greater numbers of tetraspores per frond were produced only in summer and autumn whereas more carpospores were produced in winter and spring.

If similar calculations are made for *Grateloupia filicina* using the mean mass of fertile fronds and the mean number of spores released per 0.002g of fertile thallus section per season, most spores were released per frond in spring for both phases (see Table 5.4 of chapter 5). The mean number of tetraspores released per frond ranged from a minimum of 5.4×10^6 in March 1998 to a maximum of 1.8×10^7 in September 1998. The carposporic fronds released a minimum of 1.2×10^7 carpospores in June 1998 and a maximum of 2.9×10^7 in September 1998. With the exception of March 1998, when parent thalli failed to release spores the carposporic fronds tended to release greater numbers of carpospores which may have contributed to tetrasporophyte dominance.

Values obtained for other species include 3.0×10^5 carpospores and 2.7×10^4 tetraspores in summer, and 3.4×10^4 carpospores and 1.9×10^4 tetraspores in winter per plant of a tetrasporophyte dominated population of *Gelidium robustum* (Guzmán del Próo *et al.* 1972); 8.3×10^7 carpospores per plant of *Rhodymenia pertusa* (Boney 1978) and 7×10^5 carpospores and 1×10^6 tetraspores per specimen of *Chondracanthus canaliculatus* (Pacheco-Ruiz *et al* 1989). Phase dominance is not mentioned in the latter two studies.

The dominance of a particular phase may be achieved or maintained by vegetative reproduction (Braga 1990, Scrosati 1998a) and appears to be an important method of reproduction in populations where space is limiting. *Iridaea cordata* in California was dominated by the tetrasporangial stage (Hansen and Doyle 1976). Perennial basal crusts from which vegetative growths occurred maintained occupation of the substratum after blade senescence occurred in autumn thus maintaining the dominance (Hansen 1977). In the area in which the latter study was conducted open spaces were rare. This same species was gametophyte dominated further north in Washington and was also thought to be maintained by crust perennation (May 1986). In a study in which areas of the substratum were cleared in order to determine mechanisms of recolonization of *Mazzaella cornucopiae*, recruitment from spores and vegetative recruitment from fronds surrounding the cleared areas made similar contributions (Scrosati 1998b). Spore dispersal was able to cover a greater distance than was vegetative growth from perennating holdfasts. However new thalli from spores appeared to have been vulnerable during periods of high desiccation and irradiances in summer.

In an attempt to synthesize the sometimes-conflicting evidence, the following theory for phase distribution is postulated. In an environment sufficiently disturbed so that new colonizing space is continually made available, both phases are continually establishing themselves and a mixture of phases results. The haploid phase may allocate resources towards faster growth rates and higher levels of recruitment. In an environment with much competition for space, but where physical stresses are not too harsh, areas may be colonized more rapidly and dominance of the haploid stage might then be expected.

The diploid phase may be allocating more resources to strategies of survival, both mechanical and chemical. This requires more elaborate structures to be made, such as stronger mechanical support, resulting in overall growth to decrease. It would thus be expected to be slower growing but more resistant to physical stresses such as high temperatures, stronger wave action and greater environmental fluctuations. It would also be expected to be more resistant to predation. Thus, environments with a high degree of physical stress would be expected to favour the diploid phase such as in the high-intertidal areas and perhaps exposed shores, provided the stress is not so strong as to remove thalli. Once dominance is established, it may be maintained by vegetative reproduction in either phase.

This of course needs further investigation such as evidence of the diploid phase developing mechanisms to promote survival and faster growth rates of the haploid phase in environments in which they dominate. There are also contradictions in the literature and in this project as described above.

The preceding discussion assumes that if the life-history phases are ecologically similar they must possess a G:T ratio close to 1. However, demographic models have demonstrated that in certain instances it is possible for ecologically similar phases to show a G:T ratio that is not equal to 1 (Scrosati and DeWreede 1999). This occurred for annual thalli in which life history phases produced equivalent numbers of spores per frond but had initial G:T ratios not equal to one and in annual, triennial and perennial thalli that produced four tetraspores per frond for every one carpospore produced per male-female gametophyte pair. The annual thalli fluctuated between tetrasporophyte and gametophyte dominance, whereas the longer lived thalli exhibited dampening oscillations that stabilized at a G:T ratio of 2.8. The longevity of thalli and the number of spores produced per frond are thus further factors to investigate in species with phase dominance. The longevity of the thalli of the species investigated in this study is not known but the complexity of form indicates that they are not annuals. Neither species appeared to produce four tetraspores for every one carpospore although *Sarcothalia scutellata* did have a G:T ratio close to 2.8.

Comparisons between the two species

Grateloupia filicina was the dominant macroalgal species in many of the high-intertidal rock pools on the west coast. It is also sometimes found in mid-intertidal rock pools as well as subtidally but not in such abundance as in the higher pools. The mid- to low-intertidal pools of the west coast are often dominated by *Sarcothalia scutellata* and it is possible this species prevents *Grateloupia filicina* from becoming abundant in the lower pools.

In comparing the size of the two species, the individual frond masses of *Grateloupia filicina* (mean fresh mass of 5 to 10g) was comparable to that of entire clumps of *Sarcothalia scutellata* (mean fresh mass of 6 to 14g) rather than to individual frond mass (mean fresh mass of 0.2 to 4g). The fronds of *Grateloupia filicina* frequently formed a bushy mass attached to a single stipe. Much branching may provide similar benefits to that of clumping. *Sarcothalia scutellata* had a bushy appearance when many fronds were clumped together. This may protect the algae from desiccation or may act as cushioning in areas of high wave impact. However, it was noted that fronds were also able to occur singly. Correlations between wave impact or desiccation and frond clumping could be investigated. As both species occur in rock pools and so were usually permanently submerged in seawater the risk of desiccation is decreased.

In comparing fecundity, *Sarcothalia scutellata* released far more spores per reproductive structure (in the tens of thousands) than were released per 4mm^2 by *Grateloupia filicina* (in the thousands). The 4mm^2 thallus pieces contained more than one carposporophyte but it was difficult to tell if more than one released spores over the time period investigated. The germination success rate of spores of *Sarcothalia scutellata* tended to be somewhat higher than that for *Grateloupia filicina* and remained consistently high throughout the study period whereas the latter species showed a decline at certain times of the year. The spores of *Sarcothalia scutellata* were also bound by more mucilage. These differences in fecundity may contribute to *Sarcothalia scutellata* being more abundant in the lower pools than *Grateloupia filicina*.

Both species showed year round fertility with fewest cystocarps of *Sarcothalia scutellata* and tetrasporangial sori of *Grateloupia filicina* produced in winter. No seasonal variation occurred for numbers of tetrasporangial sori produced in *Sarcothalia scutellata* or in percentage of carposporic thallus that was fertile and number of carposporophytes produced per fertile thallus section in *Grateloupia filicina*. The number of carpospores released per cystocarp or per fertile thallus section indicated highest fecundity in spring whereas the number of tetraspores released did not show any seasonality for both species. Other species within South Africa including *Suhria vittata* (Anderson and Bolton 1985); *Gelidium pristoides* (Carter 1985); *Sarcothalia stiriata* and *Gigartina polycarpa* (Levitt 1998) also showed year round fertility. *Gelidium pristoides* produced more tetrasporangial sori in spring compared to late summer (Anderson *et al.* 1991). *Sarcothalia stiriata* and *Gigartina polycarpa* had similar numbers of cystocarps and tetrasporangial sori per gram of fresh weight in winter and summer (Levitt 1998).

Various studies of other species that occur within a similar latitudinal range (from around 30 to 45 degrees) including *Gelidium robustum* (Gardner) Hollenberg and Abbott (Melo and Neushul 1993 (34°N)) and *Gigartina skottsbergii* (Piriz 1996 (44°S)) also showed year round fertility. Seasonal variation in degree of fertility is often described at these latitudes. Tetrasporophytes of *Gelidium robustum* in Argentina produced more sori in spring/summer but the number of spores released per sorus was greater in winter (Melo and Neushul 1993). Species of *Gracilaria* from higher latitudes were said to show reproduction peaks in late summer but was high all year in the tropics (Kain and Destombe 1995). Cystocarpic plants of *Gigartina skottsbergii* had an autumn peak in fertility as indicated by the percentage of the thallus that was fertile (Piriz 1996).

Other studies in areas within 30 to 45 degrees of latitude found periods of infertility, but in only a few of the species studied. At Cape Cod in the west Atlantic at around 42°N, 12% (Sears and Wilce 1975) and 10% (Mathieson *et al.* 1981) of the species had periods of infertility. The latter study found that some species became reproductive when water temperatures were low, others when the water was warmer and the remaining species were reproductive all year regardless of temperature. *Gracilaria tikvahiae* from the Great Bay Estuary, New Hampshire, at 43°N, was mostly vegetative but greatest fertility and growth occurred in summer and was correlated with increased water temperature (Penniman *et al.* 1986).

Periods of infertility are common at higher latitudes. A high percentage of species (45%) had periods of infertility in Newfoundland between 49 and 50°N (Hooper *et al.* 1980). At 48°N, reproductive fronds of *Mazzaella cornucopiae* from the Pacific coast of Canada were only present in autumn and winter (Scrosati 1998a). Three out of six subtidal rhodophytes studied at the Isle of Man (54°N) were fertile year round (Kain 1986). Maximum fertility of three of these species occurred in autumn and for the remaining three in spring. *Grateloupia filicina* from the British Isles has fertile fronds of both phases throughout the year with cystocarps being most abundant in summer (Irvine 1983). At around 69°N in the Barents sea all of the nine species that were studied had periods of infertility (Schoschina *et al.* 1996). Six of the nine species had summer peaks of reproduction.

The reasons for seasonal disparity in fertility between different species are uncertain. Most of the species in the studies reviewed in Kain and Norton (1990), which occurred at various latitudes, became reproductive in short days. This was ascribed to winter being a time of greater wave action and less growth providing more space for colonization. It is more likely that plants become fertile in winter in order to produces new juveniles in late winter or early spring which may subsequently utilize favourable periods for growth. Enhanced fertility is thus triggered by short days in many species. It is also possible that different species have adapted and evolved over long periods to either colder or warmer climates. Timing of fertility depends on where that species was collected. Those adapted to cold environments that have managed to spread to lower latitudes may display winter reproduction and vice versa.

The final comparison between the two species is that of growth in culture. Both species had growth optima for sporelings at temperatures higher than those generally experienced on the west coast. Greatest growth occurred around 18 to 21°C. Light saturation occurred at 30 to 60 μ mol photons $m^{-2} s^{-1}$ for both species, a similar level obtained for sporelings of many other species.

Practical applications for mariculture

Fertile fronds of *Grateloupia filicina* may be collected throughout the year for growing the species from spores. Most of the plants encountered will be tetrasporic and fertile fronds may be identified as those possessing many terete ultimate branchlets of similar length (1 to 3 cm) that show pinnate branching and are olive in colour. Carposporic fronds are far less common but fertile fronds may be identified by dark spots spread over the thallus. Although year round fertility occurs the optimal period to collect fertile fronds appeared to be spring when greatest number of tetrasporangia occurred and the greatest number of carpospores were released. The carposporic fronds appeared less ready to release carpospores in autumn. Germination of spores in culture should be performed at 15°C rather than 20°C. Greatest frond masses occurred in summer.

Fertility was high throughout the year for *Sarcothalia scutellata* but spring/summer appears to be the optimal period to collect fertile fronds. Greater numbers of reproductive structures were produced and greater numbers of spores were released during this period. Highest frond masses occurred in summer and lowest carrageenan content in spring.

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