FIELD AND CULTURE STUDIES ON THE TILOPTERIDALES (PHAEOPHYCEAE) IN NEWFOUNDLAND

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By

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Abstract

The family Tilopteridaceae (Tilopteridales, Phaeophyceae) consists of three monospecific genera of which two species, *Tilopteris mertensii* and *Haplospora globosa*, occur throughout the North Atlantic and Arctic Ocean. The third species, *Phaeosiphoniella cryophila*, is restricted to four localities in Newfoundland and, aside from its initial description, is little known. Both *Phaeosiphoniella* and *Haplospora* develop prostrate systems when their rhizoids come in contact with the substrate. These prostrate discs are functionally, and to some degree also morphologically, equivalent to crusts of species of the Sphacelariales or Scytosiphonales and serve as perennating structures during the summer. Upright filaments are produced from the prostrate systems only under winter conditions. Combined culture and field studies showed that uprights of *Phaeosiphoniella* are restricted to water temperatures of less than 10°C and light intensities of less than 8 μmol m^{-2} s^{-1} while prostrate discs withstand up to 20°C.

Phaeosiphoniella reproduces exclusively through fragmentation and abscission of branch systems. The sporadically occurring antheridia or sporangia are functionless and the reproductive system is more reduced than in the other two species.

Quantitative DNA fluorometry confirmed that *Tilopteris* and *Phaeosiphoniella* exhibit only one level of ploidy, whereas the two generations in

Haplospora show different ploidy levels, although the number of chromosomes does not change.

The reduced life cycles shown in *Tilopteris* and *Phaeosiphoniella* might indicate that both species are derived forms and have formerly possessed a sexual life cycle with an alternation of generations such as seen in *Haplospora*. This study suggests that *Phaeosiphoniella* represents a relic species which exists only at the extremes of its former area of distribution. Members of the Tilopteridaceae, especially *Phaeosiphoniella*, might be regarded as species with weak competitive capabilities, but with the ability to live in areas of high physical disturbance where competition with other algae is low.

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Introduction

The algal order Tilopteridales (Phaeophyceae) contains a single family, the Tilopteridaceae, and three monospecific genera. *Tilopteris mertensii* (Turner in Smith) Kützing and *Haplospora globosa* Kjellman have been known for almost two centuries, whereas *Phaeosiphoniella cryophila* Hooper, Henry *et* Kuhlenkamp was discovered during benthic studies by R. Hooper in 1976 (Hooper *et al.* 1988). As the genera are monospecific they will be referred to solely by their generic name throughout this dissertation.

Tilopteris and *Haplospora*, although of sporadic occurrence, are widely distributed in the North Atlantic and Arctic Ocean. Their southern limits are near the 18°C summer surface isotherm (South & Tittley 1986). *Phaeosiphoniella* has a more restricted distribution and is currently only known from four sheltered embayments on the south coast of the island of Newfoundland (Hooper *et al.* 1988).

Dawson Turner collected the type material of *Tilopteris* in 1779 and described the species in Smith's English Botany (1802) as *Conferva mertensii* (Brebner 1896). Kützing (1849) created the genus *Tilopteris* within the family Ectocarpaceae where it remained until Thuret (1855) established the Tilopteridaceae, based primarily on the occurrence of large non-motile

'monospores'. The life history of *Tilopteris* was first described by Damman (1930) and further elucidated through subsequent culture studies by South (1972), Moestrup *et al.* (1975) and Kuhlenkamp & Müller (1985). *Tilopteris* exhibits a life cycle involving only gametophytes which reproduce by means of eggs. Although antheridia are present on most specimens, released spermatozoids are functionless and fertilization has never been observed. Eggs, therefore, develop into a new generation of identical plants through apomictosis.

The confusion about the terminology dealing with tilopteridalean life histories requires the definition of a number of terms. Apomictosis (original in German: Apomiktosis) defines the development of the gametophyte from an unfertilized gamete or egg (Winkler 1942). All modifications in the development of the gametophyte within a usually sporic life cycle are termed aposporosis (original in German: Aposporosis) and include besides apomictosis such modifications as vegetative propagation and apospory. Modifications in the development of the sporophyte are termed apomixis (original in German: Apomixis) and include parthenogenesis which describes the development of sporophytes from unfertilized eggs. Since this nomenclature by Winkler (1942) is undisputed and widely used (Stosch 1967) it is more appropriate to describe the reproduction in *Tilopteris* as apomictotic instead of parthenogenetic as in previous publications. This study, in using the term apomictosis, indicates that reproduction in *Tilopteris* is even more reduced than in *Haplospora*, because eggs reproduce the gametophyte only, eliminating the sporophyte stage.

Haplospora globosa was first described and placed within the Tilopteridaceae by Kjellman (1872) who separated the gametophyte generation as

the species *Capsicarpella speciosa* Kjellm. (Ectocarpaceae), which he later renamed *Scaphospora* and transferred to the Tilopteridaceae (Kjellman 1877). Reinke's (1889) proposal that *Scaphospora* and *Haplospora* were conspecific was supported by Brebner's (1896) field observation of specimens that bore both sporangia and oogonia with antheridia, a phenomenon named apogamy or apospory. A specimen of *Haplospora* (the sporophyte) bearing antheridia next to sporangia had been described earlier by Kjellman (1872). Past taxonomic considerations of the *Haplospora* complex have relied mostly on the interpretation of morphological observations, whereas subsequent culture studies have shown unequivocally that *Scaphospora* is the gametophyte of *Haplospora* (Sundene 1966, Kuhlenkamp & Müller 1985). Gametophytes, which bear both oogonia and antheridia, alternate with isomorphic sporophytes characterized by quadrinucleate sporangia. Sundene (1966) reported his observation of fertilization in *Haplospora*, but presented no confirmatory cytological evidence. Sexual reproduction is, however, apparently absent. Spermatozoids degenerate after release and eggs develop parthenogenetically. Chromosome numbers are identical in both the sporophyte and gametophyte phases though meiotic stages are present in the development of spores (Kuhlenkamp & Müller 1985). Meiotic stages have been reported to occur in a similar manner in unilocular sporangia of haploid sporophytes in *Ectocarpus siliculosus* (Dillw.) Lyngb. (Müller 1967a) which produced offspring with the same chromosome number as the parent. Oltmanns (1922) regarded cytological investigations as necessary in order to identify the reproductive organs present in the Tilopteridaceae. Nienburg (1923) was the first to undertake such work on *Haplospora*. He carefully described the dividing stages

in the sporangia, which he assumed were meiotic, although he could not show any reduction in the chromosome number. Cytological studies were repeated by Damman (1930) with similar results. Unfortunately no data were presented on the chromosome numbers in somatic cells which would have confirmed the failure of meiosis in sporangia. Both authors were confronted with the disadvantage of using study material obtained from the local populations of *Haplospora* at Helgoland (FRG), where the gametophyte generation is absent and spores from the quadrinucleate sporangia give rise directly to a new sporophyte generation (Kuhlenkamp & Müller 1985).

There is considerable confusion in the use of the terms oogonia, sporangia, monosporangia, eggs and monospores which appear interchangeably and uncritically in many descriptions of members of the Tilopteridales. Reinke (1889) and Brebner (1896) postulated the occurrence of sexual reproduction in *Haplospora* based on their observation on the morphology of the 'monosporangia' and the plurilocular structures. They argued that *Haplospora* is oogamous because the contents of the uninucleate 'monosporangia' (on the gametophyte) were released naked, without a cell wall, and hence were eggs not spores. However, the cells produced from the quadrinucleate 'monosporangia' (on the sporophyte) formed walls prior to release and hence should be regarded as spores. Brebner (1896) extended this theory by including the 'monosporangia' of *Tilopteris*, which release their uninucleate contents without a cell wall and should therefore be regarded as eggs. Kylin (1917) agreed with Brebner (1896) and Reinke (1889) in separating the reproductive structures in the gametophyte stage of *Haplospora* from those in the sporophyte stage. But he did not define the mode of

reproduction. In the attempt to find a plausible explanation for his observations, Sauvageau (1899) proposed the occurrence of parthenogenesis in *Haplospora*. Unfortunately, he retracted his theory and suggested that all types of 'monospores' should be regarded as propagules, thus postulating vegetative reproduction (1928). South (1972) noted that Sundene (1966) did not present any cytological studies to show fertilization in *Haplospora* and for that reason lacks evidence for oogamy. South (1975), however, proposed oogamy to be the mode of reproduction without providing any further evidence for his conclusions. Similarly, Pedersen (1984) supported the idea that oogamy is present in *Haplospora*, while expressing doubts about the evidence of fertilization supposedly observed by Sundene (1966). Pedersen (1984) uncritically maintained the interpretations of reproduction by Damman (1930) and South (1975), but he disagreed with South (1975) in describing quadrinucleate sporangia as meiosporangia because they are different from the uninuclear sporangia releasing zoospores, for which the term meiosporangia was originally introduced. In mature quadrinucleate spores all four nuclei remain active during germination and in some germlings these nuclei are not separated by cell walls and resemble undivided tetraspore mother cells of members in the Dictyotales (Robinson, 1932). Culture observations by Kuhlenkamp & Müller (1985) finally confirmed a modified oogamous reproduction with apomeiosis and parthenogenesis.

Since Damman (1930) discovered the life history of *Tilopteris*, there have been several different and confusing attempts to describe the mode of reproduction in *Tilopteris* and its position within the Tilopteridaceae. Damman (1930) constructed a life cycle based on *Tilopteris* as the sporophyte and a

hypothetical unknown gametophyte that remains to be found. Kylin (1947) introduced the term 'parthenosporangium' to describe the oogonia-like structures releasing 'monospores', which develop without fertilization. South (1975) adopted the term 'parthenosporangium', although he suggested that *Tilopteris* was equivalent to the gametophyte stage in *Haplospora* which should have implied the presence of gametangia, not sporangia.

The diverse interpretations of the life histories of members of the Tilopteridaceae are due to a combination of incomplete or unreliable observations, and the fact that isolates from different locations behave differently. The absence of fertilization, as shown cytologically, can be interpreted as a phylogenetic reduction in this line, resulting in a secondary loss of sexuality within the Tilopteridaceae. The study of Kuhlenkamp & Müller (1985) has shown conclusively that despite the absence of a sporophyte generation in *Tilopteris* and the absence of sexual reproduction in *Haplospora*, as seen in the apomeiotic development of the gametophyte and subsequent parthenogenetic development of the sporophyte, it is still reasonable to regard their reproduction as oogamous. A phylogenetic approach allows the resolution of these conflicts which otherwise emerge from a purely functional classification. Functional classifications do not take into account the chromosomal abnormalities combined with parthenogenesis and apomixis. Tilopteridalean life histories therefore have to be regarded as fluctuating or fluid systems and not as static or stable life cycles. Instability is shown for example in the *Haplospora* complex where, on occasion, uninucleate spores are released prior to the formation of the quadrinucleate stage. Such spores develop into sporophytes instead of gametophytes (Kuhlenkamp & Müller 1985).

Taxonomic opinions about the Tilopteridaceae are divergent. Wynne (1982) retains the Tilopteridaceae within the order Tilopteridales, whereas Parke and Dixon (1976) place them in the Ectocarpales. In addition, characters and definitions used by Pedersen (1984) and Christensen (1980) extend the circumscription of the order Tilopteridales to include families usually placed in the Dictyosiphonales (Wynne 1982). Most phycologists studying the Tilopteridales, however, regard them as sufficiently distinct to warrant their recognition as a separate order within the Phaeophyceae.

Phaeosiphoniella was placed in the Tilopteridaceae (Hooper *et al.* 1988) because of its thallus morphology, but the discovery of this species makes the definition of the limits of the family even more difficult. Reproduction in *Phaeosiphoniella* differs from the other two species in that it is principally by vegetative propagation. Hooper *et al.* (1988) found antheridia and plurilocular sporangia in field material and observed that oogonia may develop in culture. The reproductive structures appear to have no importance in its life history of when compared to the extensive fragmentation of filaments or the abscission of small branches, which rapidly develop rhizoids to adhere to the substrate. While *Phaeosiphoniella* undoubtedly belongs in the Tilopteridaceae, its relationship within the family has not been sufficiently addressed, nor was the evolutionary aspect of a third member of the family discussed in detail by Hooper *et al.* (1988). Further culture and field studies on *Phaeosiphoniella*, *Haplospora* and *Tilopteris* are required to re-evaluat the Tilopteridaceae and to determine its affinities to other families of the Phaeophyceae.

In this study, field, culture, and cytological investigations are integrated in

an attempt to gain further information on the biology of members of the Tilopteridaceae in Newfoundland and to further elucidate their evolutionary relationships. Russell (1973) stressed the need for combined field and culture observations in such studies, but relatively few have been undertaken in the Phaeophyceae. Reinke (1889) described pseudoparenchymatous, adhesive discs in *Haplospora* produced by the tips of rhizoids after contact with the substrate. Initial observations on *Phaeosiphoniella* revealed the presence of similar, prostrate structures formed from the rhizoids. In addition to their role in attachment, prostrate systems may be particularly important as perennating structures for both *Haplospora* and *Phaeosiphoniella* in Newfoundland. The sandy, unstable habitats in which these species are found are difficult to characterize in terms of community structure. Such habitats clearly present different environmental conditions than those of the stable rocky substrates which have received considerable attention in ecological research. The present study will therefore attempt to elucidate some of the questions concerning the life histories of *Phaeosiphoniella* and *Haplospora* with regards to their habitat and survival capabilities, especially the means of perennation during the time of the year when upright plants are absent.

The life histories of Newfoundland members of the Tilopteridaceae have been re-evaluated by applying traditional cytological methods of chromosome staining and counting, as well as quantitative DNA fluorometry to measure DNA levels of individual nuclei. Fluorometry, utilizing either mithramycin or DAPI as DNA specific fluorochromes, has been successfully applied to a number of different green and red algae (Coleman *et al.* 1981, Hull *et al.* 1982, Goff & Coleman

1984). Müller & Schmidt (1988) used the technique in brown algae to localize the change in ploidy level within *Elachista stellaris* Areschoug. The DNA fluorometry is considerably more specific and sensitive than the previous method involving the Feulgen reaction and is ideal for measuring DNA contents of single algal nuclei. DNA fluorometry easily allows the comparison of DNA values between any nuclei within a species and subsequently to determine possible changes in ploidy between generations.

Materials and Methods

2.1. Collection of algae at study sites

All algal collections were made by means of scuba diving at several sites on the Island of Newfoundland (Fig.1). Fertile *Tilopteris* was collected on three occasions in Conception Bay, at depths of approximately 3 to 6 m: in October 1985 at Bell Island (47°37'N,52°58'W), in October 1986 at Kelly's Island (47°33'N,53°01'W) and in October 1988 at Seal Cove (47°28'N,53°04'W). *Haplospora* was collected mainly at Fairhaven in Placentia Bay (47°31'N,53°54'W) at depths of 10 to 14 m and was also found at Mt. Carmel, St. Mary's Bay (47°08'N,53°30'W) in depths of 8 m. Most *Phaeosiphoniella* specimens were collected in 9 to 14 m of water at the Fairhaven site but were also found in Fortune Bay at Grand le Pierre (47°40'N,54°47'W) at 8 m depth. All depths are expressed in metres below MLW.

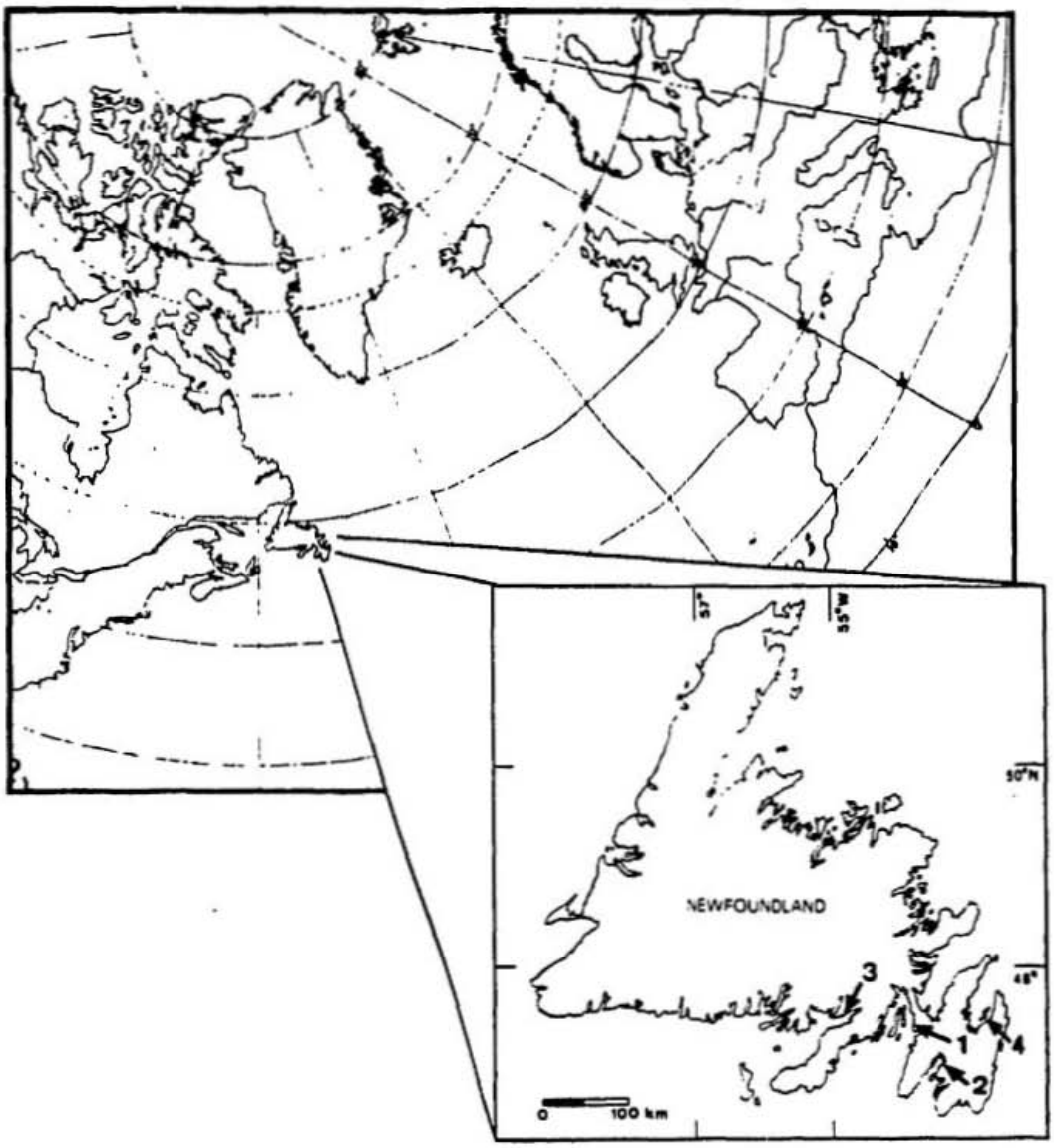


Fig. 1: Locations of study sites on the Island of Newfoundland.

1. Fairhaven (Placentia Bay).
2. Mt. Carmel (St.Mary's Bay).
3. Grand le Pierre (Fortune Bay).
4. Bell Island, Kelly's Island, Seal Cove (Conception Bay).

Fairhaven was chosen as the main study site for this work, because of the locally high abundance of *Phaeosiphoniella* and *Haplospora*. The site had been visited at almost monthly intervals since the initial discovery of *Phaeosiphoniella* in 1976 and, in consequence, there are many previous data on the species and their locations on which to develop this study (Hooper *et al.* 1988). The habitat consists of small pebbles and shell fragments embedded in a substrate of mud and sand. Most of the small stones examined were colonized only on the upper surface. There are occasional rock outcrops but the overall topography is flat and even. The study area ranged in depth from 7 m at the shoreline to 15 m at a distance of approximately 100 m. The location suffers frequently from storms and the substrate is considered unstable. The densest stand of *Phaeosiphoniella* was marked by means of anchored quadrats of one square metre; these were linked to the shore by a 100 m transect line. The line allowed rapid location of the site by scuba divers operating in the poor visibility, which frequently prevailed after storms or during phytoplankton blooms.

The site was visited regularly and collections were made at approximately two week intervals from December to June in 1985/86 and 1986/87. Sampling during the summer season was done only sporadically. Plants were collected by hand, placed in Whirlpak® model 1020 plastic bags and transported to the laboratory in ambient seawater in insulated containers. An initial microscopic screening of the material was undertaken before samples for further study were placed in incubators. Temperature recordings were made during each diving observation with a calibrated mercury thermometer placed near the substrate where the algae were collected. A continuous temperature recording was obtained

by R. Hooper (pers. comm.) by means of a Peabody-Ryan Model 'J' thermograph employed over the year 1978/79 at Fairhaven close to the main study area and 3 to 4 m above the sea floor at about 10 m depth.

2.2. Culture methods

Cultures were grown either in Provasoli ES medium as described by Starr (1978) or in filtered seawater. The medium was changed every 2-5 days with the ES-medium and glassware heat sterilized prior to use. Algae were grown in glass or polystyrene dishes incubated at temperatures of 1, 5, 10, 15, or 20°C under 8:16 (short day: SD) and 16:8 (long day: LD) light:dark photoregimes. The lighting was provided by GE Cool-White fluorescent tubes with intensities altered by varying the distance from the source or by filters. The photon flux densities were measured using a Li-Cor quantum radiometer Model 185B fitted with a quantum sensor Li 190 SB (Li-Cor, Lincoln, Nebraska, USA); all measurements are expressed in μmol m^{-2} s^{-1}. Additional photobiological studies used red (640 nm) and blue (430 nm) filters to limit the light spectrum.

Clonal cultures of *Tilopteris* and *Haplospora* were established from single eggs or spores, individually pipetted into sterile media from the initial cultures of mature field material. Cultures of *Phaeosiphoniella* were derived from vegetative fragments of field specimens. Early developmental stages and basal systems of *Haplospora* and *Phaeosiphoniella* were studied from cultures that included the substrate collected in the field, usually pebbles or fragments of scallop shells. The material was cleaned in sterile seawater with a soft brush or tooth brush prior to culture. In order to reduce contamination in these cultures most were grown in un-enriched seawater. Such substrates frequently carried small brown crusts and

basal systems from which the uprights were absent. The identity of the prostrate systems was established after the filaments developed sufficiently to allow their determination.

2.3. Fluorometry

Quantitative DNA fluorometry was initially undertaken using the procedures described in the literature (Coleman *et al.* 1981, Hull *et al.* 1982) with some modifications being developed in the course of the study. The two fluorochromes utilized in this study, DAPI (4'-6-Diamidino-2-phenylindole) and the fungal antibiotic mithramycin, both obtained from Sigma Chemical Co., St Louis, Missouri, USA, have a highly specific affinity for DNA. DAPI shows some staining of RNA as well. Stock solutions of each fluorochrome were made at concentrations of 1 mg/ml in distilled water and stored in the dark at 1°C. The working concentrations were 50 μg/ml of mithramycin in McIlvaine's buffer at pH 7.0 and 0.5 μg/ml of DAPI in McIlvaine's buffer at ph 4.0 (Hull *et al.* 1982).

In order to reduce growth artifacts, only vigorously growing plants were fixed for epifluorescence measurements of DNA levels. All specimens were fixed in absolute ethanol:glacial acetic acid (3:1) diluted 1:1 with seawater for a period of up to five hours and then transferred to 70% ethanol. This procedure also ensured that during subsequent micro-spectrophotometry, interference fluorescence from photosynthetic pigments, especially chlorophylls, was suppressed. For DAPI staining, fixed specimens were rinsed for 10 min in distilled water, placed in DAPI for 2 hr and mounted in buffer on glass slides. For mithramycin staining, fixed specimens were rinsed for 10 min in McIlvaine's buffer at pH 7 containing 10 mM of magnesium chloride, stained for 30 min in mithramycin and mounted in buffer

on glass slides. All staining manipulations were carried out on ceramic plates containing small wells and kept in the dark to avoid premature UV-light exposure. Specimens were handled by means of glass hooks to allow the preparation of short filaments in 10-30 μl of stain solution.

The prepared, mounted specimens were examined on a Zeiss photo-microscope III fitted with fluorite objectives, utilizing an oil immersion Plan Neofluar 25/0.8 for the actual fluorescence measurements. The microscope was fitted with a 50 W UV-epifluorescence system. Fluorescence emitted from the stained material was amplified through a Zeiss photo-multiplier system and PMI amplifier/digital voltmeter attached to a chart recorder. The system was operated in the range such that the readings on the voltmeter were linearly related to the amount of epifluorescence. The voltmeter readings are presented in this study as relative fluorescence units (rfus). Filter combinations in the source beam provided the required excitation wavelengths for the fluorochrome DNA complex, while another filter in the emitted light beam acted as a barrier filter to reflected UV light and other unwanted naturally occurring fluorescence of different wavelength. For DAPI, Zeiss filter combination 487702 (exciter filter: 365 nm, barrier filter: 420 nm) was used and for mithramycin 487709 (exciter filter: 450-490 nm, barrier filter: 520 nm) or 487718 (exciter filter: 395-425 nm, barrier filter: 450 nm). A diaphragm in the incident light path limited the illuminated area of the slide to about three times the area of the nucleus under examination to reduce light scatter and photochemical bleaching of adjacent nuclei. The emitted epifluorescence was restricted to the approximate size of the nuclei examined by the use of pinhole apertures in place between the specimen and the photo-

multiplier head in such a manner that they appeared in the same focal field as the specimen. All measurements of tilopteridalean nuclei used the same pinhole, even though the size of nuclei varied. Each nucleus was moved under transmitted visible light into the center of the pinhole field and then illuminated with UV light. Emitted fluorescence was measured immediately and recorded on chart. Instantaneous recording of fluorescence from mithramycin stained material provides an initial, maximum intensity, which under continued UV excitation begins to decrease within the first second and levels off after several minutes, due to rapid fading (Hull *et al.* 1982). In order to standardize the method, only the initial, maximum intensity within the first second of UV irradiation is used as a reading with both, mithramycin and DAPI. Every nuclear measurement was followed immediately by a reading of the adjacent cytoplasm to establish a background value which was subsequently subtracted from the nuclear reading.

In order to evaluate and standardize the fluorescence techniques several preliminary experiments were conducted, both with a chicken red blood cell (RBC) standard and with *Tilopteris*, which was chosen because of its ease of cultivation and handling. Chicken blood is recommended as a standard to determine the variation of the staining method over time. It is a reliable source of nuclei with constant DNA levels and is readily available. It was fixed in an identical manner to the algae and stored in 70% ethanol at 4°C (Coleman *et al.* 1981).

The minimum time required for staining was investigated and fluorescences of RBCs after staining for 5, 10, 15, 30, 60 and 120 min examined to determine the maximum level of intensity. The rate of fading was also examined using

measurements on the same individual nucleus under continuous UV illumination. The effects of storage of fixed material were evaluated measuring nuclei of the same specimen of *Tilopteris* held in 70% ethanol for varying periods of time. Measurements were only taken from large cells of the main filament which were proven to be in the same stage within the nuclear cycle, the G2 stage. Similar cells in G2 stage were used in comparative DNA measurements of separate generations in *Tilopteris*. To ensure consistent results, all fluorescence measurements have to be taken with material of the same storage time in fixative.

The data obtained were analyzed by analysis of variance and Scheffé 's multiple range test. Results shown in graphics or text are given as a mean and 95% confidence limits. All statistical calculations were by SPSS-PC+ (Norušis 1986) on an IBM clone personal computer.

2.4. Chromosome staining

Material for cytological studies involving examination of nuclear divisions and chromosome counting was fixed in an identical manner to that for DNA measurements. Whenever possible, fixing was done approximately 2 hr after the onset of darkness in order to maximize the frequency of nuclear stages in which chromosomes can be counted. This is based on the observations that in field material of some Scytosiphonaceae a peak of nuclear divisions occurs 2-4 hr after sunset (Kapraun & Boone 1987). Chromosome staining used aceto-iron-haematoxylin-chloral hydrate according to Wittmann (1965). Specimens were mounted in Euparal®.

2.5. Microscopy

Microscopical examinations were carried out on a Zeiss photomicroscope III fitted with a variety of Neofluar and Plan-apochromat objectives, a Nikon inverted microscope and a stereoscopic Wild Makroscop. All microscopes had attached 35mm camera systems. Photomicroscopy for this thesis was on Kodak Panatomic X and on Tri-X pan photographic film.

Results

3.1. Field observations

3.1.1. Water temperature

The water temperatures for the Fairhaven study site are presented in a graph (Fig.2) combining the single temperature measurements obtained in this study with the continuous measurements of the period February 1975 until March 1976 obtained by R. Hooper (pers. comm.). Both measurements coincide and show an increase of the water temperature from ca -0.5°C at the end of April to a maximum of ca 16°C in August and September. After the summer peak, there is a steady decrease in temperature and the minimum of -1°C occurs in February and March. Rapid temperature changes may be superimposed on these regular seasonal changes and daily fluctuations of 4°C were observed, presumably the result of upwelling of deeper, colder water (R. Hooper pers. comm.)

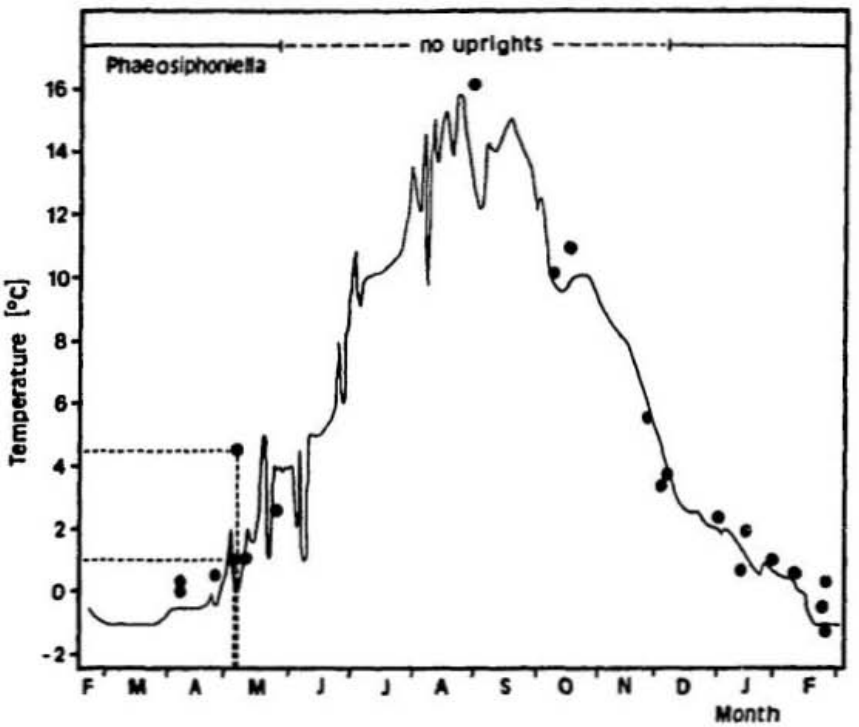


Fig. 2: Seawater temperatures at Fairhaven and appearance of *Phaeosiphoniella*.

Continuous measurements in the years 1978/79 at 9m depth near the main study area (—). Additional, single measurements for the years 1985-88 are indicated by (•). Two of the dates when *Phaeosiphoniella* was found to be senescent and the corresponding water temperatures of 1°C (6.5.88) and 4.5°C (7.5.87) are indicated by stippled lines.

The maximum seasonal appearance of *Phaeosiphoniella* uprights is shown by (—), the absence of uprights by (- -).

3.1.2. Description of field material

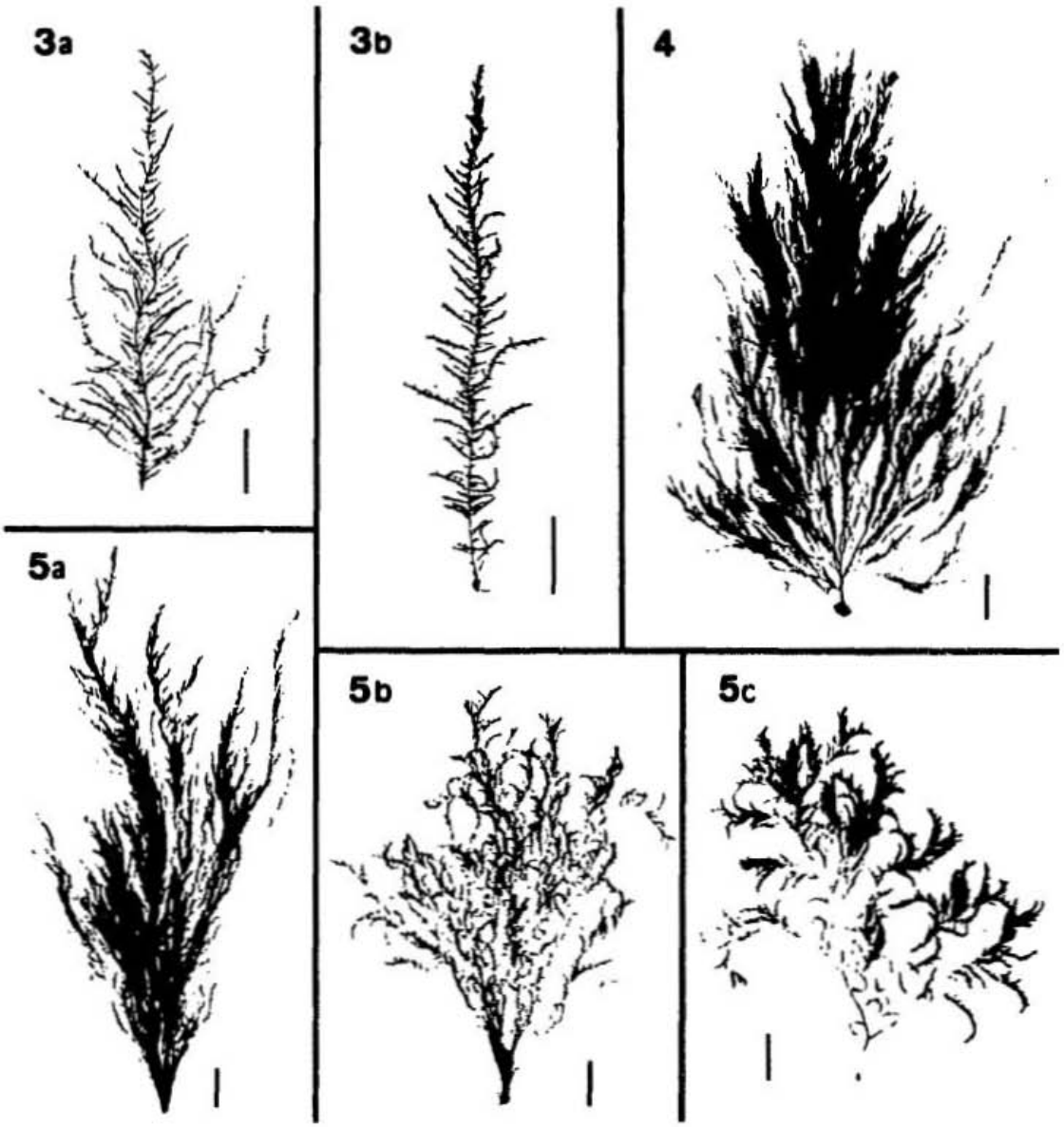
Specimens of *Tilopteris* were observed with two different forms, plants from Bell Island (Fig.3a) exhibit long primary branches and wider spaced branching points than those from Kelly's Island (Fig.3b) which have a much denser habit and shorter branches. Single, fully grown *Phaeosiphoniella* plants (Fig.4) appear very bushy due to their repeated branching. In *Haplospora*, both the sporophyte (Fig.5a) and the gametophyte (Fig.5b) attain a similar tufted appearance as found in *Phaeosiphoniella*, but only if several individuals are intertwined. Single *Haplospora* plants (Fig.5c), however, have a prominent main axis with alternate branching. The characteristic curvature of secondary branches is much less conspicuous in sporophytes than in gametophytes.

Tilopteris plants in the field reached up to 13 cm in length and bore both antheridia and oogonia when collected in October to December. Specimens were always found as scattered, single plants in relatively low abundance. *Tilopteris* was found growing on a variety of substrates including bedrock, boulders and wood.

Figs 3-5: Field specimens of the Tilopteridaceae.

Fig. 3: *Tilopteris mertensii*. (a): Loose habit. (b): Dense habit.
Fig. 4: *Phaeosiphoniella cryophila*.
Fig. 5: *Haplospora globosa*. (a): Several individual sporophytes.
(b): Several individual gametophytes. (c): Single fertile gametophyte.

All scale bars 1 cm.


3a
3b
4
5a
5b
5c

Various field observations were recorded for *Phaeosiphoniella* and *Haplospora* at the Fairhaven site throughout the sampling period (Table 1).

Table 1: Field data for the Fairhaven site.

The table lists the seawater temperatures measured at the site while field material was collected. Indicated are the corresponding abundances of uprights and/or prostrate systems of *Phaeosiphoniella cryophila* and *Haplospora globosa*. It is recorded, if the field material carried any reproductive structures, if prostrate systems had signs of formation of new uprights (initials) and if filaments expressed senescence.

		Phaeosiphoniella			*Haplospora*		
date	T[°C]	up	d	remarks	up	d	remarks
4.12.85	3.5	-	-		-	-	
29.12.	2.5	+	+		+	+	
12.01.86	0.8	+	+		+	+	
28.01.	0.9	+	+		+	+	
8.02.	0.5	+	+		+	+	
23.02.	0.3	+	+		+	+	
15.03.	0.3	+	+	sporangia	+	+	
6.04.	0.2	+	+	sporangia	+	+	
25.04.	0.5	+	+	antheridia	+	+	spo/oog
8.05.	1.0	+	+		+	+	spo/oog
22.05.	2.5	+/-	+	senescent	+	+	
7.10.	10.0	-	+		+	+	
6.12	3.8	+/-	+	initials	+/-	+	initials
15.01.87	2.0	+	+		-	+	
21.02.	-0.6	+	+		+	+	
5.04.	0.0	+	+		+	+	
7.05.	4.5	+/-	+	senescent	-	+	
28.08.	16.0	-	+		-	+	
16.10.	11.0	-	+		-	+	
26.11.	5.5	-	+		-	+	
20.02.88	-0.5	+	+		+	+	
6.05.	1.0	+/-	+	spo/senes.	+	+	spo/oog
2.05.89	2.0	+/-	+	senescent	+	+	spo/oog

T = seawater temperature / up = uprights / d = prostrate discs
(+) = present / (-) = absent / (+/-) = low abundance
spo = sporangia present / oog = oogonia and antheridia present

Haplospora occured at the Fairhaven site with up to 5-8 clusters of plants per square metre in the densest populations. The growth period starts in November and December when water temperatures are between 2 and 4°C. At maximum size, plants reach a length of ca 30 cm. The growing season ends during April to May when both mature gametophytes and sporophytes are abundant; this period coincides with the beginning of the summer increase in water temperature. All *Haplospora* specimens observed in the field exhibited a tufted appearance, because plants grow in clusters rather than as individual plants. Such clusters consisted of up to 40 individual primary axes arising from a prostrate disc system. The discs were primarily found on small stones or mollusc shells, usually either those of *Littorina littorea* (L., 1758) or of *Placopecten magellanicus* (Gmelin, 1791). Specimens could be found on boulders or bedrock provided that this substrate was free from sea urchin grazing.

At Fairhaven, *Phaeosiphoniella* produced filament initials as early as 6.December (1986) at a temperature of 3.8°C and became senescent as late as 22.May (1986) (Table 1). Prostrate discs are present throughout the year. The water temperature at the time when plants disintegrate can vary between 1.0°C (6.5.88) and 4.5°C (7.5.87). Somatic cells in senescent plants are characterized by a dark brown colouration resulting from an increased abundance of dense vesicles, presumably physodes, near the nucleus. Senescent plants, which can be of considerable length (ca 30 cm), readily fragment. Microscopic examination of such substrates as pebbles or mollusc shells revealed that *Phaeosiphoniella* uprights developed from prostrate basal systems similar to those of *Haplospora*.

3.2. Morphology

3.2.1. *Haplospora globosa*

Individual plants of *Haplospora* show a clear division into a basal parenchymatous part of the primary axis and a mostly uniseriate filamentous upright part which bears branches up to the third order. Branches of *Haplospora* frequently show a pronounced curvature with secondary branches borne secundly. In mature sporophytes (Fig.6a), secondary branches are usually short and carry sporangia in various developmental stages from uninuclear sporangium initials to mature quadrinucleate sporangia (Fig.6b). Spores are released through a wide opening (Fig.6c) and already exhibit a rigid cell wall. Secondary laterals in gametophytes (Fig.7a) are usually longer than in sporophytes and show the characteristic trichothallic meristems terminating in a hair with elongated, hyaline cells. Gametophytes bear intercalary oogonia and hollow antheridia (Fig.7b). Eggs are released without a cell wall through a narrow opening in the mature oogonia (Fig.7c). Antheridia expel their spermatozoids independently of egg-release.

3.2.2. *Tilopteris mertensii*

Tilopteris plants (Fig.8a) have a pronounced primary axis and opposite branches that are shorter than the main axis. The laterals, which terminate in a long hair originating from a trichothallic meristem, carry intercalary oogonia (Fig.8b) and antheridia. At maturity, oogonia release their uninucleate contents, which lack a cell wall, through a narrow opening (Fig.8c). Spermatozoids are released independently and the empty antheridia (Fig.8d) reveal the distinct cavity characteristic for both *Tilopteris* and *Haplospora*.

Fig. 6: *Haplospora globosa*, sporophyte.

(a): Mature sporophyte.
(b): Sporangia in different developmental stages (1, 2, or 4 nuclei).
(c): Release of quadrinucleate spore.

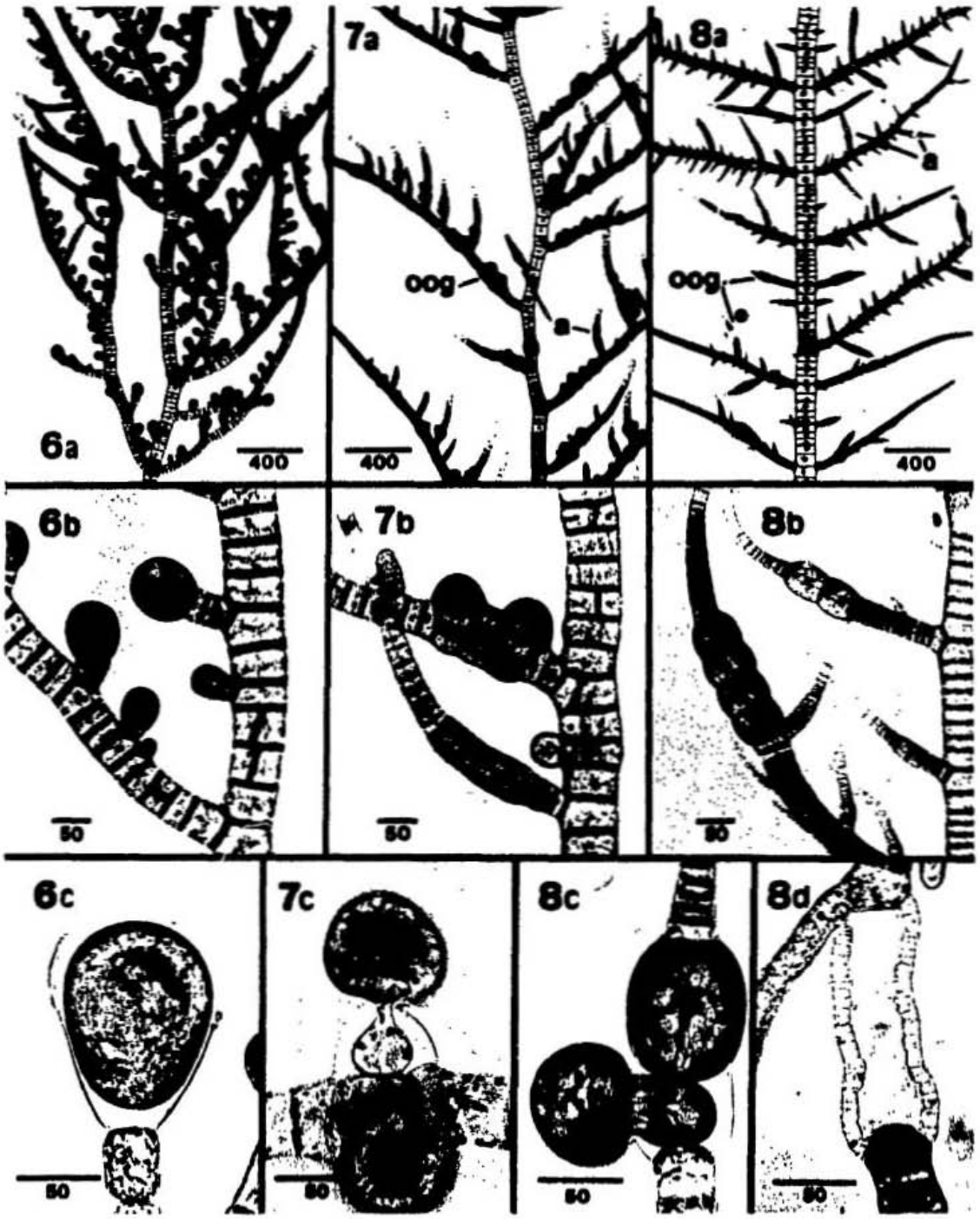
Fig. 7: *Haplospora globosa*, gametophyte.

(a): Mature gametophyte with oogonia (oog) and antheridia (a).
(b): Intercalary oogonia and antheridia.
(c): Release of egg.

Fig. 8: *Tilopteris mertensii*.

(a): Specimen with oogonia (oog) and antheridia (a).
(b): Mature catenate oogonia.
(c): Release of egg.
(d): Empty holiow antheridium.

All scale bars in μm.


6a
400
7a
oog
a
400
8a
a
oog
400
6b
50
7b
50
8b
50
6c
50
7c
50
8c
50
8d
50

3.2.3. *Phaeosiphoniella cryophila*

The general morphology of *Phaeosiphoniella* described in its diagnosis (Hooper et al. 1988) is confirmed by the present study. Young plants (Fig.9a) show a prominent main axis with shorter branches very similar to young *Haplospora* plants. The primary axis is less distinct in older specimens of *Phaeosiphoniella* (Fig.9b), and plants usually occur as multiaxial tufts, although a short main axis is found in the immediate basal region.

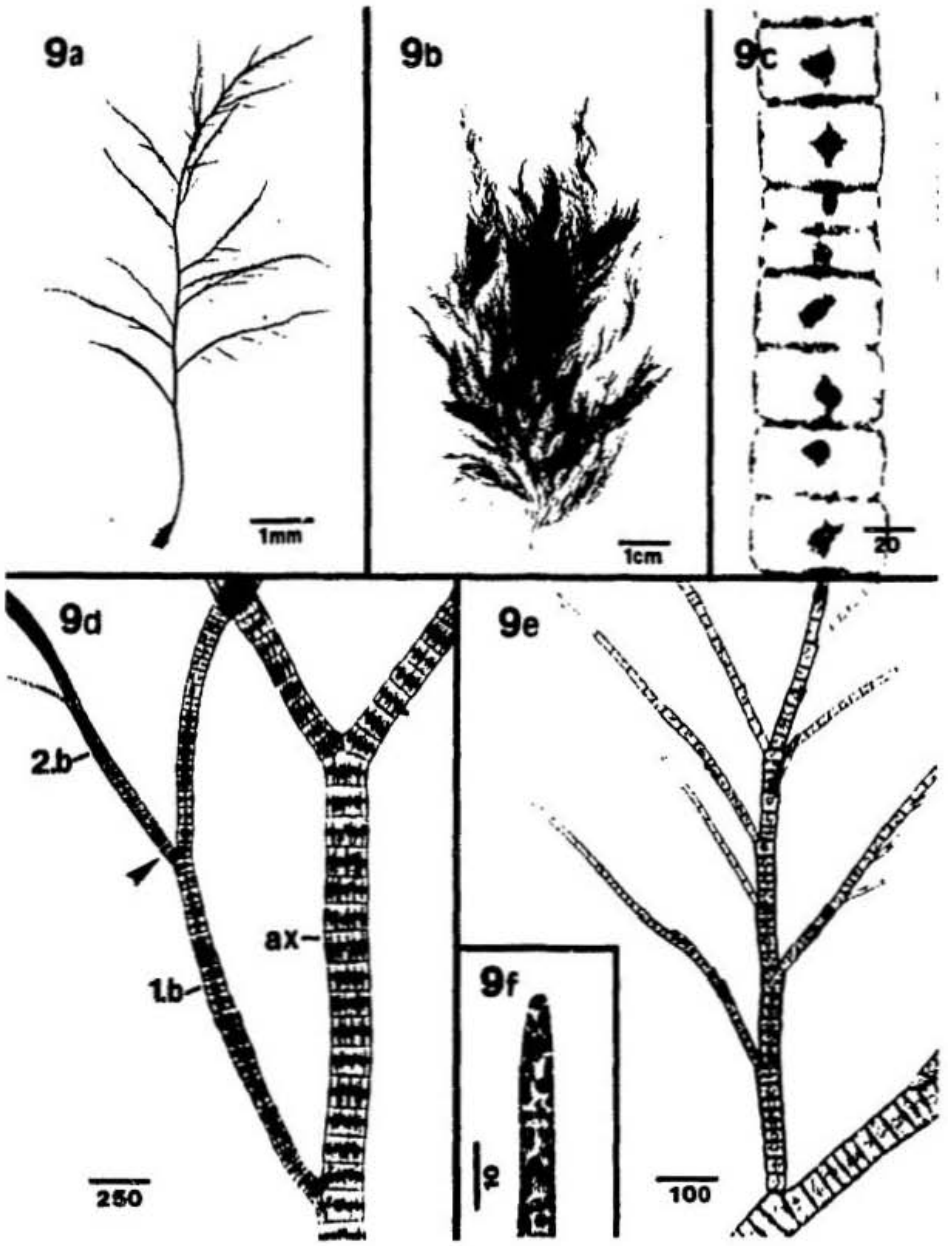
Nuclei in uniseriate filaments (Fig.9c) are aligned due to their arrangement in the centre of each cell, a characteristic feature found in all members of the Tilopteridaceae. Most primary axes become parenchymatous, with the extent and complexity of the parenchymatous structure increasing with progressing age (Fig.9d). The main axes appear polysiphonous due to repeated longitudinal cell divisions. Secondary branches or axes are frequently parenchymatous at their bases and at various intervals along the filament. Several of the cells have undergone only a single longitudinal division. Tertiary branches might have a parenchymatous thallus at the base, but otherwise they remain uniseriate throughout. There are no obvious meristematic regions in the uprights (Fig.9e). The trichothallic growth of *Haplospora* and *Tilopteris* is lacking and while the tips of the branches are somewhat acute they lack hairs (Fig.9f).

Longitudinal divisions in the parenchymatous parts of the primary axes sustain the regular septation by the initial, transversal divisions (Fig.10a), although a medulla and a cortex are distinguishable. Fewer secondary divisions occur in the central cells, which remain large and constitute the medulla, than in the peripheral cells, which divide more frequently and represent the cortex.

Fig. 9: *Phaeosiphoniella cryophila*, general morphology.

(a): Young field specimen, showing prominent main axis and secondary rhizoids.
(b): Older, tuft-like field specimen.
(c): Uniseriate filament stained with haematoxylin to show arrangement of nuclei.
(d): Parenchymatous structures: parenchymatous main axis (ax), primary branches (1.b) with longitudinal cell walls at irregular intervals and terminal, uniseriate branch systems (2.b) with few longitudinal cell walls at the base (►).
(e): Uniseriate branch with terminal branches and branch initials.
(f): Apex of terminal branch.

All scale bars in μm except for 9a and 9b.


9a
1mm
9b
1cm
9c
2b
9d
2b
ax
1b
250
9e
9f
2
100

Differentiation of cells is clearly visible in material stained with haematoxylin, in which the smaller, peripheral cells contain small nuclei with a diameter of about 6 μm (Fig.10b) and the central cells, which are twice the length of the outer cells, contain nuclei of up to 15 μm in diameter (Fig.10c).

Reproductive structures are rare. In field material, antheridia are not accompanied by oogonia, as is always the case in *Tilopteris* and *Haplospora*. Field specimens with antheridia, therefore, could be interpreted as male plants. Antheridia may be found in abundance. For example, a specimen collected in April 1986 bore antheridia on all branches (Fig.11a), and a similar specimen was described by Hooper *et. al.* (1988). The antheridia differ from those of *Haplospora* and *Tilopteris* in that they are solid rather than hollow (Fig.11b). The spermatozoids, however, are similar, being heterokont with one long anterior flagellum and a shorter posterior flagellum (Fig.11c). Spermatozoids possess a single plastid with a red eyespot, but remain inactive after release and show no responses to light. Oogonia were never found in field material. Occasionally, reproductive structures were observed in culture (Hooper *et al.* 1988) that resembled the catenate oogonia described for *Tilopteris*. The oogonia also released a single, uninucleate and non-motile cell; however, development of those eggs ceased early. From March till May, plurilocular sporangia occurred sporadically in the field as prominent structures formed along the main axis as a corticating layer (Fig.11d). Release of zoids was not observed in this study. In previous culture studies spores were released in a very few instances, but failed to continue their development beyond the initial germination (Hooper et al. 1988).

Fig. 10: *Phaeosiphoniella cryophila*, polystichous structures.

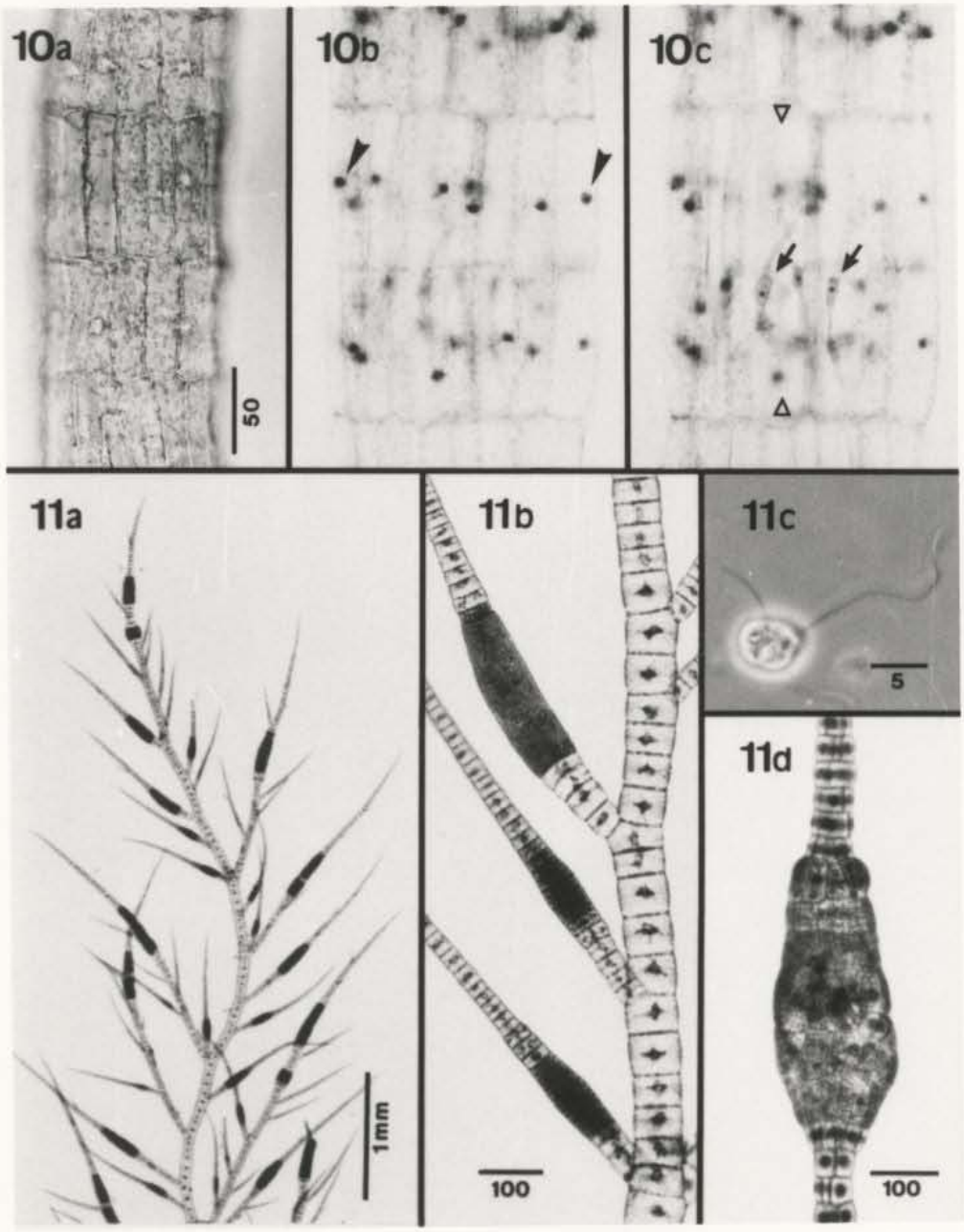
(a): Parenchymatous filament showing regular septation.
(b): Parenchymatous filament stained with haematoxylin; focal plane on peripheral cells and their small nuclei (►).
(c): Same as (b); focal plane on central cells and their large nuclei (→); length of the long cells is indicated by (Δ).

All to same scale bar 50μm.

Fig. 11: *Phaeosiphoniella cryophila*, reproductive structures.

(a): Field specimen (25.4.87) with numerous antheridia.
(b): Antheridia of solid structure in different developmental stages.
(c): Spermatozoid with heterokont flagellation; the short hind flagellum is tapering into a thin terminal filament (not visible in photograph).
(d): Plurilocular sporangia.

All scale bars in μm except for 11a.


10a
50
10b
10c
11a
1mm
11b
100
11c
5
11d
100

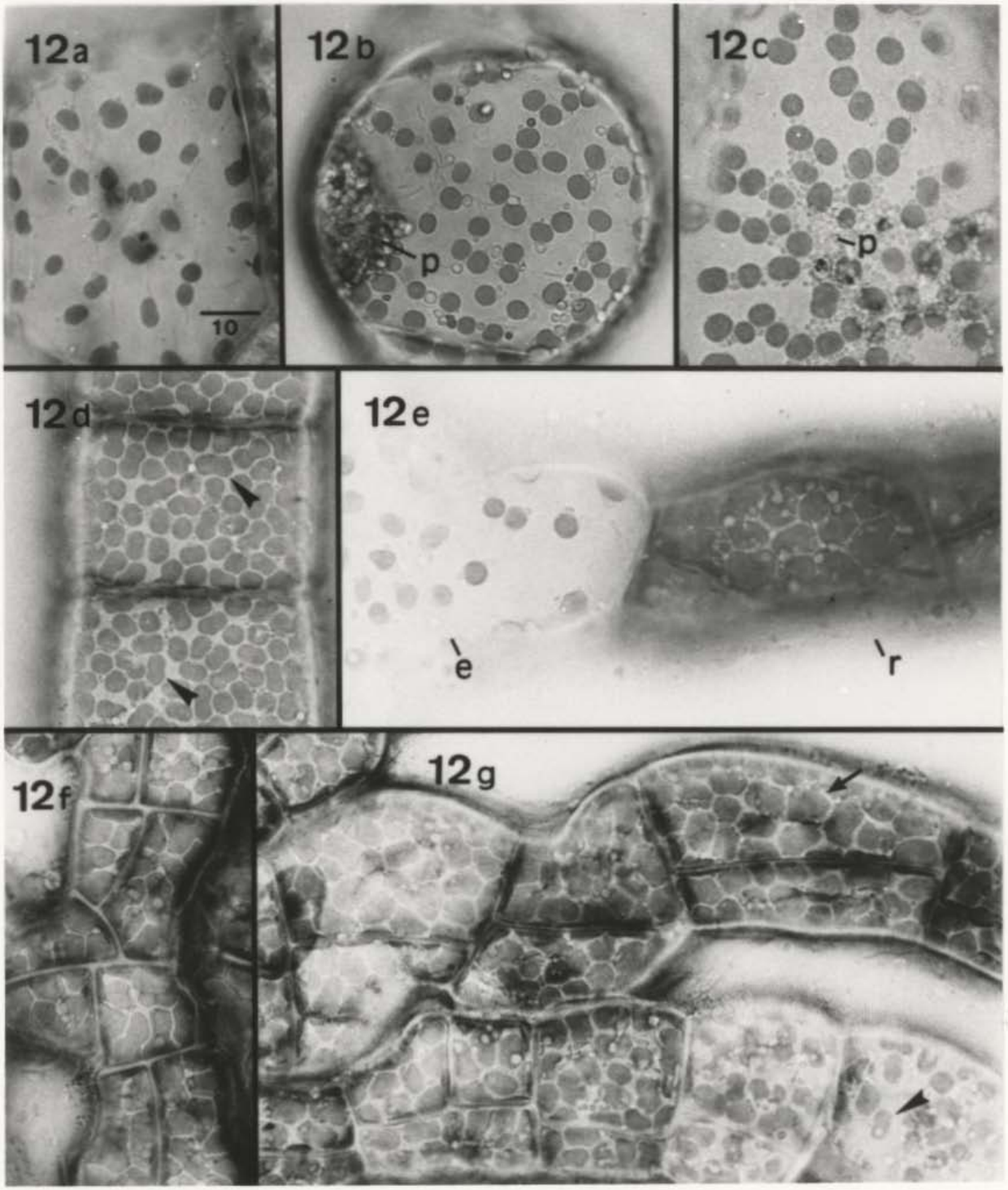
3.2.4. Chloroplast morphology

Cells of all three species of the Tilopteridaceae contain the same discoid chloroplasts, 3-4 μm in diameter, without a pyrenoid (Figs 12a,12b). Large, hyaline cells contain fewer chloroplasts (Fig.12c), whereas smaller cells, such as those in meristematic zones of the filaments in *Phaeosiphoniella* (Fig.12d), show numerous chloroplasts covering nearly the entire surface area of the cell. Many chloroplasts were found in dividing stages and, because of their high density, became tightly packed, loosing their discoid shape. A similar phenomenon was observed in the darker pigmented cells in the proximal area of rhizoids of young *Haplospora* gametophyte germlings (Fig.12e). Besides covering the surface area, the chloroplasts are exceptionally large (up to 10 μm) and can reach approximately six times the size of chloroplasts in adjacent cells. Large chloroplasts are also characteristic for prostrate discs of *Haplospora* (Fig.12f) and *Phaeosiphoniella*. Peripheral cells of the discs, however, still contain small chloroplasts comparable in size to those in cells of the filaments. The size of plastids gradually increased from the smallest chloroplasts in the peripheral cells to the largest ones in cells near the centre of the prostrate discs (Fig.12g).

Fig. 12: Chloroplast morphology.

(a): *Haplospora globosa*, filament cell.
(b): *Tilopteris mertensii*, embryo-body; accumulation of physodes (p) around nucleus.
(c): *Phaeosiphoniella cryophila*, large hyaline cell; accumulation of physodes (p) around nucleus.
(d): *Phaeosiphoniella cryophila*, small meristematic cells; some chloroplasts in dividing stages (⊢).
(e): *Haplospora globosa*; gametophyte germling with darker proximal part of the rhizoid (r); chloroplasts in the embryo-body (e) are much smaller than those in the adjacent rhizoid cells.
(f): *Phaeosiphoniella cryophila*; sector of a prostrate disc showing cells with large chloroplasts.
(g): *Haplospora globosa*; sector of a prostrate disc with the chloroplast size increasing from small chloroplasts (⊢) in young, peripheral cells to large chloroplasts (→) in older cells.

All to same scale bar in μm.


12a
10
12b
p
12c
p
12d
12e
e
r
12f
12g

3.3. Abscission and rhizoid formation

At certain times of the year many of the larger *Phaeosiphoniella* plants exhibit a distinctive fragmentation shown in the form of abundant abscission of branches. The region of abscission is predominantly at the base of branches and is caused by the separation of cells rather than by their rupture. Branch systems or branchlets in field material might develop rhizoid initials at any of the proximal cells (Fig.13a) prior to the actual abscission. In unstirred cultures, these initials eventually produced long rhizoids while the branches were still attached (Figs 13b, 13c). Abscised branchlets of *Phaeosiphoniella* loosely attached by rhizoids were found in the benthic detritus. The lateral branches of field material collected in March and April readily abscised when the plants were agitated in culture medium, though less abscission occurred in unagitated media. The abscised branch systems when left in sea water at 1 or 5 °C (SD, 5-7 μmol m^{-2} s^{-1}) produced a multi-celled rhizoid originating from their basal cell within two to four days (Fig.13d). Fragments of any size were able to develop rhizoids, even branchlets of only a few cells long (Fig.13e). In parenchymatous fragments, each terminal cell initiated a secondary rhizoid (Fig.13f). After a period of three to five days, numerous long rhizoids had developed and were able to attach the new plant to the substrate (Fig.13g).

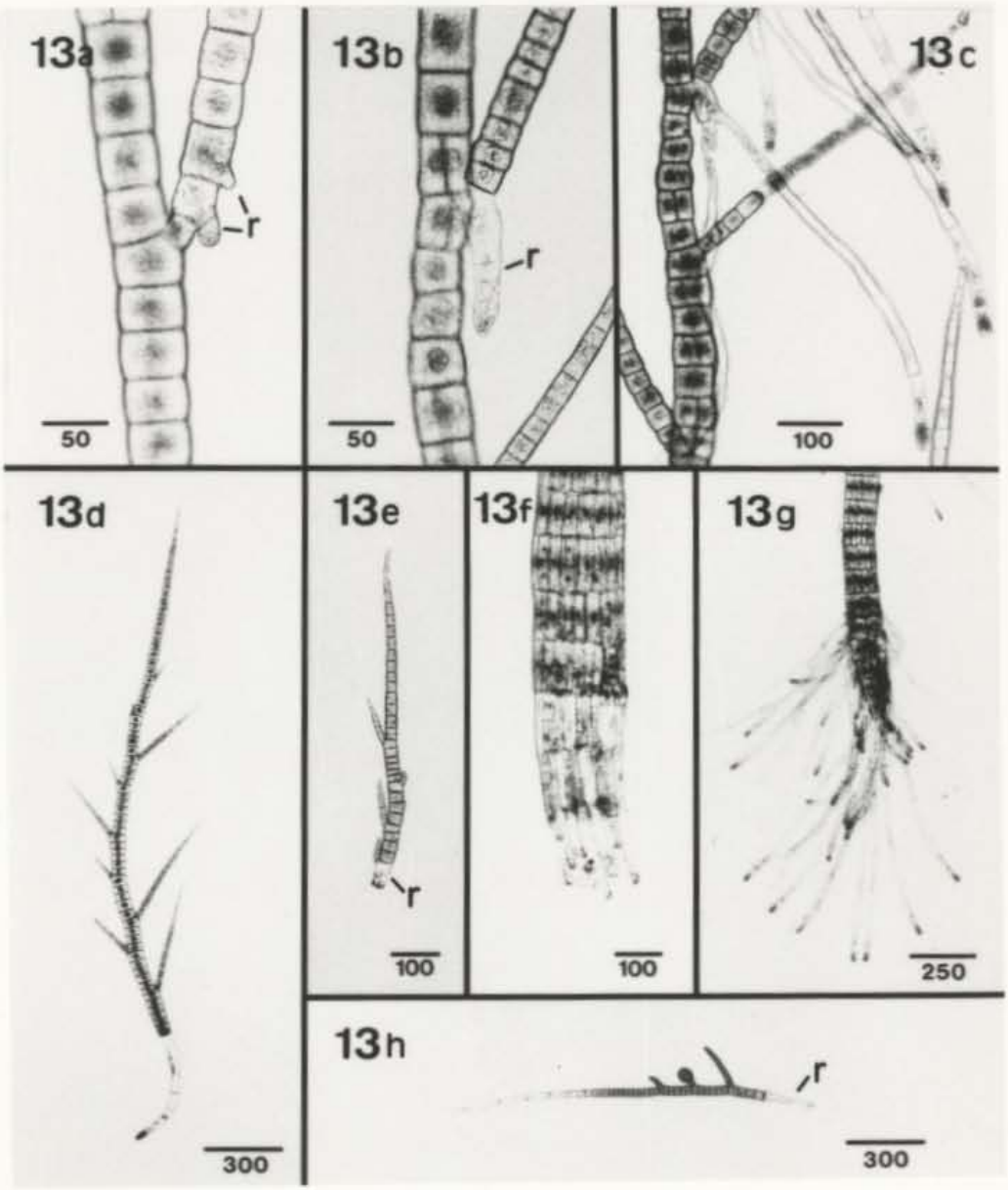
Haplospora also fragmented but not as readily as *Phaeosiphoniella* and no distinctive abscission zone was observed. The fragments produced rhizoids at their basal ends (Fig.13h) in a manner similar to that described for *Phaeosiphoniella*. All cells seem capable of producing rhizoids and this was especially noticeable in senescent material.

Fig. 13: Culture studies on abscission and rhizoid production.

(a) - (g): *Phaeosiphoniella cryophila*; (h): *Haplospora globosa*.

(a): Rhizoid initials (r) at the base of branchlets before their abscission.
(b): Branchlet in unstirred culture is still attached during rhizoid (r) development.
(c): Later stage of (b); numerous long rhizoids developed in unstirred culture.
(d): Abscised branch system which developed a rhizoid from the unruptured, basal cell.
(e): Terminal branchlet developing a rhizoid (r) after abscission.
(f): Fragment of a parenchymatous axis which produces rhizoid initials from each basal cell.
(g): Same as (f), but after numerous rhizoids developed and attached the fragment.
(h): Terminal branch system of a sporophyte developing a rhizoid (r) after abscission.

All scale bars in μm.


13a
r
50
13b
r
50
13c
100
13d
300
13e
r
100
13f
100
13g
250
13h
r
300

3.4. Prostrate systems

3.4.1. Definition

The prostrate parts of *Haplospora* and *Phaeosiphoniella* are extensions of their rhizoids and function partially as the holdfast system. According to their developmental pattern, prostrate systems in this study are called discs when they consist of a single, monostromatic extension of the rhizoid. Prostrate systems are defined as crusts when they incorporate several discs, either as a cluster of many individual discs or as one larger, multilayered system derived from coalescence of single discs.

3.4.2. Field material

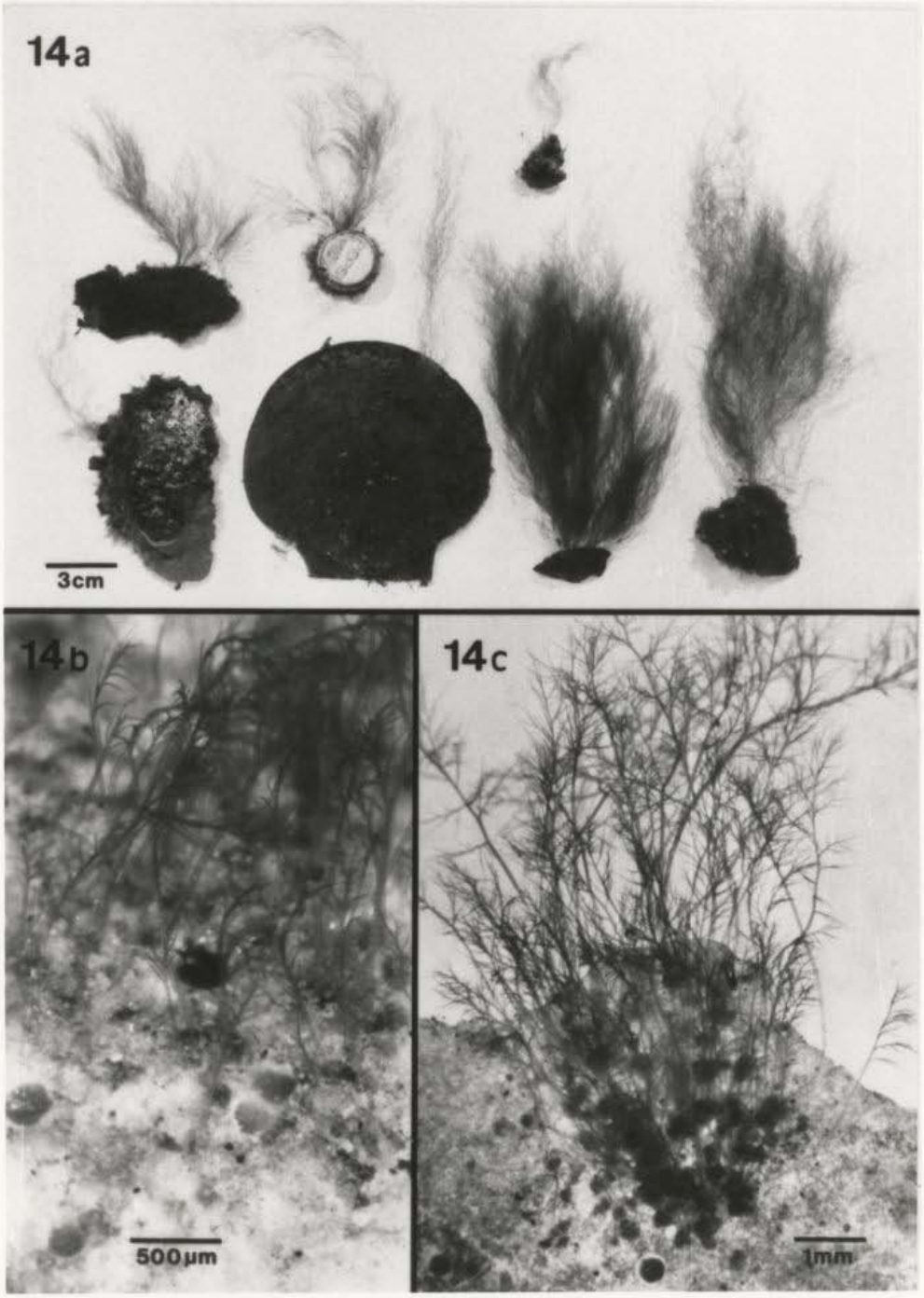
In *Tilopteris* no disc-like structures or other prostrate extensions of the rhizoids were found.

Both *Haplospora* and *Phaeosiphoniella* were found in the field to arise from prostrate, adhesive systems and examination of the various substrates (Fig.14a) showed that crusts, rather than rhizoids, were usually the primary holdfast systems of the species. Careful removal of the detritus covering the holdfast systems of uprights emerging through the detritus layer (Fig.14b) on such substrates as scallop shells revealed that all axes of a single plant cluster arose from one large discoid crust. Alternately, single uprights arose from dense clusters of numerous individual discs (Fig.14c).

In *Phaeosiphoniella* it was especially common to observe secondary rhizoids originating from clusters of several uprights to penetrate the detritus and to end in individual lighter discs (Fig.15a). The rhizoids covered the prostrate crust and

Fig. 14: *Phaeosiphoniella cryophila*, field material.

(a): Collection of substrate on which *Phaeosiphoniella* was found to grow: wood; mussel-, scallop- or *Littorina*- shells; pebbles or smaller stones; rhodoliths and artificial artifacts.
(b): Uprights emerging through detritus layer from discs on a scallop shell.
(c): Cluster of plants arising from numerous, individual prostrate discs.


14a
3cm
14b
500µm
14c
1mm

by growing beyond the area of the initial crust, they extended the holdfast area with the development of new discs .

Initially developing as a single monostromatic disc, prostrate systems can grow to 4 mm in diameter by coalescence of numerous additional discs (Fig.15b). Both *Haplospora* (Fig.15c) and *Phaeosiphoniella* (Fig.15d) have similar dark brown discs in which the oldest cells are in the centre of the disc. The peripheral cells are the largest cells and undergo anticlinal divisions to increase the diameter of the discs by increasing the lengths of the coalescent filaments, which radiate from the centre of the discs. The result is a monostromatic disc of sometimes very regular, nearly circular shape.

Samples of substrate such as scallop shells and pebbles collected during the summer and fall when *Haplospora* and *Phaeosiphoniella* uprights were absent had discs present that were morphologically similar to those bearing uprights in the winter. The substrate can show a high density of discs. One scallop shell of ca 14 cm in diameter, collected in October, bore about 32 separate crusts, which upon subsequent culture studies showed 15 to be *Haplospora* and 17 to be *Phaeosiphoniella* (Fig.16a). A few *Phaeosiphoniella* crusts consisted of several hundred individual discs (Fig.16b), while others showed a compact appearance due to coalescence and overgrowth of individual discs (Fig.16c). The dense, multilayered crusts are more common with *Haplospora* (Fig.16d) and seem to be larger than those of *Phaeosiphoniella*. Clusters of small discs, some consisting of only a few cells (4-11), are frequently observed in *Haplospora* covering areas of up to 2 cm^2 (Fig.16e). In *Phaeosiphoniella*, some of these small discs show remnants of a rhizoid usually attached at the side or centre of the disc (Fig.16f). Rhizoids

Fig. 15: Prostrate systems from field material.

(a): *Phaeosiphoniella cryophila*; prostrate crust on a scallop shell with several uprights (f) arising from the central discs; secondary rhizoids (r) developed from the uprights and extended outwards ending in numerous new prostrate discs (►).
(b): *Phaeosiphoniella cryophila*; cluster of prostrate discs beginning to merge and produce a compact disc system.
(c): *Haplospora globosa*; individual prostrate disc.
(d): *Phaeosiphoniella cryophila*; individual prostrate disc.

All scale bars in μm except for 15a.

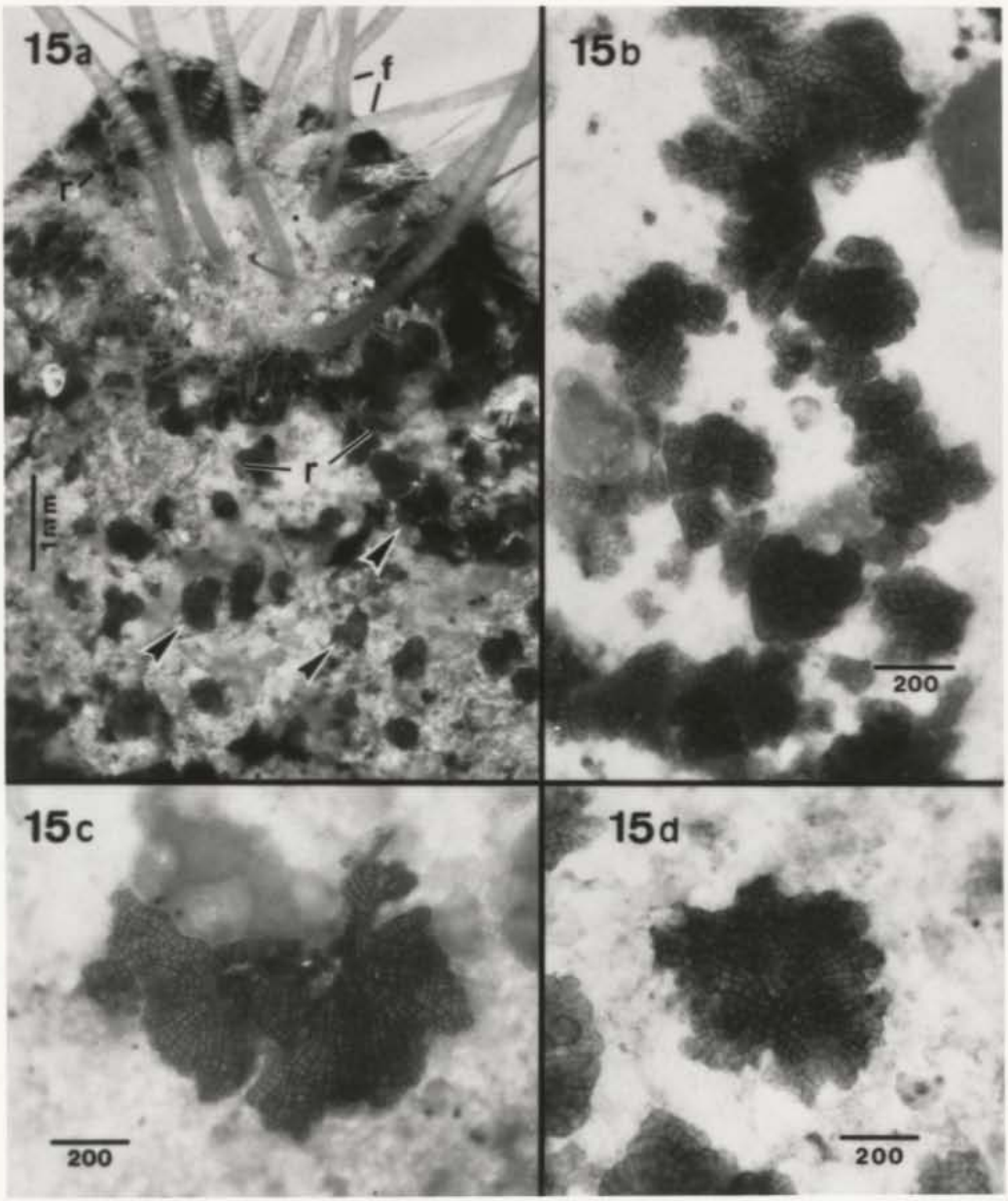
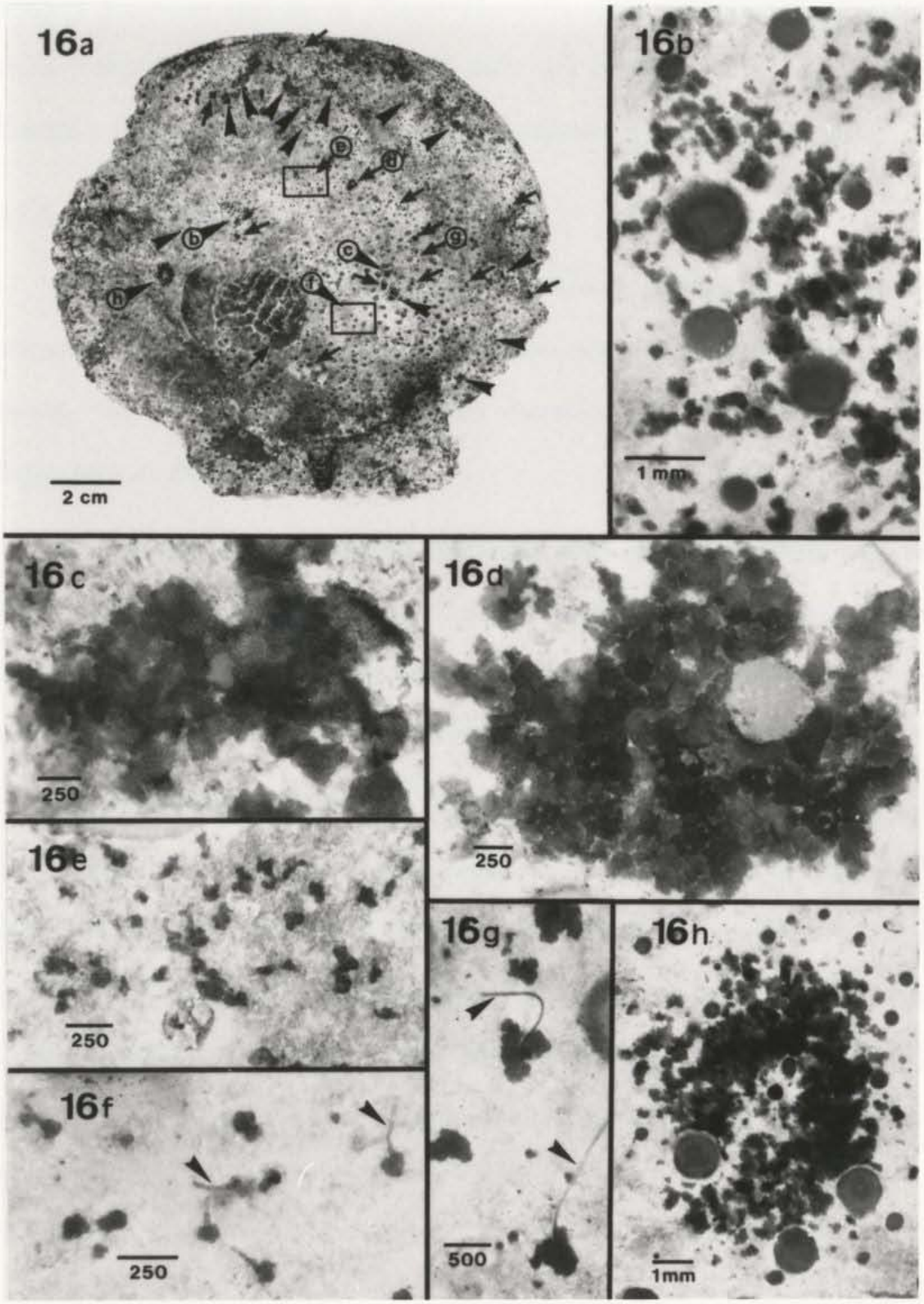

15a
f
r
r
1mm
15b
200
15c
200
15d
200

Fig. 16: Different forms of prostrate systems from field material.

(a): Scallop shell with 17 *Phaeosiphoniella cryophila* crusts (►) and 15 *Haplospora globosa* crusts (→); the letters marking some of the indicators correspond to the following figures.
(b): *Haplospora globosa*; sector of area with several hundred individual discs.
(c): *Phaeosiphoniella cryophila*; large compact crust.
(d): *Haplospora globosa*; large compact crust.
(e): *Haplospora globosa*; numerous small discs consisting of 4-11 cells each.
(f): *Phaeosiphoniella cryophila*; small discs with remnants of rhizoids (►).
(g): *Haplospora globosa*; large discs with remnants of rhizoids (►).
(h): *Phaeosiphoniella cryophila*; large crust with compact centre due to overgrowth and coalescence; many lighter individual discs surround the centre.

All scale bars in μm except for 16a,b,h.


16a
2 cm
16b
1 mm
16c
250
16d
250
16e
250
16f
250
16g
500
16h
1mm

seem to be the point of origin of the disc cells which radiate out from this point. Some large *Haplospora* discs also show rhizoid remnants (Fig.16g). The more common prostrate systems in *Phaeosiphoniella* show a characteristic pattern in which crusts, consisting of many fused discs, are surrounded by single, small discs (Fig.16h), similar to material with uprights collected in winter (Fig.15a).

3.4.3. Culture studies

It was discovered that all prostrate systems collected from field material produced new uprights if placed under suitable culture conditions. After sufficient growth, the uprights permitted specific determination of prostrate systems of *Haplospora* or *Phaeosiphoniella*.

In *Haplospora*, uprights from prostrate discs were formed in both culture medium and un-enriched seawater under temperatures of 1, 5 and 10°C with much fewer uprights produced at 15°C. Photon flux densities had been set between 0.5 and 15.0 μmol m^{-2} s^{-1}, under both long and short days.

Phaeosiphoniella discs have a more restricted response than *Haplospora* discs (Table 2) with uprights readily produced at temperatures of 1 and 5°C, but only rarely at 10°C. Combined with this low temperature requirement is a low light intensity requirement. Uprights were only formed at light intensities between 0.5 and 5 μmol m^{-2} s^{-1} while higher light levels suppressed the formation of uprights. At 8 μmol m^{-2} s^{-1} only a few occurred and at 10 μmol m^{-2} s^{-1} none were observed.

Table 2: Culture results in *Phaeosiphoniella cryophila*.

Culture results of the formation of *Phaeosiphoniella* uprights from prostrate discs under various temperature and light conditions. The culture medium was filtered seawater and results are the same for SD and LD light conditions. Indicated are a strong response of discs (+), a weak response (+/-) and no response (-) after a four week incubation period. (n.a.) = results not available.

Photon flux density [$\mu mol\, m^{-2} s^{-1}$]	Seawater temperature			
	1°C	5°C	10°C	20°C
0.5	+	+	+/-	-
3	+	+	+/-	-
8	+/-	+/-	-	-
10	-	-	-	-
15	-	-	-	-
2 (red)	n.a.	-	n.a.	n.a.
2 (blue)	n.a.	+	n.a.	n.a.

In un-enriched seawater, uprights of *Phaeosiphoniella* were visible after two weeks incubation of the crusts at 5°C, SD and 1.5 μmol m^{-2} s^{-1} (Fig.17a), reaching a length of 5 mm after four weeks. Uprights of field material did not survive culture temperatures above 10°C. Crusts, however, readily survived temperatures of 20°C for at least two months with light intensities between 1.5 and 15 μmol m^{-2} s^{-1} (SD and LD). After this treatment, crusts were cultivated at 5°C and 1.5 μmol m^{-2} s^{-1} (SD), which resulted in the production of new uprights. Both *Phaeosiphoniella* and *Haplospora* crusts were viable for more than a year. Crusts on a scallop shell collected in October produced uprights in culture at 5°C. The material was then transferred to 10°C for 9 months which

inhibited upright formation. Upon transfer back to 1°C the production of uprights resumed. All experiments were carried out under light levels of 2-3 μmol m^{-2} s^{-1} (SD) and with un-enriched seawater changed every two weeks.

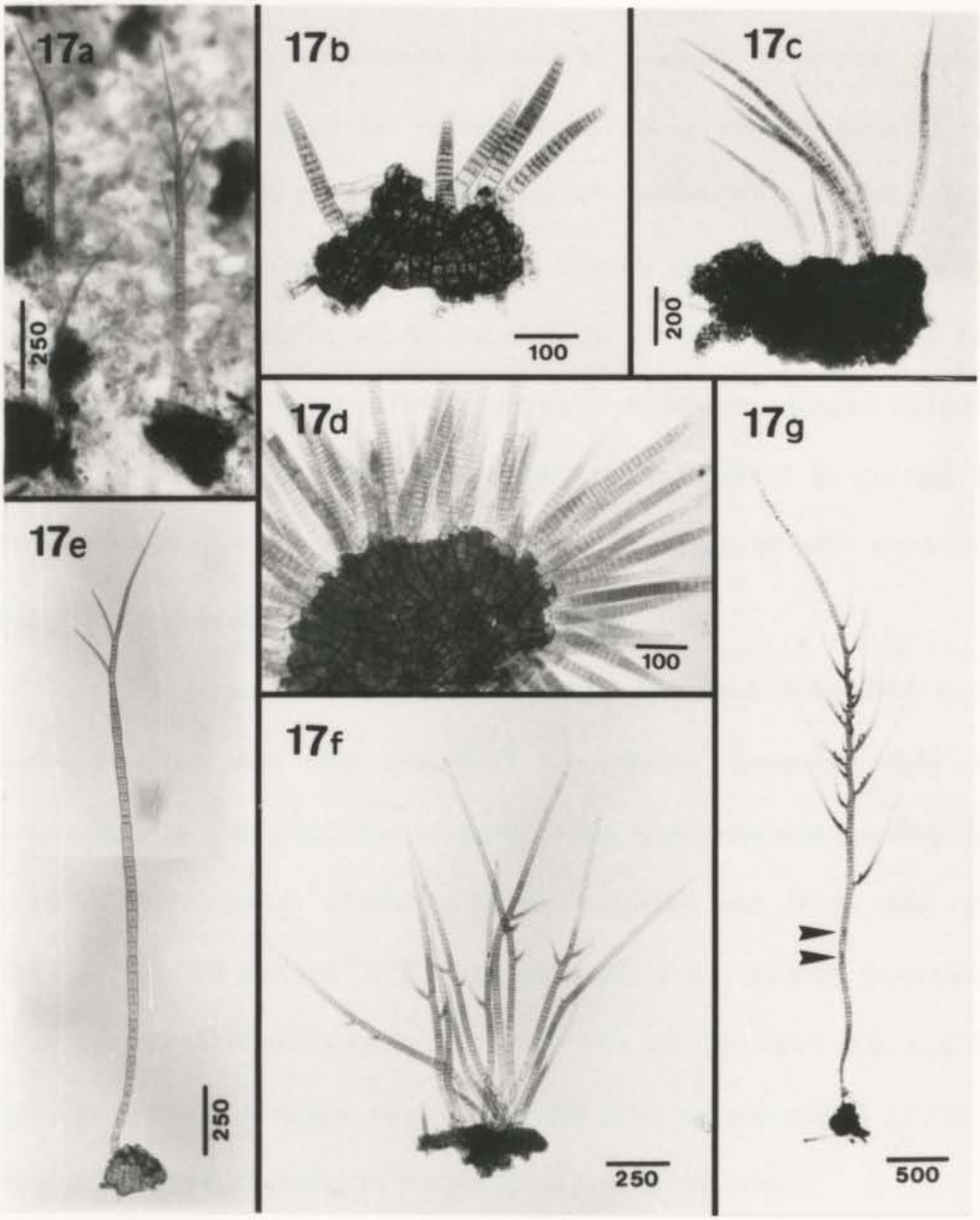
The use of filters to restrict the wave length of the incident light indicated that light quality plays a role in the development of *Phaeosiphoniella* uprights. Culture experiments were conducted at 5°C (SD) with light intensities of 2 μmol m^{-2} s^{-1} in un-enriched seawater. Under blue light, uprights were produced from prostrate discs after two weeks in culture, whereas in red light no uprights appeared.

New uprights from discs of *Haplospora* initially lack a meristematic zone and a hair (Fig.17b), while *Phaeosiphoniella* discs produce similar uprights with the exception of its typically tapered and acute tips (Fig.17c). Filaments in *Haplospora* arise primarily from cells at the periphery of the discs (Fig.17d) and their subsequent development is identical to germlings from either eggs or spores (Kuhlenkamp & Müller 1985). During further growth, *Phaeosiphoniella* uprights initiate branches in the upper region (Fig.17e), but unlike *Haplospora*, which differentiates a distinct trichothallic meristem (Fig.17f), no meristem or tilopteridean hair is developed in *Phaeosiphoniella*. In both species, the first longitudinal cell divisions appear in cells of the main axis, predominantly in the basal part (Fig.17g).

Fig. 17: Culture studies on the development of uprights from discs.

(a): *Phaeosiphoniella cryophila*; discs from field material developed new uprights in culture.
(b): *Haplospora globosa*; young uprights from a prostrate disc, no hair cells differentiated.
(c): *Phaeosiphoniella cryophila*; young uprights from a prostrate disc, filaments attenuated.
(d): *Haplospora globosa*; disc developed new uprights predominantly at the periphery.
(e): *Phaeosiphoniella cryophila*; young plant with two branches, but no meristematic zone or hair.
(f): *Haplospora globosa*; young filaments from a prostrate disc showing the characteristic trichothallic meristem.
(g): *Haplospora globosa*; plant forming the first longitudinal cell walls (⊢) in the primary axis, aside from some in the basal part.

All scale bars in μm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3.5. Life history studies

3.5.1. *Phaeosiphoniella cryophila*

3.5.1.1. General observations

Culture of *Phaeosiphoniella* showed no evidence of spores, antheridia or eggs being produced under the variety of conditions which supported vegetative growth of uprights and prostrate systems (1-5°C under SD and LD conditions with photon flux densities of 1-3 μmol m^{-2} s^{-1}). Besides vegetative propagation through fragmentation and abscission, no other form of reproduction could be found. Plants survived well in un-enriched seawater which encouraged uprights to be produced in greater abundance than culture in ES medium. In general, however, growth was slow in culture and plants often showed signs of early senescence.

3.5.1.2. Senescence and rhizoid production

The phenomenon of senescence already described from field material of *Phaeosiphoniella* was also examined in culture. Senescent field material, characterized by the possession of dark brown inclusions surrounding the nuclei (Fig.18a) and the ready abscission of the branches was taken into culture at 5 or 10°C (SD, 1.0 μmol m^{-2} s^{-1}). Such material, if not already possessing them, readily developed rhizoids from numerous cells of the main axis and branches (Fig.18b). Usually, abscised branch systems from non-senescent plants develop such rhizoids predominantly at the proximal end of the axes.

3.5.1.3. Development of prostrate discs

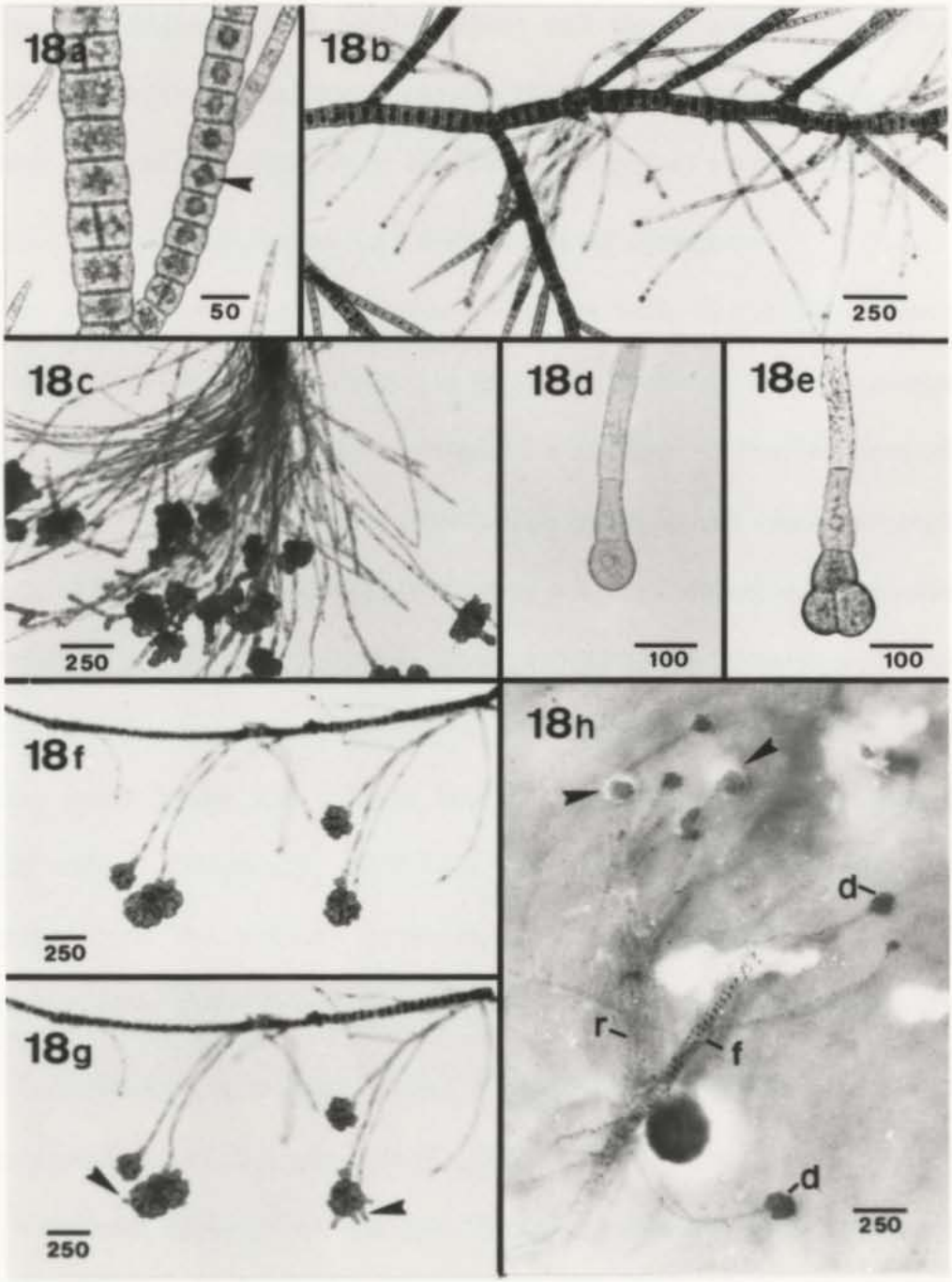
Occasionally, in culture experiments, specimens of *Phaeosiphoniella* develop knot-like cell accumulations at the distal ends of their rhizoids if these are not in contact with the substrate (Fig.18c). When rhizoids touch the substrate, however, they develop prostrate discs at the point of contact. The development of discs begins with a characteristic swelling of the terminal rhizoid cell (Fig.18d) which then divides transversely and longitudinally (Fig.18e). Peripheral cells begin to grow and divide until a nearly circular, monostromatic disc is formed. Most cell divisions are anticlinal. Small fragments of senescent filaments tend to produce numerous rhizoids which then attach to the substrate and subsequently develop prostrate discs (Fig.18f). While kept in un-enriched seawater (5°C, SD, 8 μmol m^{-2} s^{-1}), discs only increase in size, but after transfer to fresh seawater and lower light intensity (5°C, SD, 2 μmol m^{-2} s^{-1}), rhizoid or filament initials develop (Fig.18g).

A few of the rhizoidal discs formed in culture on the surface of the coralline alga *Lithothamnion glaciale* caused a bleaching of the surface cells surrounding the brown algal disc (Fig.18h). Other discs, rhizoids and filaments in contact with the algal surface did not provoke the same reaction. Such areas of coralline algae bleached by the discs have also been observed in field material.

Fig. 18: *Phaeosiphoniella cryophila*, senescence and disc development in culture.

(a): Senescent, uniseriate branch system with dense vesicles around the nuclei (►).
(b): Senescent plant which developed in culture numerous rhizoids along the entire filament.
(c): Secondary rhizoids from a specimen kept in a stagnant culture developed rhizoidal knots.
(d): Swelling of the rhizoid tip after contact with the substrate (polystyrene culture dish).
(e): Same as (d), but after first transverse and anticlinal divisions of the tip cell occured to initiate the development of a prostrate disc.
(f): Discs which were produced from rhizoid tips after contact with the polystyrene culture dish; rhizoids had been produced from senescent fragments (f) of field material.
(g): Same as (f), but after transfer to fresh seawater which triggers development of rhizoid or filament initials (►) from the prostrate discs.
(h): Bleaching of cells of *Lithothamnion glaciale* by prostrate discs of *Phaeosiphoniella cryophila* (►); rhizoids (r) from a senescent filament (f) developed new discs upon contact with the coralline surface; filaments, rhizoids and some discs (d) do not cause bleaching.

All scale bars in μm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3.5.2. *Haplospora globosa*

3.5.2.1. General observations

The life history of this species has already been extensively studied in culture by Kuhlenkamp & Müller (1985) and the information reported here is therefore restricted to matters relating to the prostrate system. It should be noted, however, that no deviations from the life cycle reported previously were observed.

3.5.2.2. Disc development and formation of uprights

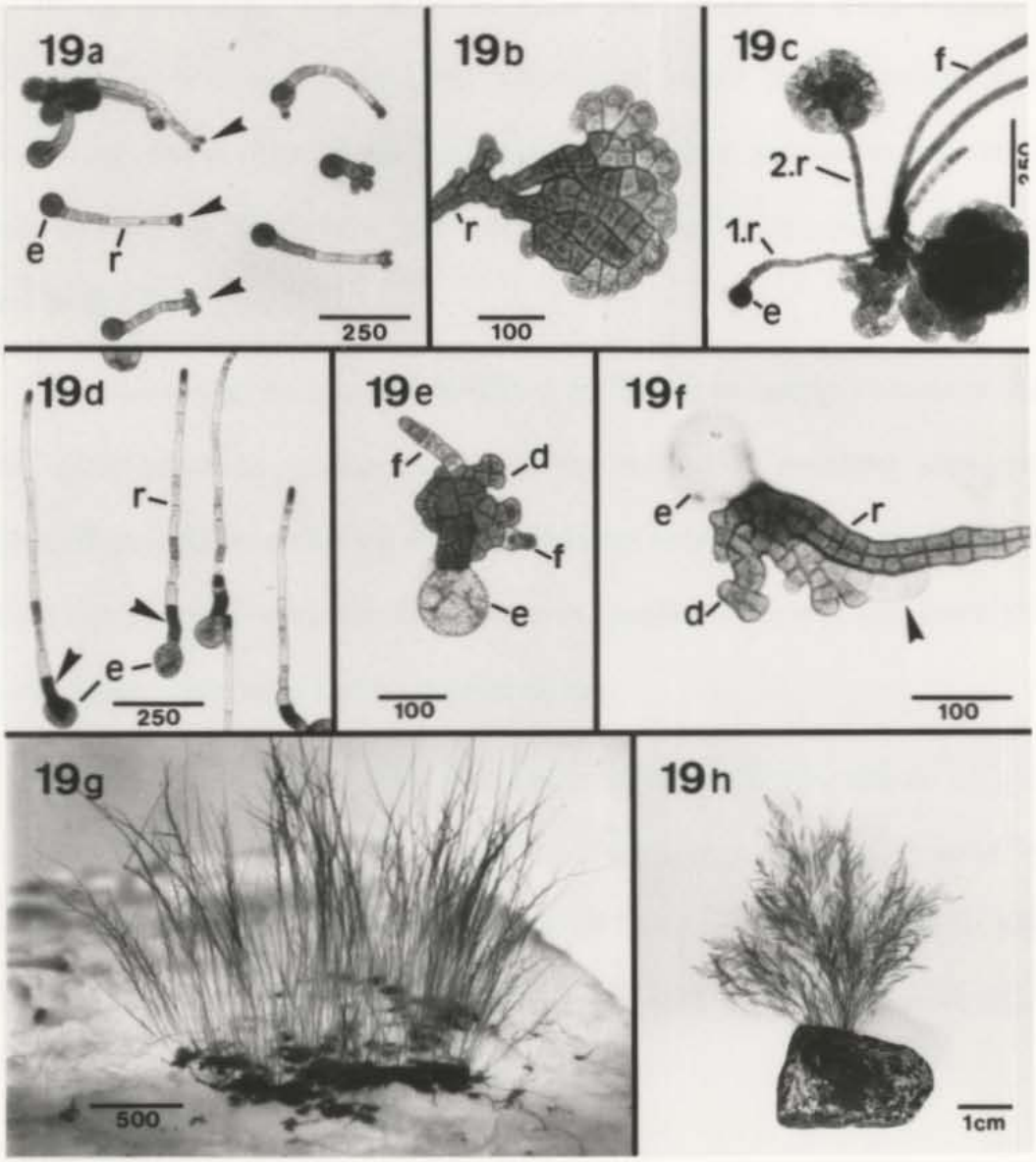
In *Haplospora*, eggs or spores germinate with the initial production of a rhizoid. At 5°C (SD, LD), many sporophyte germlings from *Haplospora* eggs, after a short period of growth, developed a swelling of the terminal cell of the rhizoid (Fig.19a), which became multi-lobed prior to the commencement of cell division. Alternation of anticlinal divisions with cell enlargement resulted in the formation of a monostromatic disc which expanded by increasing the size of the peripheral cells prior to their anticlinal cell divisions (Fig.19b). Peripheral cells are hyaline, much larger and contain fewer or smaller chloroplasts than the more central cells. Frequently, additional discs which were initiated by rhizoids emerging from the primary prostrate system, overgrew the older discs and eventually, after about two to three weeks, developed the first uprights (Fig.19c).

Gametophyte germlings exhibit a characteristic, darker pigmented area at the proximal part of their rhizoids (Fig.19d). If the culture medium is not changed, and nutrients presumably become depleted, rhizoids frequently produce a prostrate disc early in their development (Fig.19e). Not only do they form prostrate discs from apical cells, but they also form them as extensions of the proximal, darker portion of their rhizoids (Fig.19f). Prostrate discs of *Haplospora*

Fig. 19: *Haplospora globosa*, culture studies with discs and uprights.

(a): Sporophyte germlings with multi-lobed terminal rhizoid cells (►) which through further cell growth and repeated cell divisions initiate a prostrate disc.
(b): Prostrate disc which had originated from a multiseriate rhizoid (r) consists of rows of cells radiating outwards; peripheral cells expand the disc by increasing their size and subsequent anticlinal cell divisions.
(c): Prostrate system produced by the rhizoid (1.r) of a sporophyte germling; a secondary rhizoid (2.r), originating from the first disc, developed a second disc at its tip while the main crust produced two filamentous uprights (f).
(d): Gametophyte germlings exhibiting the darker proximal area of their rhizoids (►).
(e): Gametophyte germling which produced a prostrate disc (d) and upright initials (f) very early in the rhizoid development.
(f): Gametophyte germling similar to 19d which initiated a prostrate disc (d) from the dark pigmented, proximal area of its multiseriate rhizoid (r). Peripheral cells of the disc (►) are large and hyaline.
(g): Young gametophytes arising from numerous discs which had been developed by spores settled on the surface of a scallop shell.
(h): Field material; tuft of numerous young plants arising from prostrate crusts on a pebble.

e = embryo-body of sporophytes or gametophytes.
All scale bars in μm except for 19h.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germlings situated in close proximity overgrew each other and the resulting coalescence produced extensive polystromatic crusts similar to the ones found in field material described earlier (Fig.16d).

When germlings with prostrate discs were placed in fresh culture medium they produced filamentous uprights. Clusters of single discs resulting from spores germinating on scallop shells ultimately developed numerous plants (Fig.19g) which collectively resembled the tuft-like multiaxial specimens of *Haplospora* found in the field (Fig.19h).

Disc development may be modified by the attachment substrate. All spores which germinated on scallop shells, on the surface of coralline algae or on the bottom of polystyrene culture dishes produced extensive discs soon after the first cells of the rhizoids were formed. However, spores were not observed to develop discs on glass surfaces of microscopical slides.

Spore and egg germination may occur in red light (2 μmol m^{-2} s^{-1}, SD) or in complete darkness (both at 5°C) and such germlings may stay viable for up to 10 months in a state of arrested growth. If the plants are moved to white light (5°C, 5 μmol m^{-2} s^{-1}, SD), they resume development and produce uprights.

3.5.2.3. Creeping rhizoids

Haplospora has the ability to produce multiseriate rhizoids which creep along the surface of the substrate. Such rhizoids were found in field material (Fig.20a) and produced, when placed in un-enriched seawater at 5°C (SD, 3 μmol m^{-2} s^{-1}) numerous uprights (Fig.20b). In culture, production of creeping rhizoids is commonly observed under low nutrient conditions or during germination in darkness. After new culture medium was added or cultures were placed in white light, uprights were produced along the whole length of the rhizoid (Fig.20c).

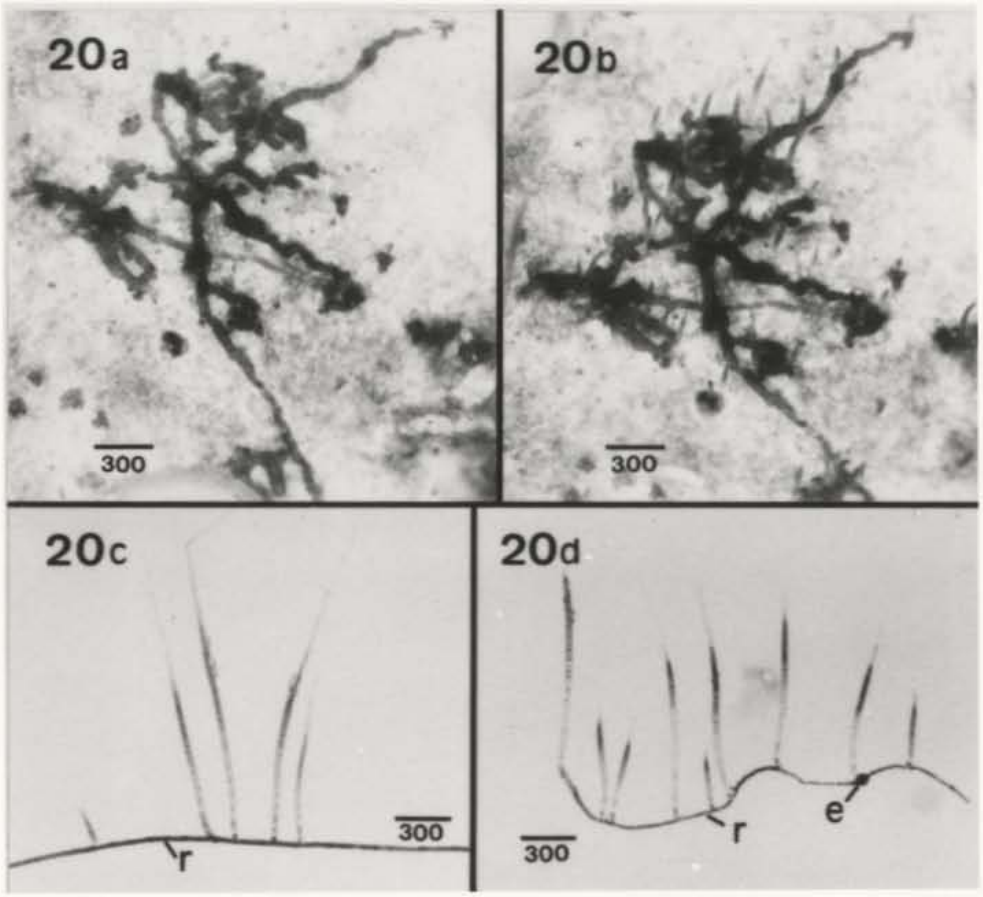
3.5.3. *Tilopteris*

Clonal cultures were observed over several generations and their vegetative and reproductive development is in accordance with that reported by Kuhlenkamp & Müller (1985). Specific developmental patterns in the germination and photomorphogenetic responses of the germling have been described previously (Kuhlenkamp 1989). There is no evidence in these studies to indicate that *Tilopteris* produced any form of prostrate disc system. The only new result of this study is the observation on the ability of germlings to develop creeping rhizoids and subsequent uprights (Fig.20d) in a similar manner to *Haplospora*.

Fig. 20: Creeping rhizoids.

(a): Field material of a prostrate system of *Haplospora globosa*; multiseriate rhizoids adhere to the surface of a scallop shell.
(b): Same as (a); after culture in seawater, numerous filamentous uprights were produced.
(c): Long, multiseriate rhizoid in some *Haplospora globosa* germlings produced several uprights after transfer from nutrient depleted medium to fresh seawater.
(d): Rhizoids from *Tilopteris mertensii* eggs, which had germinated under red light conditions, produced several uprights after transfer to white light.

e = embryo-body, r = rhizoid. All scale bars in μm.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3.6. Cytology

3.6.1. *Phaeosiphoniella cryophila*

Staining with haematoxylin revealed several cytological features. Monofilamentous parts of the thallus show the typical arrangement of a single nucleus in the centre of each cell (see fig.8c). The nuclear size is variable, ranging from a diameter of 15 μm in large cells of the main axis to a diameter of 4 μm in the smallest nuclei found in apical cells of branches.

Nuclei in branches are increasingly smaller towards the apex and seem to loose their nucleoli, usually a prominent feature in all other cells.

3.6.2. Chromosome numbers

Haplospora has a chromosome number of n=50 (48-53, six counts) in Newfoundland material irrespective of whether the source is from the sporophyte or gametophyte generation. These results confirm the previous study (Kuhlenkamp & Müller 1985) which illustrates in detail the sequence of the meiosis-like stages in sporangial initials and the metaphase stages in which chromosomes were counted. The chromosome number in *Tilopteris* is n=62 as obtained from the previous study (Kuhlenkamp & Müller 1985). Five chromosome counts on *Phaeosiphoniella* were obtained from five different plants and range from 41 to 46 with an average of n=42.

3.7. Fluorometry

3.7.1. Tests with red blood cells

Fixed chicken red blood cells (RBCs) were stained for different periods to obtain a time curve. With DAPI (Fig.21a), the minimum time to reach a constant fluorescence level was one min, whereas with mithramycin (Fig.21b) increase in fluorescence occurred until the material had been stained for 15 min. However, the final fluorescence shows less variation than that of DAPI stained cells.

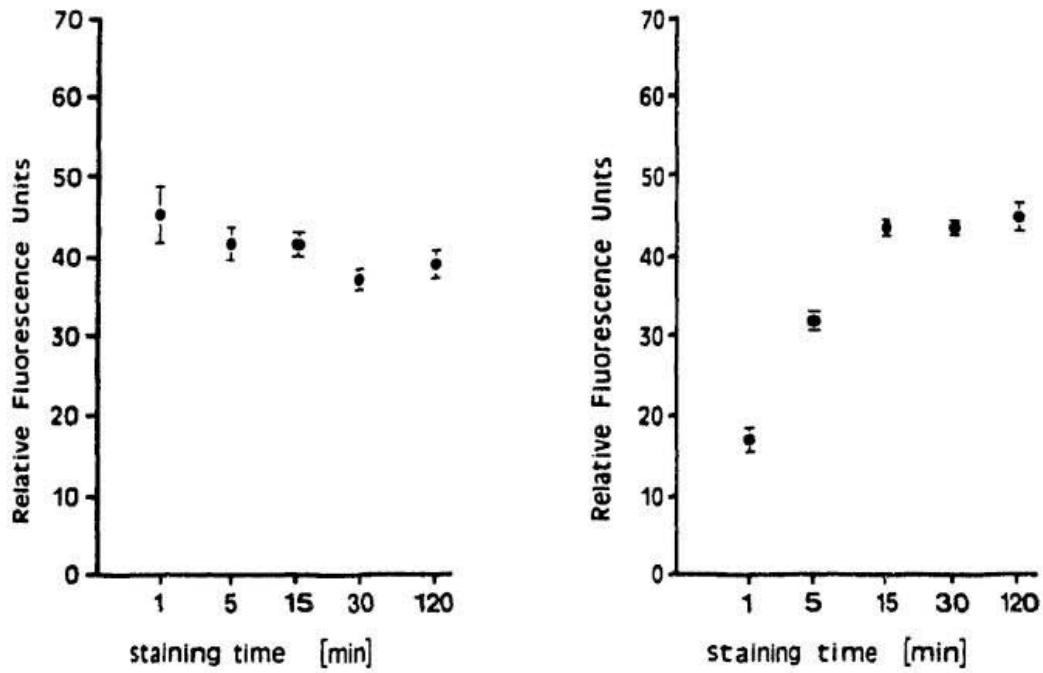


Fig. 21: Fluorescence values for RBCs.

(a): Mean rfus for nuclei of chicken RBCs stained with DAPI measured over time.
(b): Mean rfus for nuclei of chicken RBCs stained with mithramycin measured over time.

Chicken RBCs were also used as a standard throughout the study to ensure that staining was consistent and that the epifluorescence measurements did not change due to changes in the set up or sensitivity of the measuring instruments. Over the one year period in which these measurements were made, the RBCs stained with DAPI measured between 36.8 +/-1.2 and 38.4 +/-3.4 relative fluorescence units (rfus) and those stained with mithramycin varied between 43.4 +/-1.0 and 46.0 +/-1.6 rfus. Analysis of variance showed that no significant differences occurred between the beginning and the end of the measurement period for DAPI, whereas the two mithramycin results are found to be different. While the two mithramycin results do not overlap, the interval of 42.47 to 44.47 rfus for the first measurement is separated from the second measurement with an interval of 44.58 to 47.41 rfus by only 0.1 rfus. Confidence levels of mithramycin results are tighter than with DAPI stained material because mithramycin staining is more consistent. Consequently, the mithramycin groups, even if only slightly different, are found to be significantly different groups under statistical analysis. This difference, however, should have no methodological or biological consequences and the mithramycin staining method is, therefore, considered to be consistent over the period of experimentation.

3.7.2. *Tilopteris mertensii*

Because of its known asexual life cycle involving a single reproductive phase with constant chromosome numbers and because of its ease of culture, *Tilopteris* was selected as the species for the initial fluorometric study. Exposure of mithramycin stained cells to UV-light produced rapid fading presumably due to photochemical bleaching of the DNA-dye complex. An experiment which involved measuring the fluorescence of a single nucleus over a 20 min period (Fig.22) showed that 50% loss of fluorescence occurred during the first 5 min with the curve leveling off after 15 min at approximately the 30% value. Similar results were obtained with RBCs, except the final fluorescence level was at 60% of the maximum level. It is apparent therefore that measurements must be taken at the maximum, initial level or after the leveling out of the fluorescence values.

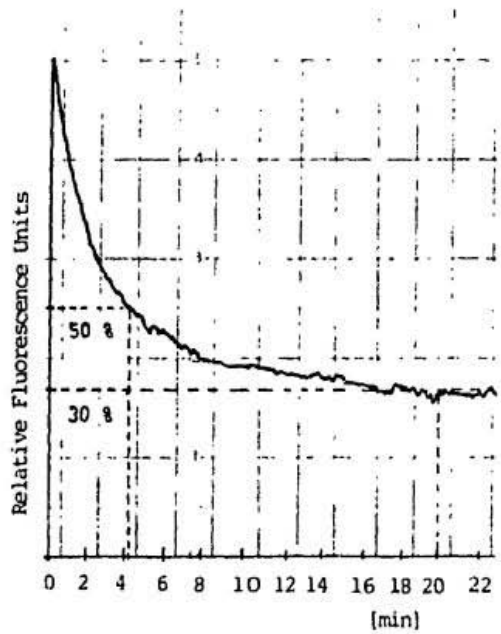


Fig. 22: Fading of fluorescence.

Fluorescence measured in a mithramycin stained nucleus of a G2 cell in *Tilopteris mertensii*. The fading occurs over a period of 20 min and indicated are the 50% and 30% levels of the maximum fluorescence value.

It is clear through results on *Tilopteris* (Fig.23) that storage in ethanol has a profound effect on the level of fluorescence obtained. The graph shows that the greater part of the loss of activity occurred within the first seven days of storage in fixative with the rate of loss decreasing with subsequent storage. The rfu levels dropped after four weeks from a day one value of 66 rfus to 32 rfus, a loss of over 50%. The background fluorescence levels, however, did not change even after 240 days of storage.

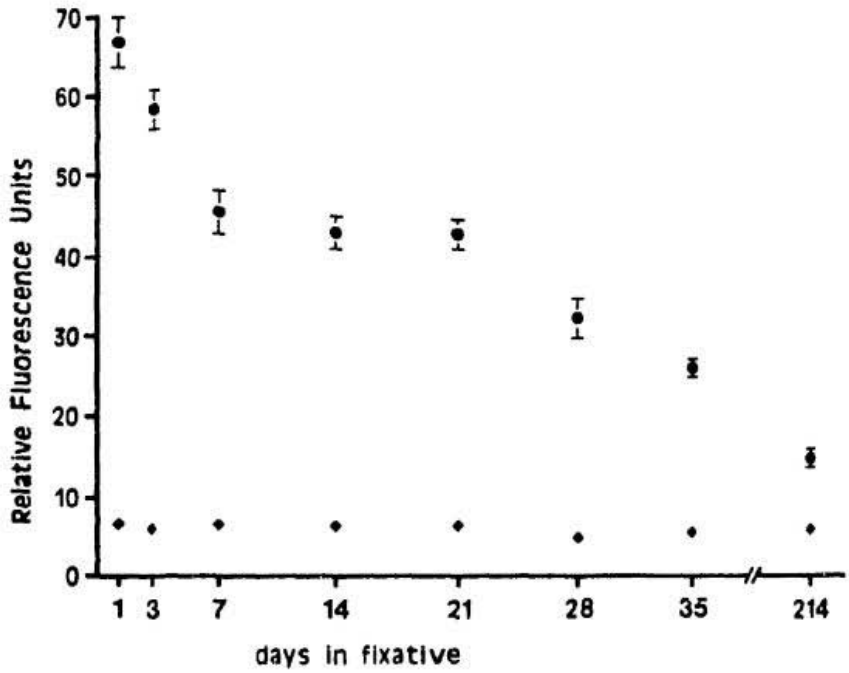


Fig. 23: Effect of storage in ethanol on rfus.

Mean rfus for *Tilopteris mertensii* nuclei of G2-cells (•) and their respective background values (•) measured at consecutive times during storage in fixative.

Measurements of nuclei in different stages and different cells permitted to obtain the range of nuclear C-levels present in *Tilopteris* (Fig.24). Nuclei in telophase or early interphase determined the G1 level of 18.5 +/-2.4 rfus, while nuclei in cells of the meristematic zone or in large cells of the main axis just prior to division determined the G2 level of 48.0 +/-2.5 rfus. Fluorescence obtained from oogonia are, with a mean of 13.8 +/-1.1 rfus, lower than the G1 level, probably reflecting problems in staining and accurate measurement of fluorescence in these cytoplasmically dense bodies.

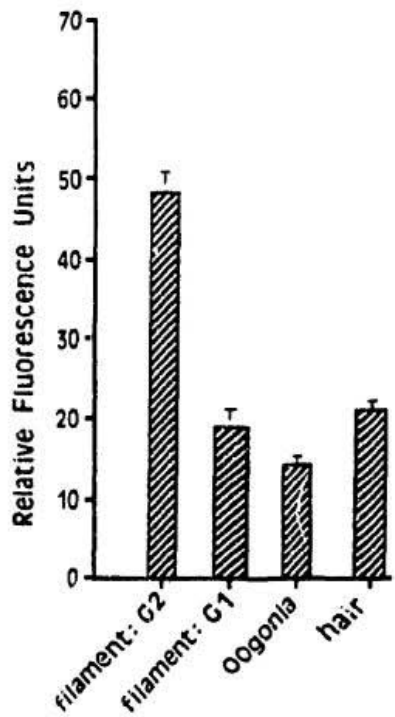


Fig. 24: Range of fluorescence values found in *Tilopteris mertensii*.

Mean rfus for nuclei in cells of different functions or cell cycle stages within a single *Tilopteris mertensii* specimen.

Since *Tilopteris* shows no alternation of generations and is presumed, based on its production of eggs, to be a gametophyte, the G1 stage is the 1C level and the G2 stage the 2C level.

Another series of fluorescence measurements verified that *Tilopteris* does not change the level of DNA over three successive generations (Fig.25). The respective rfu levels of 61.8 +/-3.8, 58.2 +/-2.2, and 58.2 +/-2.8 are statistically within the same group.

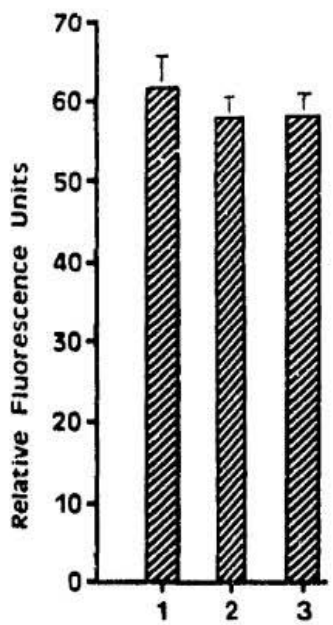


Fig. 25: Fluorescence values in different *Tilopteris mertensii* generations.

Mean rfus for *Tilopteris* nuclei of G2-cells in three successive generations (1,2,3), each derived from eggs of the preceding generation.

3.7.3. *Haplospora globosa*

Nuclear fluorescence levels were examined in cells of both the sporophyte and the gametophyte generation in *Haplospora* (Fig.26). Gametophyte cells in G1 phase with nuclei in telophase or in very early interphase determine the nuclear 1C value of 23.3 +/-1.5 rfus. Similar values were found in oogonia and hair cells suggesting that, as expected, these also have 1C levels of DNA.

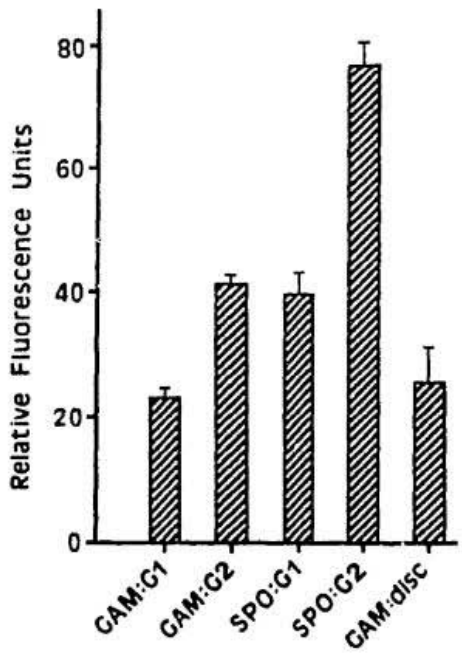


Fig. 26: Fluorescence values in both *Haplospora globosa* generations.

Mean rfus for *Haplospora* nuclei in gametophytes (GAM) and sporophytes (SPO). Fluorescence values were measured in nuclei of cells in G1 stage (G1), G2 stage (G2) and in prostrate discs (disc).

The 2C value of 41.6 +/-1.2 rfus was obtained from nuclei just prior to division either in cells within the trichothallic zone or in large cells in the parenchymatous, basal zone of the main filaments. A similar DNA value of 40.0 +/-3.2 rfus, obtained from the sporophyte generation, proved not to be statistically different from the gametophyte 2C value of 41.6 rfus. However, in the sporophyte this value was found in nuclei of cells in G1 phase such as in late telophase, in interphase immediately after cell division or in hair cells. Nuclei in sporophyte cells equivalent to gametophyte cells in G2 stage establish a 4C value with 77.2 +/-3.3 rfus, nearly twice the level of the 2C value.

Nuclei of cells in unknown G-stages in a prostrate disc of a *Haplospora* gametophyte have a mean of 26.0 +/-5.5 rfus, a value not different from DNA levels in cells of the upright parts. The relatively low value of 26 rfus might be due to the difficulties in staining and fluorescence measurements of nuclei within dense cytoplasm and thick cell walls of these discs.

The difference in ploidy levels in *Haplospora* was further confirmed by measurements on nuclei of a sporophyte and its offspring gametophyte generation (Fig.27). Quadrinucleate spores had germinated within their sporangia and thus allowed the application of the fluorometry procedures simultaneously to both stages under identical conditions.

All measurements on the two generations were separately pooled and the mean level of 47.8 +/-2.5 rfus in the parent sporophyte is clearly double that seen in the offspring gametophyte with 21.7 +/-1.9 rfus. Fluorescence values of nuclei in large, hyaline cells of the main filament which were obtained from the same individual *Haplospora* sporophyte, but at a different stage in its development,

show a clear drop of C-levels when the plant exhibits senescence. As a young, well growing plant, the rfus of 68.7 +/-5.5 compare well to the 4C values found in other specimens of *Haplospora*, while the value of 47.8 +/-2.5 rfus for similar cells in the senescent stage of the sporophyte are closer to the 2C value.

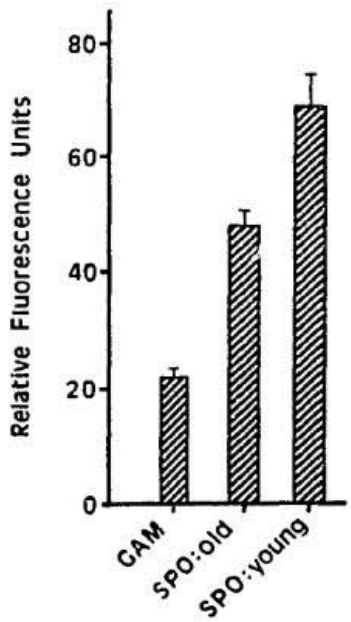


Fig. 27: Fluorescence values in *Haplospora globosa*.

Mean rfus for *Haplospora* nuclei in a gametophyte (GAM) derived from spores of the senescent parent sporophyte (SPO:old). The mean rfus for G2-cells in the same sporophyte, but at an earlier developmental stage, are shown as well (SPO:young).

A histogram including all *Haplospora* DNA fluorescence measurements (Fig.28) shows two overlapping, but statistically separate groups, the gametophytes with 15 to 52 rfus and the sporophytes with 30 to 98 rfus. These rfu ranges obtained from all cells are more extended than the respective ranges for the C values of each *Haplospora* generation which are 23 to 41 rfus (1C-2C) for the gametophytes and 40 to 77 rfus (2C-4C) for the sporophytes.

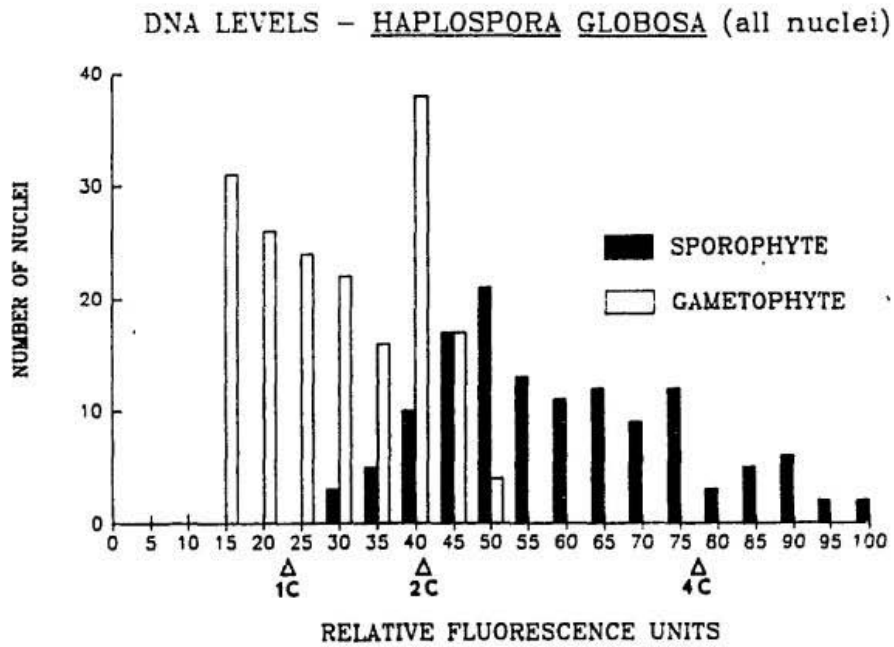


Fig. 28: Histogram of rfus for *Haplospora globosa*.

Fluorescence values were obtained from all the nuclei measured in either the gametophytes or sporophytes. Indicated are also the mean rfus representing the 1C, 2C and 4C levels for *Haplospora* (Δ).

3.7.4. *Phaeosiphoniella cryophila*

Field and culture observations revealed that *Phaeosiphoniella* exhibits no alternation of generations or any form of sexual reproduction. Fluorometry, therefore, was performed with different clonal cultures (Fig.29) and fluorescence values were obtained from large hyaline cells in the primary filaments. Such cells given the data from *Tilopteris* and *Haplospora* are mostly likely in G2 phase. Their mean values are 49.0 +/-3.7, 48.6 +/-1.8 and 45.6 +/-2.0, showing no statistical difference between DNA values of G2 cells in different clones of *Phaeosiphoniella*.

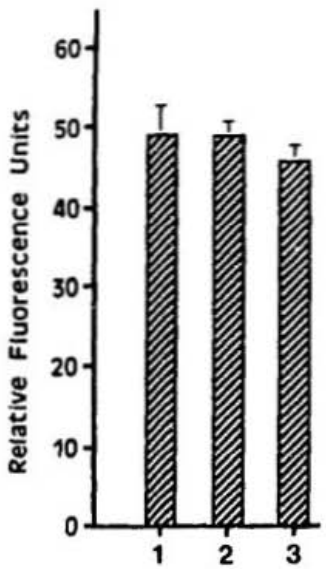


Fig. 29: Fluorescence values in different *Phaeosiphoniella cryophila* clones.

Mean rfus for *Phaeosiphoniella* nuclei of G2-cells in three different clones (1,2,3) from field material.

In one specimen, besides measuring the G2 level, cells in late telophase or early interphase were tested (Fig.30). The G1 value obtained is 27.3 +/-2.7 and slightly higher than would have been predicted, however, this is probably still within the range of variation expected. Terminal cells of branches of this species show a low rfu of 17.4 +/-1.6.

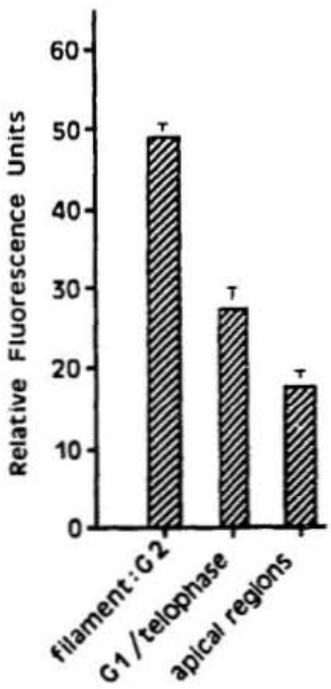


Fig. 30: Range of fluorescence values found in *Phaeosiphoniella cryophila*.

Mean rfus for nuclei in cells of different functions or cell cycle stages within a single *Phaeosiphoniella* specimen.

Attempts were made to measure the fluorescence values for nuclei in the perennating discs of *Phaeosiphoniella* (Fig.31), but it was difficult to obtain consistent results due to the dense cytoplasm and relatively thick cell walls. The mean value of 37.9 +/-4.5 rfus is pooled from all cells as it was impossible to determine their stage in the cell cycle. Nevertheless, this fluorescence value lies clearly within the range of the 27 to 45 rfus obtained from cells in the filaments.

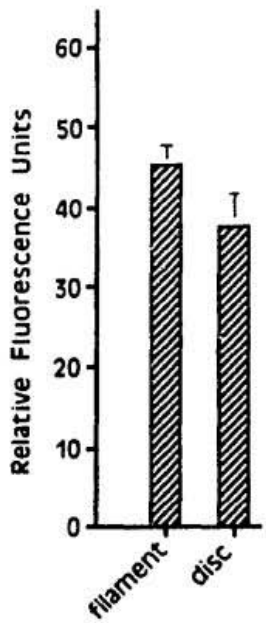


Fig. 31: Fluorescence values in *Phaeosiphoniella cryophila*.

Mean rfus for nuclei in cells of a prostrate disc and in G2-cells of its upright filament.

3.7.5. Distribution of rfus within single specimens

Fluorescence levels of every nucleus were measured in a young *Tilopteris* plant, grown in culture at 10°C (Fig.32a). The values show the expected range from 1C to 2C. Repeated measurements show the same result and allow the recognition of two major meristematic regions in the filament as indicated by nuclei at the 2C level. One such zone is referred to as the trichothallic meristem, the other occurs towards the base of the filament and is characterized by the possession of the largest cells of the filament. These cells usually undergo longitudinal cell divisions to form the parenchymatous zone prominent in the basal region of the main axis in older plants. Other cells which are not in meristematic zones show levels between 1C and 2C and include cells in the basal region of the main filament, the embryo-body (the former egg) and the rhizoid. Fluorescence values for nuclei in the hair are consistently around 1C.

A young, uniseriate *Phaeosiphoniella* plant, grown at 1°C from a prostrate disc, reveals a similar pattern in the distribution of rfus along the entire filament (Fig.32b) to that seen in *Tilopteris*. One of the regions of high DNA levels corresponds with the trichothallic meristem in *Tilopteris*, although *Phaeosiphoniella* does not morphologically differentiate any obvious meristematic zone. The same is true for the terminal part of the *Phaeosiphoniella* thallus, which shows DNA values around 1C, but does not exhibit the morphological characteristics of a hair as in *Tilopteris* or *Haplospora*. Higher DNA values are again found in the large, hyaline cells of the prospective parenchymatous region in the lower part of the filament, which indicates an equivalent situation to that found in *Tilopteris*, in which longitudinal cell divisions will lead to the development of a parenchyma.

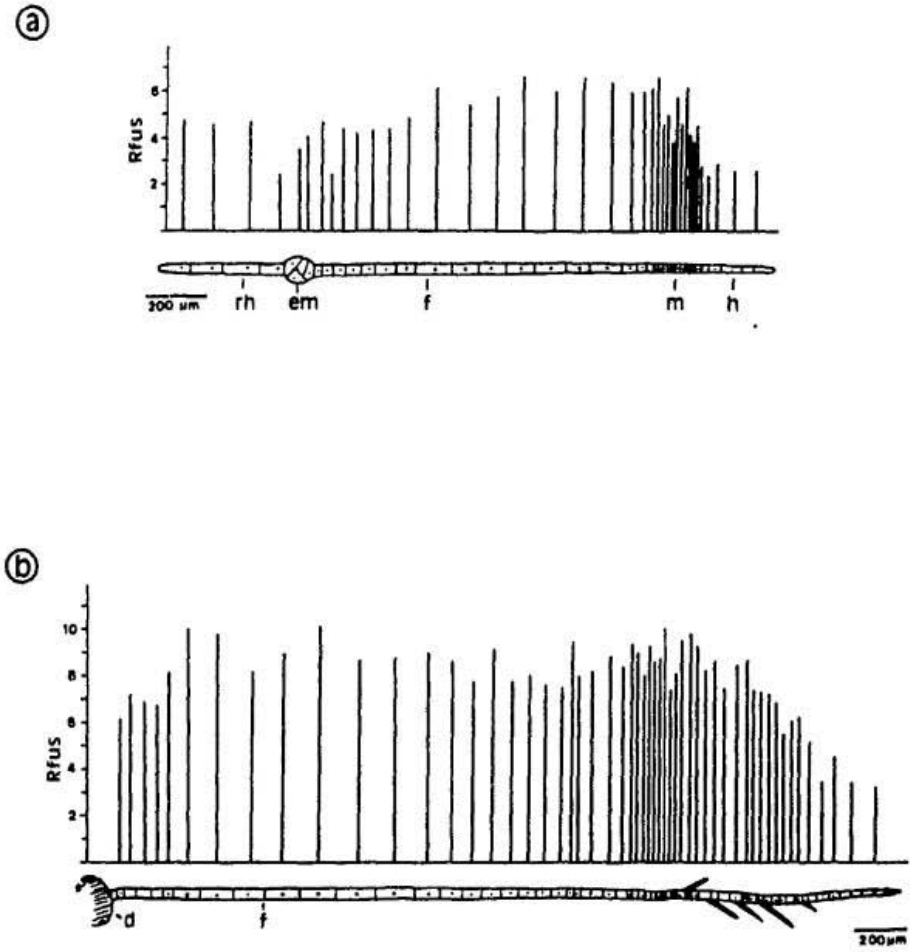


Fig. 32: Distribution of rfus for each cell in young plants.

(a): *Tilopteris mertensii* (b): *Phaeosiphoniella cryophila*

Each bar corresponds to the nucleus underneath and represents the actual rfu measured, corrected for the background value.
rh = rhizoid, em = embryo-body, f = filament, m = meristem, h = hair, d = disc.

3.7.6. Nuclear morphology from fluorochrome stained material

During measurement of rfu values from mithramycin stained material it was possible to examine the nuclear morphology of all the three species studied. Nuclei stained with fluorochromes show no differences in morphology from nuclei stained with haematoxylin. The nuclei vary considerably in size from 4 to 15 μm with the smallest being found in the hair cells of *Tilopteris* or *Haplospora* and the equivalent terminal cells of branches of *Phaeosiphoniella*. Large cells in the proximal parts of the primary uniseriate filaments in all three species have the largest nuclei which corresponds with the highest DNA levels found in those cells in all of the three species. Figure 33a shows the size range of nuclei in *Tilopteris*, with large nuclei in cells of the main filament decreasing in size towards the tip of the branches and hair cells. The same sequence of sizes is also seen in *Haplospora*. As *Phaeosiphoniella* lacks hair cells the smallest nuclei are found in the terminal cells of the branches.

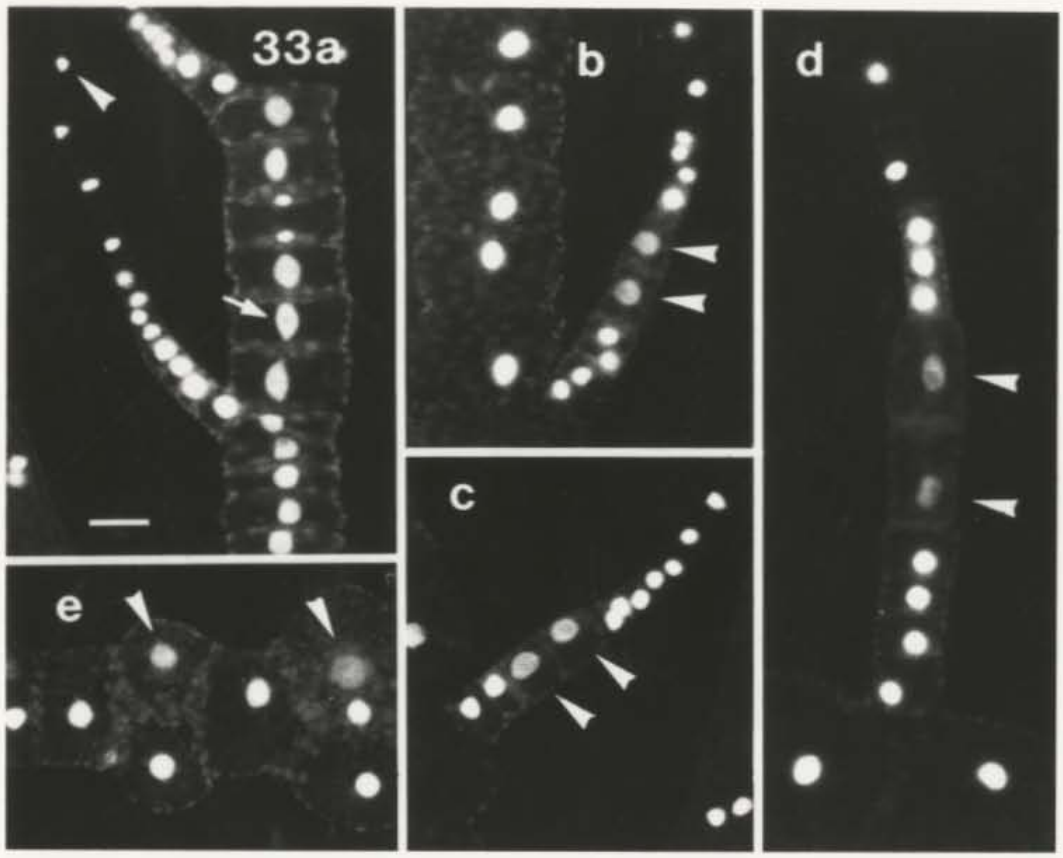
Reproductive structures were more difficult to observe because of dense cytoplasm or thicker cell walls in sporangia and oogonia. However, oogonia in *Tilopteris* were examined in a very early stage of development when nuclear staining with haematoxylin or microscopical observations did not show any differences between the oogonia initials and the adjacent vegetative cells. However, fluorescence as in the mature oogonia, was lower than the rfus obtained from the adjacent cells (Fig.33b). During subsequent development, oogonia were morphologically distinct from the vegetative cells while the differences between the respective rfus were even lower (Fig.33c). Nuclei in mature oogonia have even lower fluorescence values (Fig.33d), not only due to their low 1C DNA level, but

also due to the dense cytoplasm inhibiting proper staining and measuring of rfus. Nuclei in young oogonia in *Haplospora* are easily distinguished by their lower fluorescence values from nuclei of the same size in adjacent cells (Fig.33e).

Fig. 33: Fluorescence in mithramycin stained material.

(a): Size range of nuclei in *Tilopteris mertensii*: large nuclei in cells of the main filament (→); smaller nuclei in branches with the smallest nuclei in hair cells (--).
(b): Weak fluorescence in nuclei of oogonia initials (►) in *Tilopteris mertensii*, the oogonia are at that stage morphologically indistinguishable from the adjacent cells.
(c): Same as (b) in a later stage; the oogonia are morphologically distinguishable and have clearly lower fluorescence (►).
(d): Mature oogonia derived from initials similar to those in 33b, difference in fluorescence levels (►) is even stronger due to dense cell material in oogonia.
(e): Oogonia initials in *Haplospora globosa* show lower fluorescence in their nuclei (►) than the adjacent cells.

All to same scale bar 20 μm.


33a
b
c
d
e

Discussion

4.1. Occurrence of field material

Hooper *et al.* (1988) suggested that water temperature would be the most likely factor restricting the occurrence of *Phaeosiphoniella* uprights to the colder months from December to May. Uprights were observed until the seawater temperature reached 5°C, which was, therefore, assumed to be the upper temperature limit for *Phaeosiphoniella* uprights in the field. However, such temperature measurements must be interpreted with caution because upwelling or water currents may bring about changes of up to 4°C within hours. If one attempts to postulate a causal relationship between environmental factors and growth of the algae, as noted above, the appearance of senescence in upright filaments should be used as an indicator for the decline of the species, rather than its total disappearance. Plants of *Phaeosiphoniella* with signs of senescence were found several times in June at water temperatures around 1°C. The senescence was possibly due to previous exposure to high water temperatures or due to other factors, as shown by culture experiments. Light intensity could equally as well explain the restricted appearance of *Phaeosiphoniella*, since upright filaments do not survive photon flux densities higher than 8 μmol m^{-2} s^{-1}. Although measurements of the available light in the field were not made, light intensity is

likely at its lowest during the winter months. The response to light in culture, whereby the prostrate discs produce uprights only under low light intensity of less than 3 μmol m^{-2} s^{-1} but at temperatures of up to 5°C and occasionally at 10°C, indicates that light rather than temperature may well be the main controlling factor restricting the seasonal appearance of *Phaeosiphoniella* uprights at Fairhaven. In accordance with this theory are the results of microscopical examinations that permitted to detect the first upright initials of *Phaeosiphoniella* in the field when water temperatures were still approximately 5°C, whereas Hooper *et al.* (1988) reported the first uprights to be visible at 2°C and lower.

The low light intensity preference for the development of uprights is in strong contrast to the observation of increased growth of *Phaeosiphoniella* plants during periods of high light intensity such as sunny days and high water visibility described for the Fairhaven study site by Hooper *et al.* (1988). An explanation for this might lie in a differential response of upright filaments to light intensity at different times during their development. One has also to consider the possibility of different light level requirements for inducing the development of uprights than for the actual growth period. Another possibility is the failure of culture experiments to realistically reproduce the necessary environmental conditions, especially in a species so difficult to grow as *Phaeosiphoniella*. There is no doubt, however, that *Phaeosiphoniella* is regulated by light quality and temperature combined. Other, more extensive studies on the phenology of seaweeds (Mathieson 1989) describe how seasonal patterns in growth or reproduction in algae along the New England coast (USA) depend upon a diversity of environmental factors that determine the appearance of a certain alga at a certain

location and time of the year. Additionally, different developmental stages of the algae, such as prostrate systems, initiation of uprights, growth of uprights and senescence, might respond differently to the same environmental factors. Responses to light intensity or quality in *Phaeosiphoniella* might be a reflection of the natural conditions in which the basal systems could be experiencing light and other conditions very different from the uprights.

4.2. Prostrate systems: heterotrichy and perennation

This study is the first confirmation of the occurrence of pseudoparenchymatous, prostrate discs derived from rhizoids first described by Reinke (1889) in *Haplospora* and by Hooper *et al.* (1988) in *Phaeosiphoniella*. Sauvageau's (1928) description of prostrate discs in *Tilopteris* could not be confirmed. The discovery that both *Phaeosiphoniella* and *Haplospora* exist as prostrate, monostromatic discs or crusts during the time when uprights are absent from the field explains how these algae survive the summer season from May to December. These discs were found throughout the year and at any time were capable of producing upright filaments if placed under the appropriate culture conditions. Such conditions included low light intensity and, for *Phaeosiphoniella*, temperatures of 5°C or less. The same discs were capable of withstanding temperatures of 20°C and high light intensities for prolonged periods, which confirms that they are capable of acting as perennating structures.

Phaeosiphoniella and *Haplospora* are heterotrichous species, as defined by Fritsch (1939), in that their thallus is differentiated into a prostrate and an upright system. Both species, and also possibly *Tilopteris*, are pseudoperennial plants (Knight & Parke 1931, Sears & Wilce 1975), characterized by their

prostrate crusts persisting throughout the year and producing the typical vegetative uprights only under favourable conditions.

Many members of the Phaeophyceae have prostrate or microthallial stages capable of surviving temperatures greater than those tolerated by the macrothallus. Another apparently cold water species, *Papenfussiella callitricha* (Rosenv.) Kylin, has a microthallus which can survive in a wide range of conditions, whereas the macrothallus is limited to temperatures less than 8°C (Peters 1984). Many members of the Scytosiphonales are perennial with extensive crusts, which produce uprights in response to specific environmental factors (Kristiansen 1984, Edelstein *et al.* 1970, Lüning 1980).

Crusts of the Ralfsiaceae have been extensively studied and some former members are now known to be the prostrate systems of species within the Scytosiphonales (Fletcher 1978). The production of uprights as a seasonal response of crusts of *Scytosiphon lomentaria* (Lyngb.) Link to temperature and daylength has been studied in detail and several ecotypes have been established (Kristiansen & Pedersen 1979, Dieck 1987). Environmental factors play a similarly important role in the seasonal appearance of uprights in members of the Tilopteridaceae, although their prostrate systems are anatomically less complex than those of the Scytosiphonales. Instead of a photoperiodic control, as found in some *Scytosiphon* strains (Dring & Lüning, 1975), *Phaeosiphoniella* discs react to light quality and develop uprights under blue light, but not in red light. A similar light quality response has an influence on *Laminaria* species which require blue light to induce gametogenesis (Lüning & Dring 1975b). In certain isolates of *Scytosiphon*, however, the presence of red light is necessary to stimulate their

prostrate crusts to produce erect thalli (Lüning & Dring 1973). Development in all *Scytosiphon* strains, either in day-neutral or photoperiodic ecotypes, responds to temperature (Dieck 1987). Some strains produce erect thalli only below 15°C, regardless of photoperiod (Correa *et al.* 1986). All the *Scytosiphon* crusts, however, withstand higher temperatures than their uprights, similar to the prostrate systems of the Tilopteridaceae.

Structurally, the prostrate systems of *Haplospora* and *Phaeosiphoniella* resemble crusts in some members of the Sphacelariales. Almost identical to the *Phaeosiphoniella* discs observed in this study are the basal discs produced by rhizoids of *Sphacelaria radicans* (Dillw.) C.Ag., which through marginal growth of creeping, coalescent filaments with pseudodichotomous branching develop a discoid, monostromatic crust (Prud'homme van Reine 1982).

Tilopteris has not been studied in sufficient detail in the field to determine the importance of any prostrate system that it might possess. However, observations in culture show that rhizoids might function as stolons when produced in nutrient depleted cultures or during germination in darkness. On replenishment of the culture medium or on transfer to light the stolons develop numerous uprights. Similar stolons, which are commonly found in members of the Sphacelariales, such as *Sphacelaria nana* Näg. ex Kütz. (Prud'homme van Reine 1982), function as an extension of the initial discs and aid in the further colonization of the substrate with subsequent production of uprights. *Haplospora* and *Tilopteris* might achieve a similar increase in substrate cover with the formation of stolons when conditions for the production of uprights are unfavourable. All prostrate structures in the Tilopteridaceae constitute, besides

their important role as holdfasts, an effective system for vegetative reproduction, which facilitates many more uprights than would otherwise develop from a single spore or egg.

All observations on the Tilopteridaceae indicate that growth is regulated by primary environmental factors such as temperature or light intensity (type 1) rather than by more complex signals such as daylength (type 2). Lüning and Dieck (1989) characterized these two types of growth regulating factors and attributed a higher degree of independence to algae of the second type. *Phaeosiphoniella* clearly depends on primary factors, considering its light and temperature restriction. However, since it is no doubt that other localities than the few in which *Phaeosiphoniella* is found provide similar primary conditions suitable for growth of uprights (Hooper *et al.* 1988), there must be additional factors restricting *Phaeosiphoniella* to those few sites.

The morphological characteristics and perennating abilities of discs in *Phaeosiphoniella* and *Haplospora* appear to be well suited for the unstable substrate conditions on which these plants occur. For example, dense clusters of *Haplospora* were collected by Reinke (1889) from cobble shoals in 12 to 20 m depth near the coast of the western Baltic Sea (FRG). Davis and Wilce (1987a) described the flora of an unstable cobble habitat in Massachusetts and showed that there was a high abundance of crustose species in contrast to the flora normally associated with stable, unstressed habitats where large, fleshy algae occured in relatively high biomass. Many of their crustose species were not the heterotrichous bases of plants as examined in this study and were presumably capable of reproduction by spores or similar reproductive bodies, but such crusts

should have the same functional significance in both instances. Davis & Wilce (1987b) suggest that physical disturbance of the cobbles through water movement is the reason for the abundance of crustose species. At the Fairhaven site, the observation that crusts were predominantly confined to the upper surfaces of the pebbles suggests that these are only infrequently overturned by water movement. Another possible disturbance is grazing by herbivores, which has received considerable attention in the last two decades and is generally accepted as a major factor in structuring seaweed communities. Herbivory is also considered as an evolutionary force that has led some algae to develop crustose systems at some period in their life cycles (Littler & Kauker 1984, Lubchenco & Cubit 1980, Dethier 1981, Menge *et al.* 1985). Several crustose life forms are seen as an adaptation to grazing pressure, because they are more resistant to grazing than filamentous or upright stages (Steneck & Watling 1982). However, the loss of uprights of both *Phaeosiphoniella* and *Haplospora* in this study occurs through decay rather than grazing.

Waern (1958) suggested that substrates with small surface area, such as smaller stones, are a refugium supporting species capable of tolerating the potential instability, but having low competitive abilities. In a similar manner, Sears and Wilce (1975) found a higher diversity of species on small stones than on adjacent larger ones. The same was true for the cobble substrates studied by Davis and Wilce (1987a). The generally very low abundances and rare occurrences of members of the Tilopteridaceae are likely an expression of low competitive capabilities. Only in certain habitats, such as in Fairhaven, do *Phaeosiphoniella* and *Haplospora* show high population densities possibly due to similar refugial characteristics of their substrates.

Culture studies of both *Phaeosiphoniella* and *Haplospora* indicate that the prostrate systems and their uprights show different environmental tolerances and physiological responses. In stagnant cultures, with presumably low nutrient concentrations, plants cease axial growth and instead tend to produce prostrate discs from their rhizoids. Hooper *et al.* (1988) observed similar effects of overcrowded cultures or very high temperatures that induced senescence in *Phaeosiphoniella* filaments, but 'rhizoidal knots' remained viable under a much wider temperature range than uprights are able to withstand. Basal discs or crusts respond with the production of uprights when the culture medium is renewed and the light intensity is kept low. The prostrate systems continued to grow or stay viable under low nutrient conditions and high light intensities, within the full range of the temperatures tested. High environmental tolerances of the prostrate discs seem to be necessary for these algae to survive in the field during the summer period with its adverse conditions for the growth of uprights. Some morphological differences between the discs and uprights might also explain different physiological responses. Chloroplasts are much larger in cells of the discs and cover the whole cell surface, possibly an adaptation to existence at low light levels. Nultsch *et al.* (1981) reported higher photosynthetic activity in algal cells that have their chloroplasts exposed to the incoming light than cells with their chloroplasts aligned with cell walls facing away from the incident light. Similarly, larger and more numerous chloroplasts may be responsible for a higher photosynthetic yield compared to cells with fewer and smaller chloroplasts. This would allow survival on substrates frequently covered with sediment as found at the Fairhaven site.

There is also an indication that prostrate discs of *Phaeosiphoniella* growing on *Lithothamnion glaciale* emitted a substance which caused bleaching of cells of the coralline alga. This phenomenon is named heteroantagonism by Fletcher (1975) and describes the negative influence of substances emitted by one species on the growth of another, nearby species. In *Phaeosiphoniella*, prostrate discs might achieve higher competitive capabilities through heteroantagonism, in order to secure space and resist overgrowth by other, faster growing species.

4.3. Reproduction

4.3.1. *Tilopteris* and *Haplospora*

The observed life histories of *Tilopteris* and *Haplospora* were identical to those described by Kuhlenkamp & Müller (1985) and will not be discussed here in any detail. The absence of sexual reproduction in both species, as defined by meiosis and syngamy, but the presence of structures normally associated with sexual reproduction, such as gametes, suggests a derived condition of their life histories based on former sexual life forms. Most *Haplospora* populations show a morphological alternation between a gametophyte and sporophyte phase, whereas *Tilopteris* is only known to have a gametophyte stage.

4.3.2. Vegetative reproduction

All three species exhibit the ability to fragment and regenerate new plants. The production of uprights and rhizoids from filament fragments was described by South (1975) for *Tilopteris*, by Reinke (1889) for *Haplospora*. *Phaeosiphoniella* apparently shows an even greater loss of sexual reproduction as oogonia were never found in the field and antheridia and sporangia were only infrequently noted. This species appears to depend exclusively on fragmentation, or upon its prostrate system for propagation. *Phaeosiphoniella* shows a much greater propensity to fragment than the other two species. An obvious abscission of branch systems readily establishes new plants after release. Disintegration of plants seems to depend on the stage of senescence. Specimens with obvious accumulation of dark bodies, possibly physodes, in the nuclear regions readily break apart and can produce rhizoids from every cell. However, young vigorously growing plants do not readily break apart, even when mechanically agitated. A variety of factors may lead to senescence in marine macrophytes. Senescence is mostly associated with nutrient depletion, as in *Schottera nicaenesis* (Lamour. ex Duby) Guiry *et* Hollenberg (Rhodophyceae), which lost its upright blades and produced terete proliferations that developed into prostrate, creeping and perennating axes from which new blades ultimately arose when favourable conditions returned (Perrone & Fellicini 1988). Breeman and Hoeksema (1987) report the frequent regeneration of fragments of *Rhodochorton purpureum* (= *Audouinella purpurea* [Lightf.] Woelk. [South & Tittley 1986]) excreted from animals grazing the algal turf. Not only were the fragments highly resistant to digestion, but they also regenerated filaments under a wide range of temperature

and light conditions including complete darkness. As in *Phaeosiphoniella*, this species is capable of producing new filaments in detrital environments under heavy grazing pressure. Pearlmutter and Vadas (1978) describe the potential of *Rhodochorton purpureum* to regenerate rhizoids and new filaments from fragments consisting of only a few cells. A similar situation occurs in the three tilopteridalean species. Another similarity between the Tilopteridaceae and the rhodophytes cited above is the reduced level of sexual reproduction. Breeman & Hocksema (1987) only found one gametophyte during their study of *Rhodochorton purpureum*; in *Schottera nicaeensis*, sterile fronds that reproduced through basal systems were dominant, although a low ratio of sexual reproduction regularly occurs in this species (Perrone & Fellicini 1988).

Sexual reproduction in *Phaeosiphoniella* might also have been reduced in favour of vegetative propagation as a more effective way to survive grazing or stress related to an unstable substrate. The rapid establishment of new plants from abscised branches and the production of the prostrate, crustose discs might also be effective in maintaining *Phaeosiphoniella* at the extremes of its range. The few sites in Newfoundland might represent the last remaining areas of a once wider distribution. There are other situations known in which seaweeds maintain themselves at the extremes of their distribution ranges exclusively by vegetative reproduction (Dixon 1965). In Newfoundland, two members of the Ceramiaceae, *Callithamnion corymbosum* (Sm.) Lyngb. (Whittick 1978) and *Antithamnion cruciatum* (C.Ag.) Näg. (Whittick & Hooper 1977), at the northern limits of their ranges, reproduce through vegetative fragmentation only.

Hooper *et al.* (1988) described the occurrence of large, hyaline cells in

Phaeosiphoniella that supposedly facilitate the abscission of branches. Microscopical observations could not confirm the presence of these cells, instead branches were observed to produce rhizoids before abscission and the break occurred between adjacent cells without cellular rupture. The stimulus and mechanisms of this rupture remains unknown, but presumably some biochemical change occurs within the cell wall at the point of abscission.

In addition to fragmentation, *Haplospora* and in particular *Phaeosiphoniella*, reproduce through prostrate discs, which are produced by any of their rhizoids upon contact with the substrate. Each disc becomes the source of numerous new uprights which explains in *Haplospora* the phenological character of a very tufted appearance as described by Reinke (1889).

4.3.3. Physiology of prostrate discs

The development of the prostrate discs in *Phaeosiphoniella* and *Haplospora*, as revealed through culture studies, follows a pattern already described by Dring & Lüning (1975) for *Scytosiphon* sporelings. The apical cell of a rhizoid enlarges and divides repeatedly which the authors compare to the transition from filamentous to prothallial growth in fern gametophytes. Discs are the only structures in the Tilopteridaceae that have apical growth, as expressed in the peripheral cells of the radial rows originating in the centre of the disc.

Laboratory studies reveal that prostrate discs in *Phaeosiphoniella* are initiated only if rhizoids touch the surface of the substrate. A similar requirement was described by Müller (1984) for *Adenocystis utricularis* (Bory) Scottsberg. The surface properties of the substrate clearly play a role in whether a disc is

formed or not. *Phaeosiphoniella* produces discs on both the surface of polystyrene culture dishes and on shell fragments but not on the surface of glass culture dishes. The same discovery using tetraspore germlings of *Polysiphonia urceolata* (Lightf. ex Dillw.) Grev. was made by Fletcher (1976) who attributed the surface effect to differences in surface texture or surface tension. Both factors have been found to have a direct effect on the development of discs from rhizoids. Variation in surface texture was found by Linskens (1966) to be responsible for the varying intensities of adhesion of algal spores, while a high critical surface energy initiated the development of a compact, rhizoidal base in *Enteromorpha intestinalis* (L.) Link (Fletcher & Baier 1984). In nature, rhizoids in *Phaeosiphoniella* grow through a layer of detritus, sand and mud that covers the rock and pebble substrate at the Fairhaven site. It would therefore seem to be useful for rhizoids to produce a disc only in contact with a rock surface which is perhaps recognized by its surface structure and tension or other detectable surface properties. Filament fragments, after release from the parent plant, are found to be deposited on the top of the detrital/sand layer. The immediate development of photonegative rhizoids and a prostrate disc serving as a holdfast is an efficient mechanism for attachment, perennation and reproduction of the plant as it reduces the danger of loss through grazing or water movement. Mechanical cleaning of field material with a brush did not dislodge prostrate systems of either *Phaeosiphoniella* or *Haplospora*, which indicates a high adhesion of these discs to their substrate. Fletcher (1976) observed in the discoid stages grown in culture the same effectiveness of adhesion, which he assumesed also attributed to the perennating abilities of those stages.

There is insufficient data to determine if any prostrate system is important for reproduction and perennation of *Tilopteris* since prostrate discs never developed in culture. Instead, *Tilopteris* may utilize stolons or germlings that are able to develop and survive for an extended period of time in darkness or under low light and nutrient conditions for perennation (Kuhlenkamp 1989). In a comparable manner, young germlings in *Dictyota dichotoma* (Huds.) Lamour. persist through the unfavourable winter season and respond to improved conditions with new growth (Richardson 1979). Spores or germlings in *Saccorhiza* (de la Pylaie) express a high survival rate in prolonged darkness and this is suggested to be of an advantage for species living at high latitudes because of long, dark winter periods (Norton 1977), a likely advantage for *Tilopteris* as well. Under unfavourable conditions, young *Haplospora* germlings that produce prostrate discs almost directly from eggs or spores function as equivalents to germlings of *Tilopteris*. Additional support for the idea that young germlings with prostrate discs have important perennating functions is provided by the observation by Reinke (1889) who described similar *Haplospora* germlings from cobble habitats of the Baltic Sea and speculated that such discs were perennating structures for the summer period, since uprights were only found in spring. If discs are usually produced by spores and eggs of *Haplospora*, it would explain the high number of single discs detected in the field compared to the multiple, clustered discs of *Phaeosiphoniella* that are derived solely from the numerous rhizoids of whole plants or abscised fragments.

4.4. DNA fluorometry

4.4.1. Tests with chicken blood

Fluorescence measurements with fixed chicken red blood cells (RBCs) revealed the minimum staining time necessary to reach a constant level of fluorescence intensity. In accordance with Hull *et al.* (1982), this study found that material stained with DAPI reached the maximum intensity much faster than material stained with mithramycin, but mithramycin staining is more consistent and therefore more appropriate for quantitative work. Due to their constant DNA level, RBCs are useful as a standard (Coleman *et al.* 1981) and ensured the uniformity of the staining method during this study.

4.4.2. *Tilopteris mertensii*

Tilopteris is known to exist only as the gametophyte stage. There is no alternation of generations and hence it would be expected that the DNA level in the same nuclear phase would not vary significantly between generations. Chromosome counts in *Tilopteris* show no change over successive generations (Kuhlenkamp & Müller 1985). This lack of change in ploidy is confirmed by the DNA measurements on nuclei in the G2 phase, which are statistically inseparable between the three successive generations examined in this study.

Considerable loss of fluorescence was found to occur in fixed material which had been stored in 70% ethanol prior to fluorescence staining and the fluorescence values depended on the time in storage. After 4 weeks of storage, rfus decreased by 50% over those determined after one day in storage. Apart from the loss of fluorescence, nuclei appeared identical in appearance to material stained

immediately after fixation. The background staining of the test material remained constant over the test period and does not contribute to this dramatic loss in fluorescence. In contrast, staining with haematoxylin by visual comparison appears to be of constant density and is independent of storage time. This suggests that storage affects the specific DNA binding sites for mithramycin. None of the previous studies utilizing DNA fluorometry mention any similar effect and do not specify the length of time the material was stored prior to staining. It is clear, however, that more work is necessary and results obtained from ethanol stored or fixed material should be interpreted with caution.

Fluorescence measurements within one specimen of *Tilopteris* with mean values ranging from 18 to 48 rfus reflect changing DNA levels from 1C to 2C, while the cells undergo the cycle from G1 to G2. Again, this confirms that *Tilopteris* contains only one ploidy level. The apparent difference in the G2 stage fluorescence levels between this specimen and the levels measured in the three successive generations is perhaps due to an error in staining. As mentioned above, rfus depend on the time specimens were stored in ethanol and although measurements were always done after 3 days in storage, an error is not to be excluded. Furthermore, differences in the size of the nuclei might account for variability in results, since the same pinhole was used for all the fluorescence measurements and small nuclei with dense chromatin would more likely cause rfus to be lower than the actual level.

Comparing rfus of nuclei within a single specimen allows the determination of areas of predominantly high or low nuclear activity. Two major groups of cells in the G1 stage with 1C nuclei are hair cells and oogonia. A consistent 1C level in

oogonia supports the observation that these structures are gametangia. Eggs from oogonia would normally be fertilized by a male gamete and regain the 2C DNA level before entering the S-phase to produce a typical diploid 4C nucleus. Presumably, *Tilopteris* eggs are released at the 1C stage and early in their parthenogenetic development enter the cell cycle to eventually regain the nuclear range from 1C to 2C found in somatic cells. The 1C DNA level measured in hairs is consistent with their appearance as terminal structures unlikely to undergo any further development. They are frequently described as colourless, inactive and hyaline cells, however, the word colourless is not strictly appropriate as they usually contain a few plastids.

Two meristematic zones with predominantly high DNA levels around 2C are found in young *Tilopteris* plants. The most obvious one is in the upper part of the plant in the trichothallic zone. The other area is characterized by large cells in the main axis which will ultimately divide to produce a parenchymatous zone. More proximal cells in this parenchymatous zone are less active and their DNA level remains at 1C. The examination of every cell along the main axis of a plant therefore results in a curve which shows two 2C peaks.

4.4.3. *Haplospora globosa*

The life cycle of *Haplospora* from Newfoundland includes an alternation of morphological generations, but cytological examination showed no difference in chromosome number between the phases (Kuhlenkamp & Müller 1985). Thus, one would expect to find the same DNA levels in the sporophyte and the gametophyte generations when both were compared by DNA fluorometry. In fact, the DNA level in the sporophyte is twice that of the gametophyte. The histogram including

all rfus measured in both *Haplospora* generations shows two distinct groups, one in the range of values from 1C to 2C for the gametophytes and one in the range from 2C to 4C for the sporophytes. When successive generations were measured, such as the gametophyte germlings from quadrinucleate spores still attached to the parent sporophyte, it was again confirmed that the gametophyte had only half the nuclear DNA level of the sporophyte. This result is consistent with the interpretation of *Haplospora* as an oogamous species with an initially sexual life cycle. However, it is known that the spermatozoids are functionless, eggs develop parthenogenetically and meiosis in the sporangia is incomplete (Kuhlenkamp & Müller 1985).

Both interpretations of *Haplospora*, either as a species still reproducing sexually or as a species with a very reduced oogamous reproduction, do not explain the extraordinary feature that cells in both generations have equal numbers of chromosomes but a clear difference in ploidy level. Chromosome numbers in *Haplospora* are high in comparison to other filamentous brown algae (Kuhlenkamp & Müller) and the ploidy levels may thus be higher than the diploid levels usually found in filamentous Phaeophyceae. This still does not explain the difference in DNA levels while the chromosome numbers are identical. Moreover, quadrinucleate sporangia express genetic flexibility, since spores in material from Helgoland (FRG) produce sporophytes instead of gametophytes and exhibit only half the chromosome number of isolates of *Haplospora* from other localities (Kuhlenkamp & Müller 1985). These deviations from a sexual sporic life cycle lend no explanation to the contradictory information obtained from DNA measurements and chromosome counts. Since all the gametophyte cells, including

oogonia, have single DNA levels and all the sporophyte cells double DNA levels, doubling of the DNA presumably occurs during the parthenogenetic germination of the eggs. This phenomenon is clearly one of importance which requires further study with other isolates and detailed examination of the egg development perhaps with ultrastructural analysis. It might also be interesting to look at other pleiomorphic species such as members of the *Scytosiphon - Ralfsia* complex which have also foregone sexual reproduction, but show an alternation of morphological generations (Fletcher 1978).

4.4.4. *Phaeosiphoniella cryophila*

This study could only show that different isolates of *Phaeosiphoniella* had the same DNA levels as no life history involving reproductive structures such as spores or oogonia was obtained. The range of DNA values from 1C to 2C includes very low values for apical cells of the branchlets. Nuclei of these cells are also smaller and do not have visible nucleoli, an indication of low RNA activity and therefore low cell activity (Busch & Smetana 1970). Within the same species, smaller nuclei are generally characterized by lower DNA levels and a G1 stage compared to larger nuclei (Nagl 1970). These are the same characteristics seen in hair cells of *Tilopteris* and *Haplospora*. Terminal cells in *Phaeosiphoniella* do not exhibit morphological features distinguishing them from other somatic cells, but nuclear observations suggest that they can be regarded as being equivalent to hair cells. The DNA measurements of the prostrate discs of *Phaeosiphoniella* produce levels between 1C and 2C, excluding genetic alternation between the two morphological different phases, discs and uprights.

4.5. Phylogenetic relationships of the Tilopteridaceae

The characterization of this group of brown algae, as presented in the recent accounts on the Tilopteridales (South 1975, Wynne 1982, Pedersen 1984) is only partially correct. The main misconception lies in the interpretation of the oogonia as 'monosporangia'. With the discovery of the third member of the order, *Phaeosiphoniella*, unfortunately no new characters for classification are provided since it is the most degenerate member of the order. Hooper *et al.* (1988) restricted the discussion about its place in the order to listing features separating it from *Haplospora* and *Tilopteris*, but did not give a description of a family or order which included *Phaeosiphoniella*. It is apparent that the circumscription of the Tilopteridaceae and by extension of the Tilopteridales is modified by the inclusion of *Phaeosiphoniella*.

The family Tilopteridaceae now contains entities which possess:

- a filamentous habit
- pronounced primary axis
- intercalary meristematic zones
- uniseriate axes with multiseriate parenchymatous basal parts
- discoid plastids which lack pyrenoids
- branching either alternate or opposite
- intercalary oogonia with one uninucleate egg
- antheridia containing many spermatozoids with heterokont flagellation
- sporangia in some stages
- perennating structures (prostrate discs) produced by rhizoids

Certain features only found in *Haplospora* and *Tilopteris*:

- discrete trichothallic meristematic zones terminating in true hairs
- hollow antheridia
- isomorphic gametophyte and sporophyte generations in *Haplospora*
- quadrinucleate sporangia in *Haplospora*

Unique features in *Phaeosiphoniella*:

- cortication of plurilocular sporangia
- vegetative reproduction through branch abscission
- lack of hairs and lack of a trichothallic meristem

Phaeosiphoniella is readily distinguished from *Haplospora* and *Tilopteris* by the lack of trichothallic hairs, which have been previously regarded as one of the distinctive features of the Tilopteridales (South 1975). Studies show that in some algae hairs develop in response to environmental factors such as the nitrate levels in *Gracilaria verrucosa* (Huds.) Papenf. (Rueness *et al.* 1987) or phosphate in some members of the Chaetophorales (Gibson & Whitton 1987). Light quality has an effect in *Petalonia fascia* (O.F.Müll.) Kuntze or *P.zosterifolia* (Reinke) Kuntze and *Scytosiphon*, which produce hairs only in the presence of blue light (Lüning & Dring 1973). A different response is seen in *Tilopteris* where blue light mediates the production of upright filaments, but has no effect on hair production (Kuhlenkamp 1989). Development of a true tilopteridalean hair which consists of hyaline cells with a distal meristem (Pedersen 1984) is a constant character for *Tilopteris* and *Haplospora*, although influences on the development by environmental factors cannot be excluded due to lack of detailed studies. Nuclei in the hairs of *Tilopteris* or *Haplospora* and in terminal cells of *Phaeosiphoniella* are very small and contain a 1C level of DNA, suggesting that all terminal cells in the Tilopteridaceae are metabolically inactive. Taxonomically useful features should be constant and unaffected by environmental factors, but as Mathieson *et al.* (1981) also point out, features with phenotypic plasticity might still be valuable for classification provided that characters are judged on sufficient knowledge. In the case of *Phaeosiphoniella*, DNA fluorescence studies enabled to extend the taxonomic evaluation and to consider, despite the lack of hair-like morphological features, the terminal cells as homologous to hairs in both *Tilopteris* and *Haplospora*. It is not clear, however, whether these are potential hair initials or remnants of hairs from the evolutionary past of *Phaeosiphoniella*.

The introduction to this thesis attempted to clarify some of the potentially misleading terms used in the literature in connection with life cycle studies of members of the Tilopteridales. This study has shown that the term 'parthenosporangia', used to describe oogonia in *Tilopteris* (South 1975) is incorrect. Spores by definition are reproductive bodies developing without fertilization and would therefore not develop parthenogenetically. Only in sexual reproduction with gametes or bodies derived from gametes can parthenogenesis occur (Müller 1967b). The problem of confused terminology and the misusage of terms is not restricted to the Tilopteridaceae amongst the brown algae. Russell (1973) suggests that it is difficult to be consistent in terminology when dealing with complex life cycles such as those exhibited by many members of the Phaeophyceae. The Tilopteridaceae provide a good example of this problem where besides the final morphological appearance, developmental and evolutionary processes must be taken into account when classifying reproductive structures. *Tilopteris* exists only as a gametophyte reproducing through apomictosis in which unfertilized eggs develop into gametophytes. Although this might superficially resemble reproduction through spores, developmental studies and a comparison with *Haplospora* clearly indicate that both *Tilopteris* and *Haplospora* are fundamentally oogamous. The Tilopteridaceae thus combine a number of advanced and primitive features. Oogamy is advanced, but heterotrichy and isomorphic alternation of generations is considered a primitive feature (Fritsch 1942) and both are well developed in the family. Polystichous species are considered more advanced than haplostichous ones (Fritsch 1943). All three species of the Tilopteridaceae develop a partly parenchymatous filament, recalling the

regular septation characteristic of members of the Sphacelariales. In *Phaeosiphoniella* the filament is polystichous with a distinction between the large cells comprising an inner cylinder or medulla and smaller cells, restricted to the cortex. Similarly structured filaments are found in supposedly evolutionary advanced orders such as the Sphacelariales (Prud'homme van Reine 1982) or Dictyosiphonales (Fritsch 1945). The difficulty in the discussion of primitive and advanced characters at the species level within the family only re-enforces the even greater difficulty in determining their relationships to the other families or orders of the Phaeophyceae. Many of the characters such as oogamy and the polystichous habit probably arose independently on a number of occasions in different algal evolutionary lines. This aspect of phylogenetic relationships is greatly emphasized by Clayton (1984). Although her discussion deals particularly with the Fucales, she does not leave any doubt that if oogamy is present in two different algal groups these species did not necessarily derive from the same ancestors.

4.6. General conclusions

The Tilopteridaceae show complex and highly diverse forms of reproduction. This includes failure of meiosis and absence of syngamy. DNA levels are different between sporophyte and gametophyte generations in *Haplospora* although they show equal chromosome numbers. Evidence of apomeiotic events in the Phaeophyceae is increasing. *Pilayella littoralis* (L.) Kjellm. show meiotic patterns in the unilocular sporangial initials but no subsequent chromosome reduction (Müller & Stache 1989). Pedersen (1984) describes apomeiotic development in *Isthmoplea sphaerophora* (Carm. ex Harv. in Hook.) Kjellm. and Nygren (1987) in *Climacosorus mediterraneus* Sauvageau. Besides apomeiosis, other deviations from a strict sporic life cycle frequently occur. Unfused gametes in several physiologically anisogamous species develop into sporophytes as in *Ectocarpus siliculosus* (Müller 1967a) and *Scytosiphon lomentaria* (Clayton 1980). Parthenogenesis in oogamous species, as it is seen in *Tilopteris* and *Haplospora* may also occur in members of the Laminariales such as *Chorda tomentosa* Lyngb. (Maier 1984) and *Alaria crassifolia* Kjellm. (Nakahara & Nakamura, 1973) as well as in *Perithalia caudata* (Labillardiere) Womersley, a member of the Sporochnales (Müller *et al.* 1985). Some dioecious Phaeophyceae, such as an oogamous, ligulate *Desmarestia* from Chile (Ramirez *et al.* 1986) show a particular kind of apomixis in the development of sporophytes directly from the vegetative filaments of the gametophytes.

Algae from many families of the Phaeophyceae exhibit a variety of asexual reproductions apparently based on the modification of reproductive bodies normally associated with sexual reproduction. Whether a species reproduces

sexually or asexually might depend on environmental conditions. In *Stilophora rhizoides* (Turn.) J.Ag. it is determined by water temperature (Peters & Müller 1986). Asexual reproduction in this isogamous species is similar to that seen in *Tilopteris* in that gametophytes can reproduce apomictotically through unfertilized gametes. Another example is reported in *Sphacelaria rigidula* Kütz. (= *S. furcigera* [Prud'homme van Reine 1982]) in which gametangia are formed only at temperatures lower than 12°C, whereas plants at temperatures higher than 12°C switch to vegetative reproduction by propagules (Hoek & Flinterman 1968). Male gametophytes of this species react to stressfull conditions of very long photoperiods and high irradiances with decreasing the number of gametangia while increasing vegetative growth (Hoopen *et al.* 1983).

Environmental factors obviously not only limit the distribution of species, they may also determine the mode of reproduction which in turn might lead to isolation of populations and development of clones with restricted reproductive capabilities. This might be responsible for the diversity of reproduction and isolation of populations apparent in the members of the Tilopteridaceae. Kornmann (1954) discussed this process based on the theory of life history differentiation by Feldmann (Kornmann 1954) in which generations or stages of a life cycle become separated and develop into independent species. In a further elaboration of this theory, Kornmann (1957) considered the decrease of sexuality in some families of the Phaeophyceae, especially within the Ectocarpaceae, to be linked to the process of separation of life cycles as well. An example of this phenomenon is seen in *Acinetospora crinata* (Carm. ex Harv. in Hook.) Kornm., an ectocarpalean species with some similarities to the species of the Tilopteridales.

This species has apparently lost the ability to undergo sexual reproduction and reproduces through apomeiotically produced spores (Müller 1986). *Acinetospora* is considered to be the sporophyte generation of a formerly complete sexual life cycle. There is considerable heterogeneity in existing *Acinetospora* populations which is presumably a consequence of clonal development in isolated populations.

Similar plasticity of life histories is expressed in the Tilopteridaceae based on different modifications of their former sexual reproduction and the development of perennating structures such as prostrate crusts. *Haplospora* still shows an alternation of generations in Newfoundland although eggs develop exclusively through parthenogenesis, whereas in Helgoland it is known only in its sporophyte stage with a reduced chromosome number (Kuhlenkamp & Müller 1985). It seems evident that *Tilopteris* is the surviving gametophyte of a former biphasic life cycle, whereas *Phaeosiphoniella* is even further reduced with reproduction exclusively through fragmentation and prostrate discs.

It is known from a variety of different algae that populations at the extremes of their ranges tend to lose sexual reproduction. Environmental conditions necessary to maintain vegetative growth are often wider than those for reproduction (Whittick *et al.* 1989, Breeman 1988, Hoek 1982). As discussed for *Tilopteris* by South (1987), *Phaeosiphoniella* could have survived the cold period during the Pleistocene and represents a relic species. The life histories shown by members of the Tilopteridaceae are best discussed with those environmental constraints in mind. Especially in *Phaeosiphoniella*, which Hooper *et al.* (1988) suggest is a refugial relic of a previously more widely distributed species, it might be possible to find populations exhibiting their former sexual reproduction in other locations.

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