MOLECULAR AND FIELD STUDIES OF THE LIFE HISTORY OF *ACROSIPHONIA* (CODIOLALES) CHLOROPHYTA

by

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ABSTRACT

This study employs molecular and field sampling techniques to understand the complex life history of the filamentous green alga, *Acrosiphonia*, in southern British Columbia, Canada. The DNA sequences of the nuclear ribosomal internal transcribed spacer (ITS) regions conclusively identify the unicellular green algal endophytes, 'Chlorochytrium inclusum' and 'Codiolum petrocelidis', as the alternate life history phases of one or more *Acrosiphonia* species. 'Chlorochytrium inclusum', a spherical unicell, was found abundantly in the foliose red alga *Mazzaella splendens*, whereas 'Codiolum petrocelidis', a stalked unicell, densely colonises 'Petrocelis franciscana' (= crustose tetrasporophytic phase of *Mastocarpus papillauts*). The DNA sequence data supported previous culture studies and Kornmann's hypothesis that the two morphologically different endophytes are alternate phenotypes of the sporophyte of a single *Acrosiphonia* species. The relationship of *Acrosiphonia*'s endophytic sporophyte and filamentous, free-living gametophyte in nature revealed similar dynamics for three environmentally variable field sites. Filamentous *Acrosiphonia* plants were seasonally abundant (spring and summer) with fertile cells developing almost immediately after *Acrosiphonia*'s appearance in the rocky intertidal zone. The unicellular sporophytes colonised *M. splendens* and 'Petrocelis' one to three months later, and showed higher tolerance to abiotic factors than *Acrosiphonia*'s gametophyte: high summer temperatures (which correlated with death of the filamentous free-living plants) were survived and they overwintered in their hosts. Endophytes matured primarily in winter, zoospore release occurred throughout winter and spring and *Acrosiphonia*'s life cycle is completed with subsequent zoospore germination and establishment of filamentous gametophytic plants. The two red algal hosts, *M. splendens* and 'Petrocelis', were abundantly available for endophyte colonisation in spring and summer. A number of factors, *e.g.* herbivory, winter storms and senescence, however, were identified to produce fluctuating seasonal abundance patterns of the hosts, thus potentially affecting endophyte survival. I suggest the endophytes have evolved a strategy whereby duration in the host is synchronised with seasonality of the host. An investigation of possible hosts for *Acrosiphonia*'s sporophyte established a wide range of hosts. However, 'Codiolum' showed a greater affinity for 'Petrocelis' than for other crusts, and 'Chlorochytrium' colonised foliose red algae characterised by loosely compacted cells in the cortex and medulla and carrageenansand carragars as cell wall

constituents *e.g. M. splendens, M. heterocarpa* and *Schizymenia pacifica*, more readily than others.

A bet-hedging strategy is proposed for *Acrosiphonia*'s life history. Not only have two morphologically different phases adapted to a seasonally variable environment, but the sporophytic phase of at least one *Acrosiphonia* species can colonise two alternate hosts (crustose and foliose red algae), and low host specificity is evident for both endophytes.

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THESIS INTRODUCTION

The genus *Acrosiphonia* was established by J. G. Agardh in 1846 for a group of marine green algae composed of branched uniseriate filaments, each cell having a reticulate chloroplast. Filaments of plants tend to be bound together by hooked branchlets or by rhizoids to form large tangled masses, and are abundant on rocks in the low to mid intertidal zone (Fig. 0.1). Difficulty in separating the genus from *Spongomorpha* has long existed (Wille 1900, Collins 1909, Kornmann 1965, 1970a; Scagel 1966, Jónsson 1991). Agardh (1846) originally delimited the two genera on the basis of cell length to width ratios. Later Wille (1900) suggested that multinucleate plants be called *Acrosiphonia* and uninucleate ones *Spongomorpha*. Since culture studies of *Acrosiphonia* revealed extreme polymorphism in response to temperature and light intensity, Kornmann (1965, 1970a) proposed that reproductive behaviour rather than vegetative characteristics such as length of cells be used for separation of the two genera. Based on his studies, *Acrosiphonia* was characterised by vegetative reproduction and *Spongomorpha* by a heteromorphic life history. Jónsson (1959a, 1962), however, claimed that *Acrosiphonia* in France is heteromorphic, and pointed out that Kornmann's failure to obtain 'Codiolum' from zygotes of *A. arcta* (Dillwyn) J.G. Agardh [= *Acrosiphonia spinescens* (Kützing) Kjellman according to Hudson (1974) and Jónsson (1986)] may have been due to a complete failure of karyogamy (Jónsson reported that karyogamy in *A. arcta* is facultative). In the northeast Pacific culture studies (Hollenberg 1958, Fan 1959, Chihara 1969, Hudson 1974) also suggested a heteromorphic life history for *Acrosiphonia*. Both Kornmann (1962a) and Jónsson (1959a) agreed that the presence of opercula on the gametangia is a distinctive feature of *Acrosiphonia*. Scagel (1966) questioned the value of Wille's separation into uninucleate and multinucleate genera and Collins (1909) believed that such a delimitation de-emphasised the similarities in thallus structure, other cytological characteristics and reproductive behaviour. Nonetheless, despite these criticisms, Wille's proposal is widely accepted by taxonomists today. *Spongomorpha* is absent from the northeast Pacific, having only been reported in Scandinavia, Iceland, the Arctic Ocean, Germany and France.

There is also a lack of agreement on species delimitation of *Acrosiphonia*. Scagel (1966) reported five species of *Acrosiphonia* present in northern Washington and British Columbia. However, Hudson's (1974) intensive culture and taxonomic studies from the Puget Sound region

Figure 0.1 *Acrosiphonia coalita* in the low intertidal zone at Sooke, Vancouver Island.

suggest that only two well-defined entities, *A. coalita* (Ruprecht) Scagel, Garbary, Golden & Hawkes and *A. arcta* are represented on our coast. *Acrosiphonia coalita* appears to be present only in the northeast Pacific, whereas *A. arcta* has been recorded in both the northern and southern hemispheres, including the Northwest Atlantic, the northeast Pacific, the North Sea, Greenland, southern Chile and Antarctica. Other *Acrosiphonia* species, apparently not present in North America, have been identified occurring in Japan, Germany, France, Scandinavia and Greenland. Criteria commonly used to distinguish species of *Acrosiphonia* are primarily vegetative characteristics (Setchell & Gardner 1920, Scagel 1966), many of which are variable and responsive to environmental conditions (Kornmann 1965, 1970a; Hudson 1974).

In addition to taxonomic uncertainties, the life history of *Acrosiphonia* is poorly understood. Extensive culture studies conducted primarily in the 1960s and 1970s (Hollenberg 1958, Fan 1959, Jónsson 1959a, 1959b, 1962, 1963, 1966, 1970; Kornmann 1961a, 1964, 1970a, 1972; Chihara 1969, Hudson 1974, Miyaji & Kurogi 1976, Miyaji 1984, 1996) implicated

unicellular green endophytes of the Chlorococcales in the life histories of *Acrosiphonia* and *Spongomorpha*. Endophytic algae are those which are found within the tissues or cells of other algae / plants, but do not elicit symptoms of disease (Wilson 1995). *Chlorochytrium inclusum* Kjellman, endophytic in foliose red algae and *Codiolum petrocelidis* Kuckuck, found within red algal crusts, are suspected to be the sporophytic alternate life history phases of the gametophytic *Acrosiphonia*. (Fig. 0.2). For this reason, all reference to the Codiolum and Chlorochytrium phases appears in single quotes in this thesis.

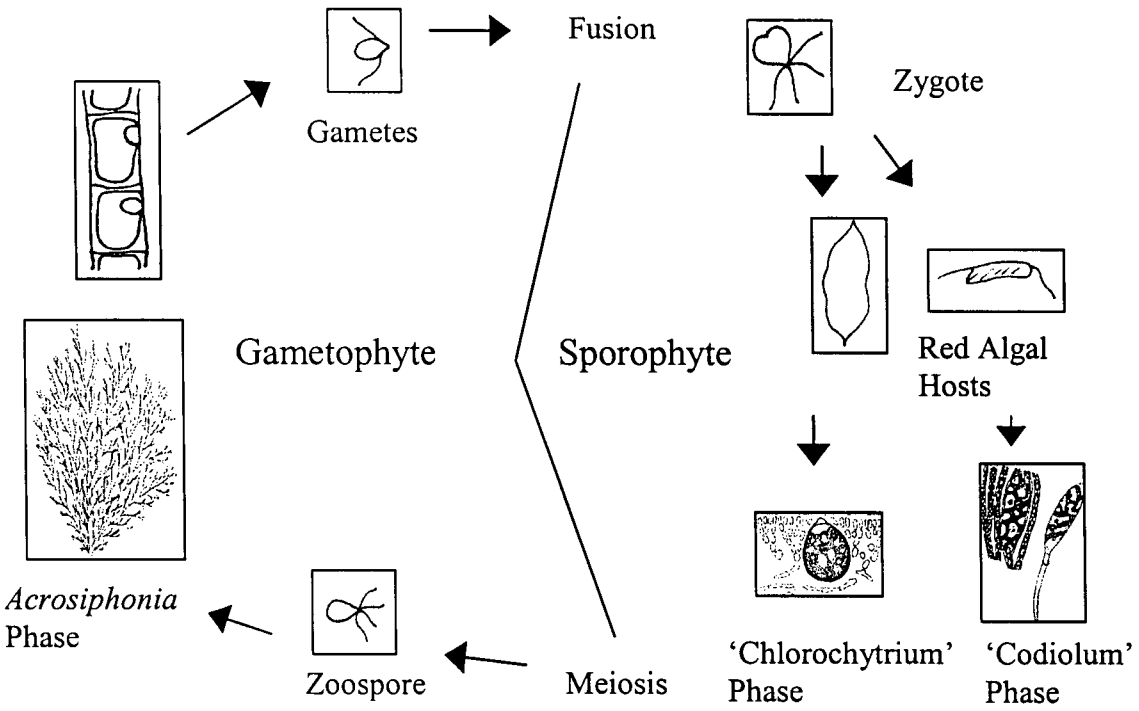

Figure 0.2 Life cycle of *Acrosiphonia*.

Several alternative life histories (other than a heteromorphic alternation of generations) have been reported for *Acrosiphonia* in culture. In *A. grandis* Kjellman (type locality Norway) the zygote develops into a 'Codiolum' or 'Chlorochytrium' phase which grows directly into a filamentous plant without formation of zoospores (Kornmann 1970b). Meiosis has not been observed, but Kornmann (1970a) suggested it might occur within the 'Codiolum' vesicle. In *A. sonderi* (Kützing) Kornmann (type locality Helgoland, Germany) and *A. arcta* filamentous plants of unknown ploidy were found to recycle by means of biflagellate zoospores, sexual reproduction not having been observed (Kornmann 1962a). Filamentous plants of unknown ploidy of *A. arcta* were also found to recycle by means of isogametes (Kornmann 1962a). Meiosis was not observed, but was suggested to occur during germination of the zygote or possibly in gametogenesis. No evidence exists that any of these alternate life histories occurs in nature.

'Chlorochytrium inclusum' is a spherical to subspherical unicell, generally found among the cells of the cortex of foliose red algal hosts such as *Schizymenia*, *Constantinea*, *Mazzaella*, *Neodilsea* and *Dilsea* (Scagel 1966, Chihara 1969, Hudson 1974). It is reported to measure 80 - 100 μm in diameter at maturity and contains one parietal chloroplast with many pyrenoids. 'Codiolum petrocelidis', on the other hand, is differentiated into an ovoid vesicle with a colourless stalk, and is commonly found embedded in the filamentous system of the red algal crust 'Petrocelis' [= tetrasporophytic phase of *Mastocarpus* (Hollenberg 1958, Fan 1959, Jónsson 1959a)]. The cell contains a single dense chloroplast and many pyrenoids, and is 125-175 μm in length and 25-50 μm in diameter in the pigmented region. Jónsson (1959a, 1966) and Kornmann (1961a, 1964) observed that when these morphologically different endophytes develop in culture, free of their host, 'Chlorochytrium' cells retain a stalk characteristic of 'Codiolum'. Kornmann (1964) thus proposed that phenotypic responses, dictated by the different nature of the two hosts, accounted for the differences in morphology of 'Codiolum' and 'Chlorochytrium', *i.e.* the morphologically distinct endophytes represent the sporophytic phase of the same organism.

'Chlorochytrium' / 'Codiolum' phases are not, however, restricted to *Acrosiphonia* and *Spongomorpha*. Representatives of the genera *Urospora*, *Ulothrix* and *Monostroma* (present in southwestern British Columbia) also possess a unicellular sporophytic phase (Kornmann 1961b, 1962b, 1963) that develops similarly to the *Acrosiphonia*- and *Spongomorpha*-related 'Codiolum' phase (hence all are members of the Codiolales *sensu* van den Hoek *et al.* 1995). Although the

past culture studies suggest that 'Chlorochytrium inclusum' and 'Codiolum petrocelidis' are likely to be a part of the heteromorphic life cycle of *Acrosiphonia*, verification is needed for which genera are most closely related to the endophytes present within an area.

The focus of my thesis is on understanding the complex life history of *Acrosiphonia* in southern British Columbia. It is comprised of two major studies: one utilising molecular work to determine whether endophytes present in an area are an alternate life history phase of *Acrosiphonia*, and the other employing field sampling techniques to investigate the natural dynamics of *Acrosiphonia* along the coast of British Columbia .

The initial objective of my research was to establish the identity of 'Chlorochytrium' and 'Codiolum' cells found in *Mazzaella splendens* (Setchell & Gardner) Fredericq blades and in the crust 'Petrocelis franciscana' Setchell & Gardner [= tetrasporophytic phase of *Mastocarpus papillatus* (Agardh) Kützing], respectively from Vancouver Island and Burrard Inlet. Rather than culturing the endophytes (in the past fraught with poor survival rates and the inability to reproduce natural conditions resulting in morphological or reproductive ambiguities in plants), molecular studies were chosen to investigate whether the endophytes and *Acrosiphonia* constitute alternating phases in the life history of a single alga. The study in Chapter 1 uses DNA sequence data from the ITS regions of the nuclear ribosomal DNA cistron to provide a phylogenetic comparison of the endophytes, 'Chlorochytrium' and 'Codiolum', and free-living genera of the Codiolales found in the region. Pairwise sequence comparisons and a proposed phylogeny of relationships among endophytes and free-living Codiolales genera establish that the endophytes are more closely associated with *Acrosiphonia* than with any of the other genera, *i.e.* the endophytes are identified as the sporophytic phase of *Acrosiphonia*. In addition, the study supports Kornmann's hypothesis that 'Chlorochytrium' and 'Codiolum' are alternate phenotypes of the sporophyte of a single *Acrosiphonia* species.

Chapters 2 to 4 focus on understanding the natural dynamics of the life history of *Acrosiphonia* elucidated in Chapter 1. More specifically, gametophyte / sporophyte and host / endophyte associations, as well as the timing of life history events in nature are investigated at three different sites in southern British Columbia: Burrard Inlet (Brockton Point) 49° 34' N, 123° 10' W in Vancouver on mainland British Columbia, and Sooke (Whiffin Spit) 47° 42' N, 123° 48' W and Bamfield (Prasiola Point) 48° 49' N, 125° 10' W on Vancouver Island (Fig. 0.3). The sites selected vary in environmental factors such as wave exposure (Burrard Inlet is relatively sheltered, Bamfield wave-exposed and Sooke of intermediate wave-exposure),

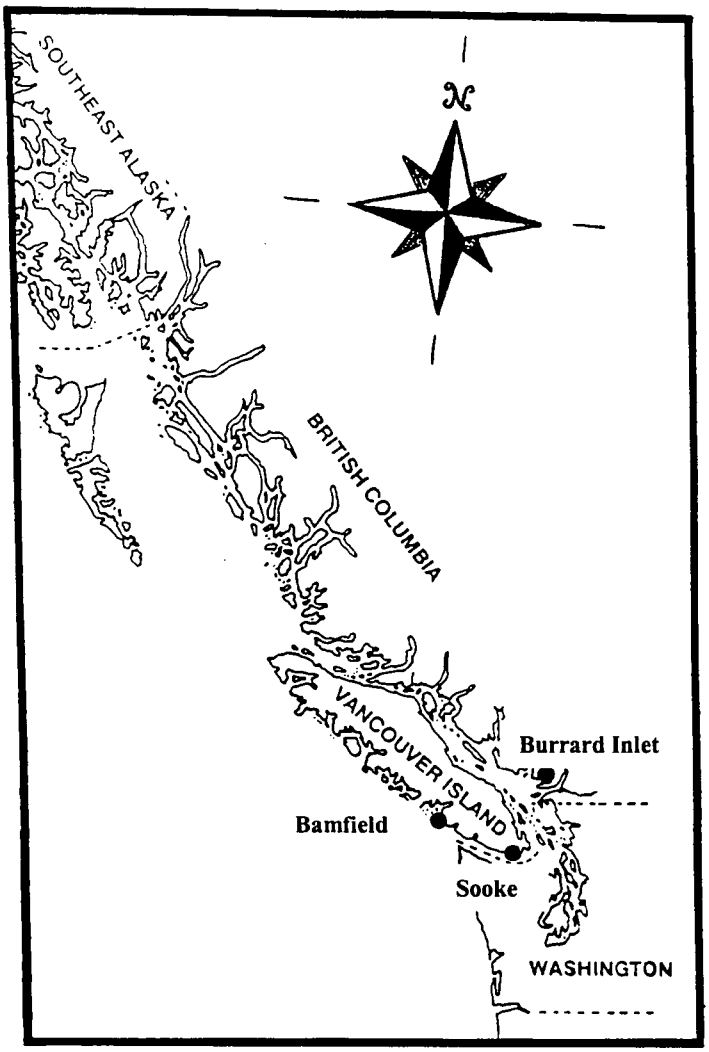

Figure 0.2 Location of the three study sites.

occurrence of summer low tides (afternoons at Burrard Inlet; early morning on Vancouver Island sites), salinity, temperature and precipitation. Burrard Inlet also differs in that human disturbance is common and can be quite destructive, *e.g.* people overturning rocks and digging in the sand for marine organisms. The effect of environmental conditions on the natural dynamics of *Acrosiphonia* is addressed.

Acrosiphonia was found growing abundantly on boulders or epiphytic on *Fucus* spp. or seagrass in the low to mid intertidal zone at all three sites, where it is the dominant filamentous green alga in spring and summer. At Burrard Inlet only one morphological species, *Acrosiphonia arcta* was present, whereas at Sooke and Bamfield the two morphological species, *A. arcta* and *A. coalita* comprised the majority of *Acrosiphonia* plants.

The algal communities of the three sites were observed to be dominated by *Fucus* spp. , *Ulva* spp., *Enteromorpha* spp., *Mastocarpus papillatus* and a number of filamentous red algae such as *Polysiphonia* spp. and *Microcladia* spp. in the mid intertidal zone and by *Mazzaella splendens* and kelps in the low intertidal zone. Generally a greater diversity of algal species were found at Sooke and Bamfield than at Burrard Inlet; species absent at Burrard Inlet included *Halosaccion glandiforme* (Gmelin) Ruprecht, *Mazzaella heterocarpa* (Postels & Ruprecht) Hommersand , *Schizymenia pacifica* (Kylin) Kylin, *Hedophyllum sessile* (Agardh) Setchell, *Cladophora* spp. and *Palmaria mollis* (Setchell & Gardner) van der Meer & Bird (= *Rhodymenia palmata* var. *mollis* Setchell & Gardner). *Constantinea subulifera* Setchell was common only at Burrard Inlet. *Urospora* sp., *Ulothrix* sp. and *Monostroma* sp. of the Codiolales were identified from Sooke and Bamfield. The encrusting algae, 'Petrocelis franciscana', *Hildenbrandia occidentalis* Setchell and *Ralfsia pacifica* Hollenberg coexisted throughout the intertidal zone at all three sites. 'Chlorochytrium inclusum' and 'Codiolum petrocelidis' had already been observed within *M. splendens* blades from Sooke and 'Petrocelis' crusts from Sooke and Burrard Inlet, respectively, prior to the field study. It was not known if the endophytes were present in other algae.

At the Sooke and Bamfield study sites the gastropods, *Thais* spp. and *Tectura* spp., and the chiton, *Katharina tunicata* Wood, were observed to be the most abundant invertebrates. Littorinid snails of the genus *Littorina* were found on *Mazzaella splendens* blades at Burrard Inlet and Bamfield. At Burrard Inlet the predominant invertebrates were the mussels, *Mytilus trossulus* Gould, and barnacles (the dominant species being *Balanus glandula* Darwin); sometimes, especially in the spring, so abundant that dense 'mats' of juvenile mussels and barnacles covered boulders. Limpets present at Burrard Inlet and Sooke were primarily *Tectura scutum* Rathke.

The studies detailed in Chapters 2 to 4 were conducted at the three sites described, Burrard Inlet, Sooke and Bamfield, and utilised field sampling techniques. The main objectives of Chapter 2 are to identify the filamentous gametophytes and endophytic sporophytes of

Acrosiphonia in nature (DNA sequences from Chapter 1 aid in the identification), and to establish the relationship of the life history phases, primarily the timing of life history events, in southwestern British Columbia. This is the first study to examine the relationship of *Acrosiphonia*'s sporophyte and gametophyte in nature. I also briefly speculate on the factors responsible for selection of this complex heteromorphic life history.

Host availability for colonisation and survival of *Acrosiphonia*'s sporophytic phase (the endophytes, 'Chlorochytrium' and 'Codiolum') is addressed in Chapter 3. The two red algal hosts, *Mazzaella splendens* and 'Petrocelis franciscana', were abundant at my three study sites, and thus seemed to represent ideal hosts for 'Chlorochytrium' and 'Codiolum', respectively. Seasonal abundance of *M. splendens* and 'Petrocelis' was determined (no study has examined seasonality of 'Petrocelis' on boulder strewn shores), and 'Chlorochytrium' and 'Codiolum' survival, as well as endophyte duration in host, assessed in relation to availability of the hosts.

Chapter 4 investigates the degree of specificity of the host / endophyte relationship for *Acrosiphonia*'s sporophytic phase. All foliose Rhodophyte species and crustose species found in the intertidal zone at Burrard Inlet, Sooke and Bamfield were examined to compare endophyte densities. A number of mechanisms and strategies are discussed for the relatively low host specificity, but differential colonisation of hosts, observed in this study. These include the role of structural and chemical characters of the host, and a bet-hedging strategy for *Acrosiphonia*'s sporophytic life history phase.

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CHAPTER 1

IDENTIFICATION OF 'CHLOROCHYTRIUM' AND 'CODIOLUM' AS THE ALTERNATE PHASE OF *ACROSIPHONIA* USING ITS1 AND ITS2 RIBOSOMAL DNA SEQUENCE DATA

INTRODUCTION

The morphologically different endophytes, 'Chlorochytrium inclusum' and 'Codiolum petrocelidis' from southwestern British Columbia were suspected to be the sporophytic alternate life history phases of free-living gametophytic algae in the Codiolales (*sensu* van den Hoek *et al.* 1995). In this study DNA sequence data were used to investigate whether these endophytes are the sporophytes of species belonging to *Acrosiphonia*.

Evidence for the implication of 'Chlorochytrium' and 'Codiolum' cells in the life histories of *Acrosiphonia* and *Spongomorpha* was established through culture studies conducted largely in the 1960s and 1970s. For example, in Europe, Kornmann (1961a, 1964) demonstrated that both 'C. inclusum' and 'C. petrocelidis' give rise to *Spongomorpha lanosa* (Roth.) Kützing [= *Spongomorpha aeruginosa* (L.) Hoek] in culture. This, and the similar appearance of the two endophyte types free-living in culture, led Kornmann (1964) to propose that the two morphologically distinct endophytes are alternate phenotypes of the sporophyte of a single *Acrosiphonia* or *Spongomorpha* species, *i.e.* the morphological variation is solely attributed to the different nature of the two hosts. Jónsson (1959b, 1962, 1966) showed zoospores of 'C. inclusum' produce *S. lanosa*, and zoospores of 'C. petrocelidis' give rise to *Acrosiphonia arcta*. *Spongomorpha* is not found in the northeast Pacific, but workers along the coasts of Washington and California have shown that 'C. inclusum' produces *A. arcta* in culture (Chihara 1969, Hudson 1974), while 'C. petrocelidis' gives rise to both *A. arcta* and *Acrosiphonia coalita* (Hollenberg 1958, Fan 1959, Hudson 1974). Similarly, in Japan, culture work by Miyaji and Kurogi (1976) and Miyaji (1984, 1996) revealed that 'C. inclusum' and 'C. petrocelidis' may be the sporophytic phase of several *Acrosiphonia* species. Figure 1.1 provides a summary of the still rather confused picture of the associations between *Acrosiphonia* / *Spongomorpha* and 'Codiolum' / 'Chlorochytrium' that have been established through culture studies.

The 'Codiolum' phases are not, however, restricted to *Acrosiphonia* and *Spongomorpha*. Representatives of the genera *Urospora*, *Ulothrix* and *Monostroma* also possess a unicellular sporophytic 'Codiolum' phase (Kornmann 1961b, 1962, 1963) that develops similarly to the

Acrosiphonia and *Spongomorpha* related 'Codiolum' phase. *Codiolum gregarium* Braun, a free-living macroscopic (1-2mm at maturity) unicell, has been associated with the life history of *Urospora* (Hanic, 1965). Kornmann (1973) even suggested a new class, Codiolophyceae, to unite all genera with a 'Codiolum' phase. Although it is possible that one or more of the 'Codiolum' phases of *Urospora*, *Ulothrix* and *Monostroma* invades red algal crusts and/or blades, none is known to exist endophytically.

While the culture work described suggests that 'Codiolum petrocelidis' and 'Chlorochytrium inclusum' are likely to be a part of the life cycle of one or more species of *Acrosiphonia*, it would be helpful to establish genotypic relatedness via phylogenetic analysis to verify which genera are most closely related to the endophytes present within an area.

Being multinucleate, the genus *Acrosiphonia* is clearly separated from uninucleate *Spongomorpha* (Wille, 1900). However, there is a lack of agreement on species boundaries within *Acrosiphonia*. Criteria commonly used to distinguish species of *Acrosiphonia* are primarily vegetative characteristics such as diameter of the filaments, length to width ratios of the cells, presence or absence of simple or compound hooks, branching patterns of the plants, shape of the tip cells and number of fertile gametangia cells in a series (Setchell and Gardner 1920, Scagel 1966). Many of these characteristics are very variable when environmental factors such as light intensity, day length and temperature differ (Kornmann 1965, 1970, Hudson 1974), making species delimitation difficult.

Due to the taxonomic uncertainty within the genus *Acrosiphonia*, the focus of this study is to establish whether the endophytes found in the northwestern Pacific region are more closely related to *Acrosiphonia* than to one of the other genera of Codiolales found in the region, rather than to predict which species of *Acrosiphonia* most likely represent the alternate phase of the endophyte life cycle. A phylogenetic comparison of the sporophytic endophytes 'Chlorochytrium' and 'Codiolum' and the gametophytes in the Codiolales, using sequences from the ITS regions of the nuclear ribosomal DNA cistron is provided in this study. Unlike the 18S, 5.8S and 26S ribosomal genes, which have been strongly conserved through time, the ITS regions (ITS1 and ITS2) provide a level of sequence variation suitable for comparisons at the genus and species level in algae (Peters *et al.* 1997, Burkhardt and Peters 1998, Blomster *et al.* 1999, Serrão *et al.* 1999) and more specifically in green algae (Bakker *et al.* 1995, van Oppen *et al.* 1993, Pillmann *et al.* 1997). Based on pairwise sequence comparisons and a proposed phylogeny of relationships among endophytes and free-living genera within the

1999, Serrão *et al.* 1999) and more specifically in green algae (Bakker *et al.* 1995, van Oppen *et al.* 1993, Pillmann *et al.* 1997). Based on pairwise sequence comparisons and a proposed phylogeny of relationships among endophytes and free-living genera within the

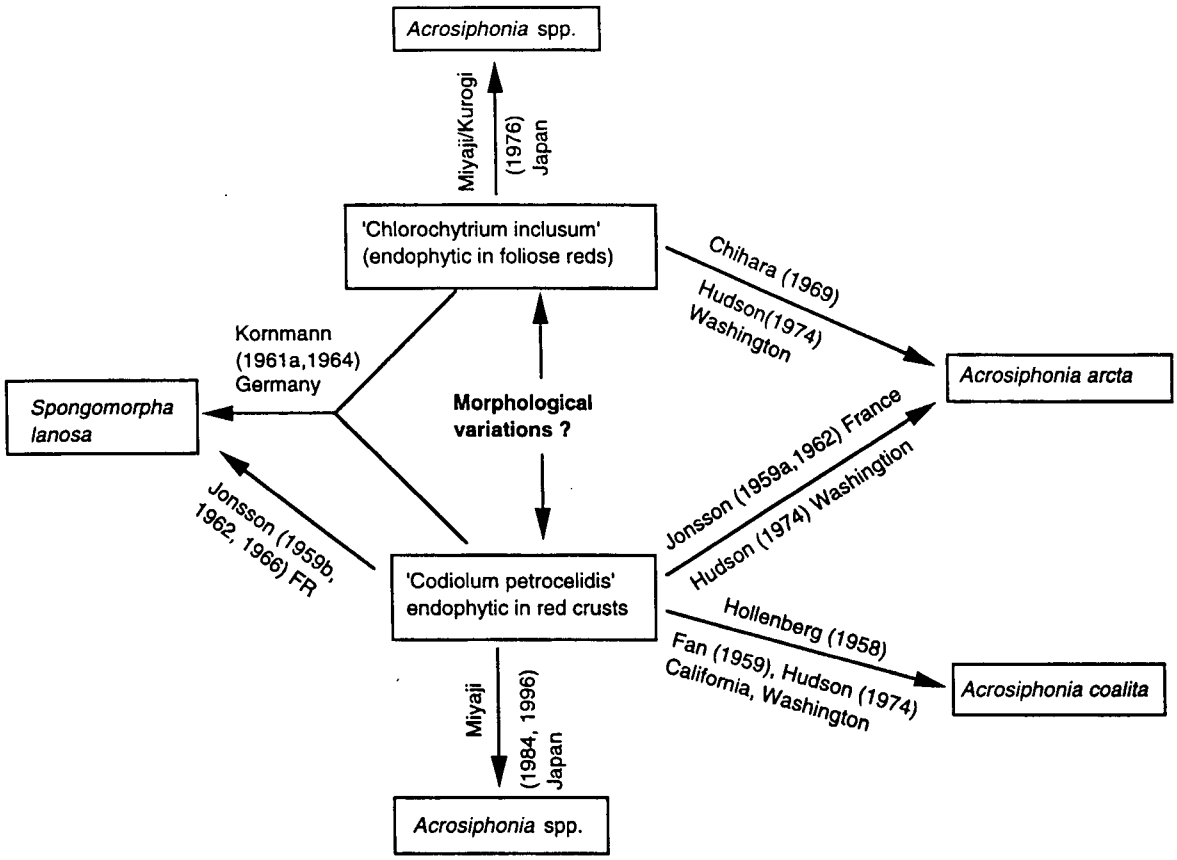

Figure 1.1 A summary of results from culture studies involving 'Chlorochytrium inclusum' and 'Codiolum petrocelidis'. Note that the northeast Pacific workers found 'Codiolum' to give rise to more than one *Acrosiphonia* species, whereas 'Chlorochytrium' only produced *A. arcta*. Both endophytes were shown to give rise to *Spongomorpha lanosa* in Europe.

Codiolales, this study 1) identifies the two morphologically different endophytes as the sporophytic phase of the free-living green algal genus *Acrosiphonia*, (2) supports Kornmann's hypothesis that 'Chlorochytrium' and 'Codiolum' are alternate phenotypes of the sporophyte of a

MATERIALS AND METHODS

Sample collection and DNA extraction. *Mazzaella splendens* blades, 'Petrocelis' crust and *Acrosiphonia* plants were collected at Sooke (Whiffin Spit) and Bamfield (Prasiola Point) on Vancouver Island, and Burrard Inlet (Brockton Point) in Vancouver on mainland British Columbia. *Acrosiphonia* species were collected in the spring of 1997 and 1998 when the plants had just appeared and were relatively free of epiphytes. Initial attempts to extract DNA from *Acrosiphonia* collected in the summer were unsuccessful due to interference from heavy epiphytisation. Van Oppen *et al.* (1993) and van Oppen (1995) used only cultured material, considering *Acrosiphonia* from the field too difficult for use in extractions. *Mazzaella splendens* blades and 'Petrocelis' crusts were collected in the summer of 1997 and 1998, when endophyte density tended to be highest (A. V. Sussmann, personal observation). In total, six 'Codiolum' isolates (two from each site), 21 'Chlorochytrium' isolates (representatives from all sites), and 34 *Acrosiphonia* isolates (also from all sites) were sequenced in this study. All 'Codiolum' isolates sequenced were from 1997 collections, whereas 'Chlorochytrium' and *Acrosiphonia* isolates from 1997 (3 and 19, respectively) and 1998 (18 and 14, respectively) were sequenced. Two additional sequences were obtained from *Acrosiphonia* isolates which were collected at Amalga Harbor 58° 29' N, 134° 47' W, Alaska and St. Lawrence Island 63° 48' N, 171° 43' W, Alaska by S. C. Lindstrom. Isolates from a particular site were assigned a number, and collection location is indicated by an abbreviated site name (Table 1.1).

Algal host specimens were refrigerated for less than 24 h, and examined for the presence of endophytic green algal unicells. *Acrosiphonia* specimens (approximately 0.1 g from each plant) collected in 1997 were lyophilised in 1.5 mL microcentrifuge tubes. The DNA from *Acrosiphonia* plants which were collected in 1998 was obtained from herbarium specimens, and the Alaskan isolates had been dried and stored in silica gel. Different protocols were initially used to extract DNA from 'Chlorochytrium' and 'Codiolum' cells due to the different nature of the host-endophyte associations. *Acrosiphonia* DNA extraction followed the protocol used for 'Codiolum'.

'Chlorochytrium' cells. In 1997 between 30 and 100 'Chlorochytrium' cells were dissected from the *Mazzaella splendens* blades and transferred to 100 µL of 5% Chelex® 100 [Biotechnology grade, Bio-Rad™, Hercules, CA (Walsh *et al.* 1991)] w/v in dH_2O with a drawn-out pipette. Centrifugation at 16,500 x g for ca. 30 s pelleted the cells. The pellet was ground

TABLE 1.1 *Species names, collection sites and abbreviations and GenBank accession numbers for isolate sequences in phylogenetic analysis. Note: host names for endophytes are given. Isolates with ambiguities have not been included.*

Species	Host	Collection site[a]	GenBank accession no.[b]
'Chlorochytrium inclusum'	*Mazzaella splendens*	Sooke, B.C. (Sk1)	AF019256
	M. splendens	Sooke, B.C. (Sk3/Sk4)	AF047681
	M. splendens	Sooke, B.C. (Sk5)	AF161595
	M. splendens	Sooke, B.C. Sk6-Sk9))	AF019255
	M. splendens	Bamfield, B.C. (Bm1)	AF047681
	M. splendens	Burrard Inlet, B.C. (BI1-BI4)	AF019255
	M. splendens	Bamfield, B.C. (Bm2-Bm4)	AF019255
'Codiolum petrocelidis'	'Petrocelis franciscana'	Sooke, B.C. (Sk1/Sk2)	AF019256
	'P. franciscana'	Bamfield, B.C. (Bm1)	AF047682
	'P. franciscana'	Bamfield, B.C. (Bm2)	AF047681
	'P. franciscana'	Burrard Inlet, B.C. (BI1/BI2)	AF019256
Acrosiphonia coalita		Monterey Bay, California (MB)	
		Friday Harbor, Washington (FH)	
		Bamfield, B.C. (Bm1-Bm5)	AF047682
		Sooke, B.C. (Sk1-Sk3)	AF047682
Acrosiphonia arcta		Helgoland, Germany (Hg)	
		Roscoff, Brittany, France (Ro)	
		Faeroe Islands (Fa)	
		Halifax, Nova Scotia, Canada (Ha)	
		Grotta, Iceland (Ic)	
		Disko Island, Greenland (Di)	
		Palmer Station, Antarctica (PS)	
		King George Isl., Antarctica (KG)	
		Friday Harbor, Washington (FH)	
		Puerto Williams, Southern Chile (SC)	
		Burrard Inlet, B.C. (BI1-BI10)	AF019256
		Sooke, B.C. (Sk1-Sk6)	AF019256
		Sooke, B.C. (Sk7-Sk9)	AF047681
		Bamfield, B.C. (Bm1-Bm4)	AF047681
Acrosiphonia sonderi		Helgoland, Germany (Hg)	
		Disko Island, Greenland (Di)	
Acrosiphonia sp.		Amalga Harbor, Alaska (AH)	AF019255
		St. Lawrence Island, Bering Sea (BS)	AF019255

TABLE 1.1 continued

Acrosiphonia sp.	Sooke, B.C. (Sk)	AF161595
Spongomorpha lanosa	Grotta, Iceland (Ic)	
Urospora penicilliformis (Roth) Areschoug	Helgoland, Germany (Hg)	
	Disko Island, Greenland (Di)	
	Otago Peninsula, New Zealand (Ot)	
	Dunedin, New Zealand (Du)	
	King George Isl., Antarctica (KG)	
Ulothrix implexa (Kützing) Kützing	King George Isl., Antarctica (KG)	
Monostroma arcticum sensu Bliding	Spitsbergen (Sp)	

[a] Abbreviated site names and isolate numbers assigned for a particular site appear in brackets.

[b] Sequences without accession numbers are all from van Oppen (1995); all those with accession numbers are new sequences.

with a sterile pestle for approximately 10 s or until green colouration indicated lysis. Samples were heated to 95-100° C for 15 min and frozen at -20° C. The samples were thawed, centrifuged again at 16,500 x g for 5 min, and then a 1:2 dilution of Chelex extract in dH_2O was used as template for PCR reactions. In 1998 the DNA extraction protocol used for 'Codiolum' was successfully applied to 'Chlorochytrium'. Several 10-25 mm^2 pieces of *M. splendens* colonised by 'Chlorochytrium' were lyophilised and ground up. Grinding endophyte and host tissue was considerably faster than dissecting individual 'Chlorochytrium' cells from *M. splendens*. The PCR product was, however, always inferior, resulting in weaker signals from sequencing results.

***'Codiolum' cells and* Acrosiphonia.** Due to the obvious difficulty in dissecting 'Codiolum' cells from filaments of the 'Petrocelis' crust, host tissue colonised by endophytic cells was used for DNA extraction. Small patches (approximately 25 mm^2) of crust were lyophilised and then ground to a fine consistency in sterile sand with a Teflon pestle-drill assembly, and 800 µL CTAB extraction buffer [0.7 M NaCl, 1% CTAB(w/v), 50 mM Tris (8.0 pH), 10 mM EDTA, 1% β-mercaptoethanol (v/v)] added. Samples were then incubated at 65° C for 1 h and extracted twice with one volume of chloroform. The DNA was precipitated with isopropanol and centrifuged at 16,500 x g for 10 min. The DNA pellet was washed with ice-cold 70% ethanol,

re-suspended in 100-150 μL TE buffer and frozen at -20° C. A 1:2 dilution was performed prior to PCR amplification. For a more detailed account of this protocol refer to McDermott *et al.* (1989).

PCR amplification and sequencing. PCR amplifications were performed in a Perkin-Elmer™ DNA thermal cycler. The initial denaturation step (95° C for 2 min) was followed by 30 cycles with a reaction profile of 1 min at 95° C, 1 min at 48° C, and 45 s at 72° C. With each successive cycle the polymerisation step was increased by 4 s. The terminal extension step was 7 min at 72° C. For each amplification 25 μL reactions were comprised of 12.5 μL of the diluted genomic DNA, 0.2 mM dNTP mixture, 2.5 μL 10x reaction buffer (Ultratherm™, Bio/Can Scientific Inc., Canada), 1.5 mM $MgCl_2$, 2.5 μL 50% glycerol, 0.9 μL dH_2O, 5 units Taq Polymerase (Ultratherm) and 0.5 μM of each primer. The primer pairs, ITS1 and ITS4, or ITS5 and ITS4 (White *et al.* 1990) were used to amplify the ITS1 and ITS2 regions and the intervening 5.8S rDNA, as well as a short segment of the 26S gene. PCR products were analysed by electrophoresis of 5 μL of reaction mixture through 1-2% agarose and visualised by ethidium bromide staining.

To target only *Acrosiphonia* sequences, an *Acrosiphonia*-specific primer, MO1, designed by van Oppen *et al.* (1993) was used in a second PCR reaction for 'Chlorochytrium' and 'Codiolum' DNA. This primer is located within the ITS1 region, 45 bp from the 5' end (Fig.1.2), and has the following sequence (5' to 3'): AGGTCTGACTTGTTGGGCGGC. In the case of the 'Chlorochytrium' DNA, the primary PCR product was diluted 100 fold and then re-amplified with the primer pair MO1 and ITS4 using the above reaction conditions but with an annealing temperature of 53° C. For the 'Codiolum' DNA, bands of appropriate length (as compared with *Acrosiphonia arcta*) were purified from the primary PCR products by electrophoresis through 1.5% agarose and ethidium bromide staining. DNA was recovered from agarose gel blocks by incubation at 65° C for 15 min in 100 μL TE. After a 100 fold dilution, re-amplification with primers MO1 and ITS4 was performed as above with an annealing temperature of 56° C.

PCR products used as templates for sequencing in 1997 were gel purified according to Qian and Wilkinson (1991). In 1998 purification of PCR products was accomplished with an ethanol spin precipitation following Applied Biosystems Inc. instructions. The DNA nucleotide sequence of the ITS1 (partial sequence due to MO1's position), ITS2 and 5.8S gene regions was determined using the AmpliTaq® Dye Terminator Cycle Sequencing kit and the 373 DNA

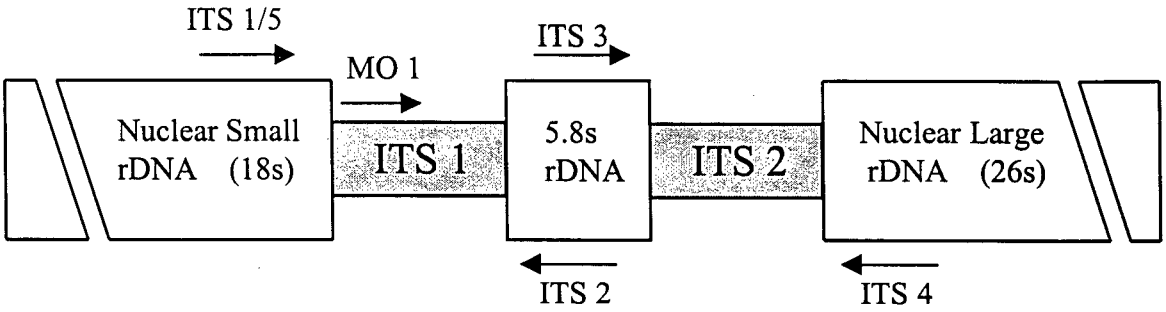

Figure 1.2 Map showing primer positions within nuclear ribosomal DNA cistron. Primer pairs ITS1(5) / ITS4 and ITS2 / ITS3 were used to amplify and sequence the ITS1, 5.8s, ITS2 and a short segment of the 26s gene. The pair MO1 / ITS4 was used to isolate endophyte from host DNA.

Sequencer (Applied Biosystems Inc.). The primer pairs MO1 / ITS4 (for the endophyte sequences), ITS1 / ITS4 (for *Acrosiphonia* sequences) and ITS2 / ITS3 (Fig. 1.2), were used so that this entire region could be sequenced from both ends.

Alignment and analysis of sequences. Clustal V (Higgins *et al.* 1992) was used to align the 'Chlorochytrium', 'Codiolum' and *Acrosiphonia* sequences to a previously published alignment of *Acrosiphonia* species from other geographic locations, and species of *Urospora*, *Ulothrix* and *Monostroma* (van Oppen, 1995; see Table 1.1 for species names and collection sites). Three kinds of phylogenetic analyses were conducted using PAUP* (Swofford, 1998). *Monostroma* was shown as the outgroup because it differed from the other species at about 44% of the sites in the alignment (without correcting for multiple hits). Other pairs of sequences in the data set all differed at fewer than 10% of the sites in the alignment. Ten heuristic searches, employing the tree bisection reconnection option and random addition of all 21 taxa, were used to find the most likely reconstructions from the data set; a branch and bound search was used to find all of the most parsimonious trees and a neighbour joining tree was found using the Jukes-Cantor model of nucleotide substitution (Jukes and Cantor, 1969). Two bootstrapping approaches were used to estimate sampling variation and support for branches: 1000 neighbour

joining bootstrap replicates with a Jukes-Cantor model of nucleotide substitution and 200 bootstrap replicates using parsimony. The number of parsimony bootstrap replicates was restricted, because the program ran out of memory when a particular bootstrap sample missed the few informative sites and generated a very high number of possible trees.

To evaluate the relative signal to noise ratio in the data set, the length of the most parsimonious tree found using parsimony was compared to the tree-length distribution generated from searches based on random permutations of the data by using the permutation-tailed-probability (PTP) test option in PAUP*.

RESULTS

DNA extraction and PCR amplification. In Coleman and Goff's (1991) review of available molecular techniques performed on algal material, it is evident that no one method for the isolation of DNA consistently yields good results. In this study the extraction and amplification of DNA from unicellular endophytes obtained from the field was especially difficult and required the use of two different methods for the two morphologically distinct endophytes. The use of 30-100 'Chlorochytrium' cells (from *Mazzaella splendens* blades) as starting material for the Chelex extraction protocol was successful for Sooke isolates. Difficulty in obtaining genomic DNA from 'Chlorochytrium' cells from Bamfield *M. splendens* blades, however, resulted in sequencing only one isolate from this site. This and the fact that dissection of individual cells from host tissue was very time consuming, prompted the use of the CTAB / chloroform extraction method for 'Chlorochytrium' / *M. splendens* tissue (25-75 'Chlorochytrium' cells present). Nonetheless, obtaining adequate amounts of DNA from PCR amplification continued to be somewhat problematic. The CTAB/chloroform extraction method was successful for 'Codiolum'/ 'Petrocelis' samples (200-1000 'Codiolum' cells present) from all sites. Goff and Moon (1993) have shown that, with red algae, it is possible to amplify DNA from as little as 2 μL of extraction supernatant of spores, which translates to ca. two spores. However, attempts to amplify DNA from a single unicell of 'Chlorochytrium' or 'Codiolum' were unsuccessful.

'Chlorochytrium' and 'Codiolum' DNA was successfully PCR amplified along with host DNA, resulting in two fragments of estimated size 0.5 kb and 0.75 kb (Fig. 1.3a). The host DNA was suspected to be the 0.75 kb fragment based on ITS sizes in other red algae (Steane *et al.*

1991). Upon agarose purification of the 'Codiolum' fragments and 100-fold dilution of 'Chlorochytrium' primary PCR product, re-amplification with the *Acrosiphonia* specific primer MO1, resulted in a single bright band of fragments for each endophyte (Fig.1.3b).

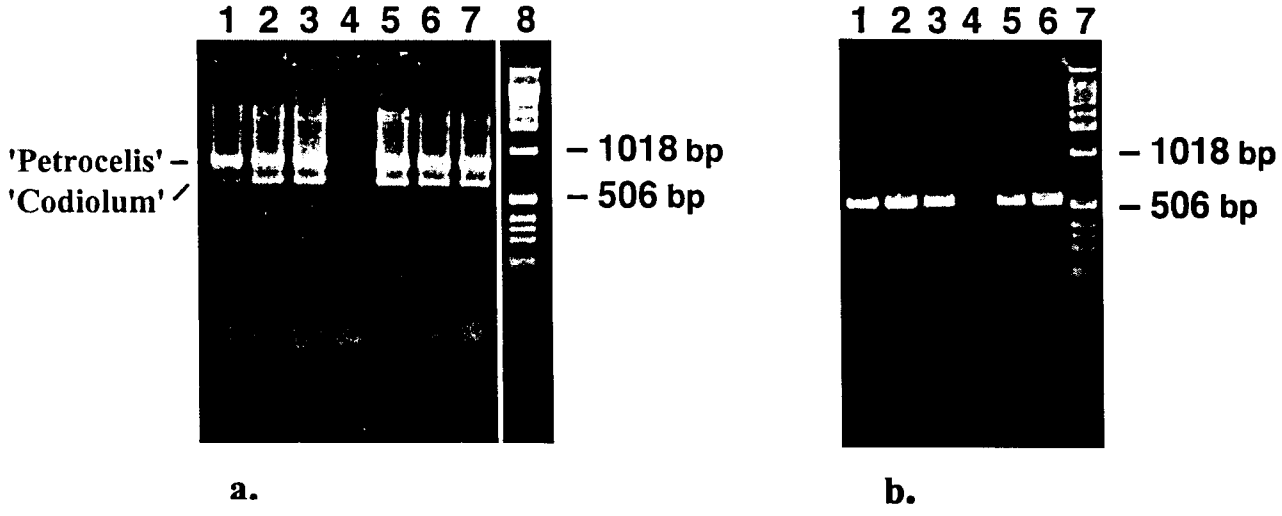

Figure 1.3a. Ethidium bromide stained agarose gel of PCR amplified ITS1, 5.8S, ITS2 and partial 26S regions from 'Petrocelis' crust colonised by 'Codiolum petrocelidis'. Note that the 0.5 kb fragment is 'Codiolum' and the 0.75 kb fragment is 'Petrocelis'. **b.** Agarose gel of 'Chlorochytrium' and 'Codiolum' PCR product re-amplified with MO1 and ITS4 primers.

DNA Sequences. The total length of the ITS1, ITS2 and 5.8S regions consists of 471 nucleotides, and it begins 66 bp from the 5' end of the ITS1 region and ends 23 bp into the 26S gene. All sequence variation was found in the ITS1 and 2 regions; 5.8S and the partial 26S sequences were identical. All unambiguous endophyte sequences (22 of the 27 isolates) were 100% identical to either *Acrosiphonia arcta*, *Acrosiphonia coalita* or *Acrosiphonia* sp. from Alaska and from Sooke. Among these 'Codiolum', 'Chlorochytrium' and *Acrosiphonia* isolates five distinct genotypes were found (Fig. 1.4). Six variable sites are present, with the exception

of the two isolates, *A.* sp. from Sooke and its 'Chlorochytrium' match, which show additional variable sites. The 'Codiolum' isolates separated into three genotypes, identical to *A. arcta* (genotype groups 1 and 2) and *A. coalita* (genotype group 3) isolates. Among the 'Chlorochytrium' isolates, four distinct genotypes were present: (1) identical to *A. arcta* isolates (genotype groups 1 and 2) (2) identical to *A.* sp. isolates from Alaska (genotype group 4) and (3) one 'Chlorochytrium' isolate from Sooke ('Chlorochytrium' Sk5) corresponded to a unique *Acrosiphonia* isolate from Sooke (genotype group 5). This sequence is interesting in that four new nucleotide changes were found within the ITS1 and ITS2 regions and one new change within the 5.8S region. An ambiguous 'Chlorochytrium' sequence obtained from a Sooke isolate (Sk2) did not match any other sequences. It should be noted here that on occasion, among 'Chlorochytrium', heterogeneity was evident at some of the sites, *i.e.* chromatograms sometimes revealed strong signal from a mixture of two nucleotides at the same position. This was the case for position 6 of the 'Chlorochytrium' Sk2 sequence and position 303 of the Bm5, Bm6, Sk10, Sk11 sequences, where both T and C were indicated (Fig. 1.4). Among the *Acrosiphonia* isolates sequenced there were two genotypes for *A. arcta* (varying by 1 bp at position 48), a single *A. coalita* genotype (different from *A. arcta* by nucleotide changes occurring at four or five of the six variable sites) and two additional genotypes for unknown *Acrosiphonia* species from Alaska and Sooke. The isolates from Alaska differed from *A. coalita* by 4 bp and from *A. arcta* by 2 or 3 bp. The *Acrosiphonia* sp. isolate from Sooke was quite distinct for this geographical region, characterised by five new nucleotide changes. It did, however, show close similarity to an *Acrosiphonia* isolate from Helgoland (10 of the 11 informative sites were identical; data not shown).

The largest amount of variation among endophyte and *Acrosiphonia* sequences was seen at Sooke, where all five genotypes were present. *A. coalita* isolates sequenced from Bamfield and Sooke were 100% identical to isolates from Friday Harbor (Washington) and Monterey Bay (California), and *A. arcta* isolates from Burrard Inlet and six of the nine *A. arcta* isolates from Sooke were identical to isolates from Friday Harbor.

Phylogenetic reconstructions. Maximum likelihood analysis of the rDNA sequences from the endophytes and from 40 isolates belonging to the Codiolales (*Acrosiphonia*, *Spongomorpha*, *Urospora*, *Ulothrix* and *Monostroma* species) found a single reconstruction

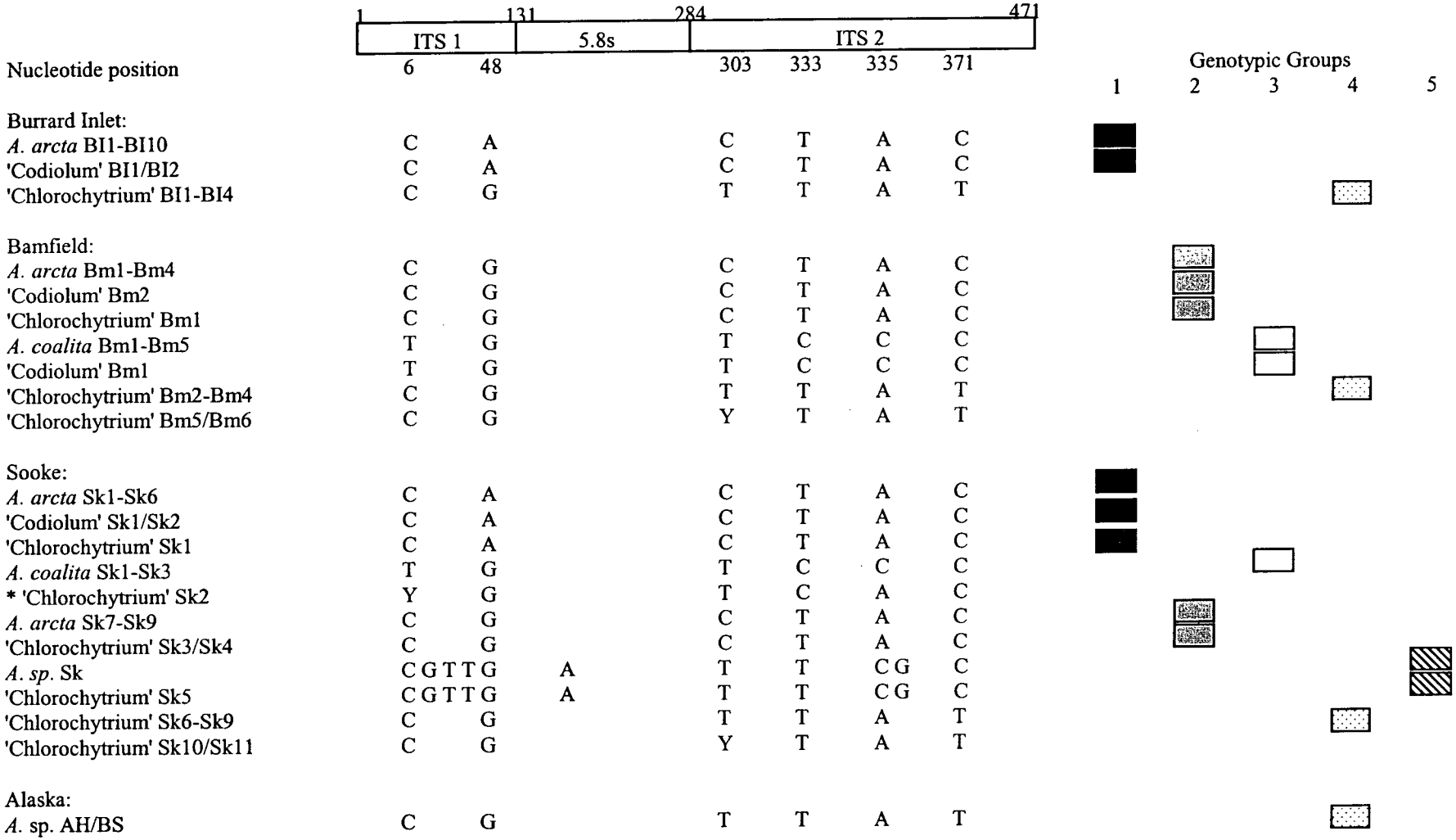

Figure 1.4 Sequence variation among 'Chlorochytrium', 'Codiolum' and *Acrosiphonia* isolates. Six variable sites occur within the ITS regions of four of the five distinct genotype groups. Group 1 differs from group 2 by a single bp change at position 48; group 3 varies from groups 1 and 2 by 4 or 5 bp changes; group 4 differs from group 2 by 2 bp changes and group 5 shows 5 new variable sites in the ITS. The * 'Chlorochytrium' is the only endophyte isolate which does not match an *Acrosiphonia* isolate.

(shown in Fig. 1.5). Six equally parsimonious trees were found with the branch and bound search, and a neighbour joining tree was found using the Jukes-Cantor model of nucleotide substitution (data not shown). The maximum likelihood, parsimony and neighbour joining reconstructions differed in the arrangement of the nearly identical species in the *Acrosiphonia* clade, for branches where bootstrap support was never more than 50%. All analyses, including the neighbour joining and parsimony bootstrap consensus, showed that the endophytes cluster in the *Acrosiphonia* clade with high bootstrap support (80% - 91%). All of the reconstructions also show *Spongomorpha* as the sister genus to the *Acrosiphonia* clade. In the maximum likelihood trees *Ulothrix* appears as the sister taxon to *Urospora* (Fig. 1.5). In the parsimony and distance trees, however, *Ulothrix* diverges after *Monostroma*, at the base of the tree.

A significant difference ($p \leq 0.01$, PTP test) was found between trees generated using parsimony and those generated from random permutations of the data, suggesting that phylogenetic signal in the data could be distinguished from random noise. A PTP test conducted using only sequences within the *Acrosiphonia* clade also showed a significant difference ($p \leq 0.01$), suggesting that although resolution within the clade was limited, there was phylogenetic signal in the data.

The strong support for the *Acrosiphonia* clade, and its low internal resolution [except for the high bootstrap support relating *Acrosiphonia* sp. (Sk) to *A. sonderi* (Kützing) Kornmann (Hg)], was due to the low sequence variation between the taxa included in this clade (Figs. 1.4 and 1.5). The relatively long branch lengths between the *Acrosiphonia* / *Spongomorpha* clade and the clade including *Urospora* and *Ulothrix* species (Fig. 1.5) clearly show that the endophytes sequenced are unambiguously more closely associated with the former group.

DISCUSSION

The 100% identity of nucleotide sequences of endophyte / *Acrosiphonia* pairs provides conclusive evidence that the unicells present within *Mazzaella splendens* and 'Petrocelis franciscana' in southern British Columbia are sporophytes of *Acrosiphonia*. Each unambiguous 471-bp sequence of the ITS regions of the 'Chlorochytrium' and 'Codiolum' isolates corresponded to the sequence of at least one of the *Acrosiphonia* isolates (Fig. 1.4). Phylogenetic clustering of the Codiolales (genera which are reported to exhibit 'Codiolum' phases in their life histories) establishes the fact that 'Chlorochytrium' and 'Codiolum',

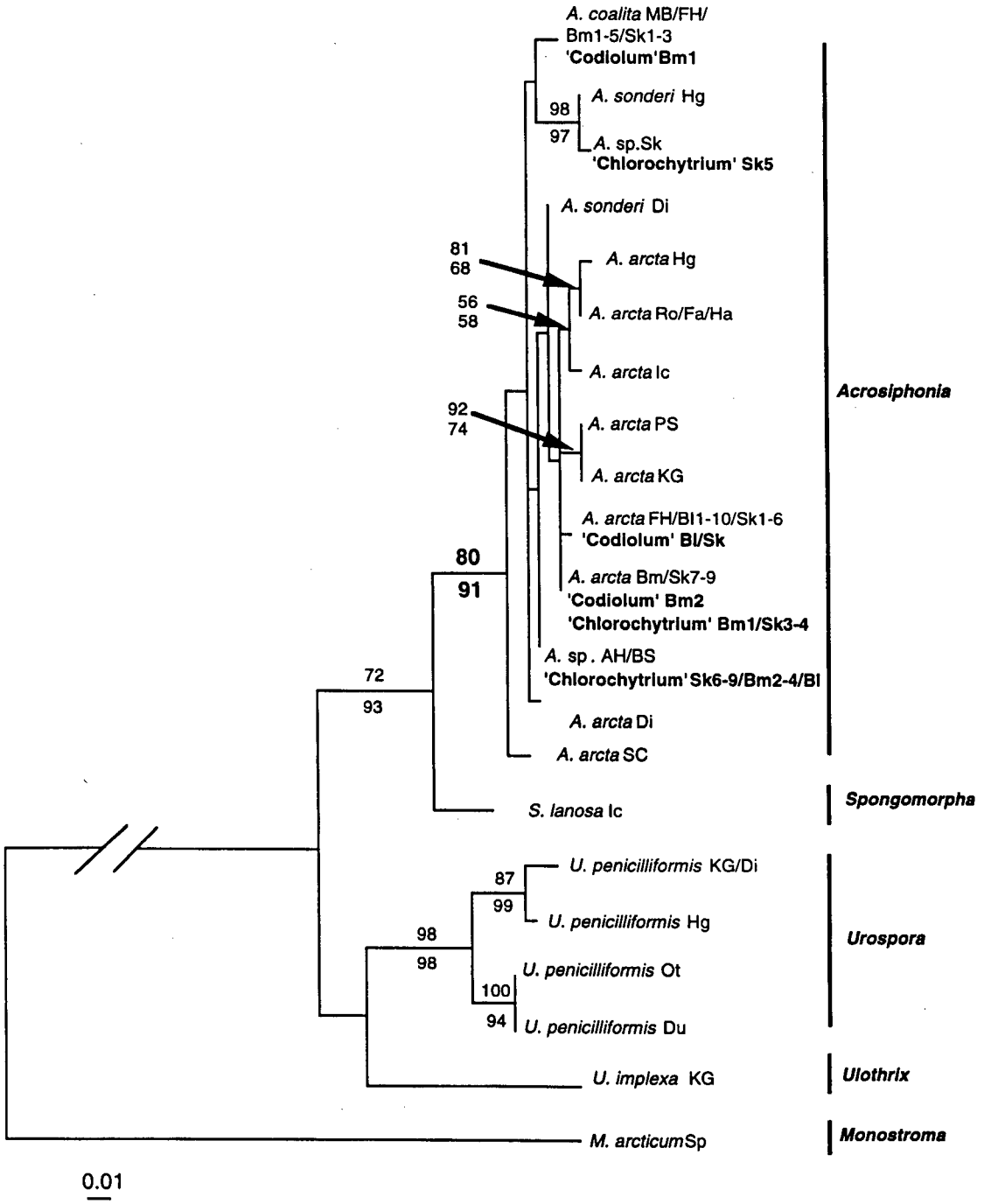

Figure 1.5 Maximum likelihood tree from ITS1 and ITS2 sequences. *Monostroma arcticum* was used as the outgroup. Numbers above the branches are neighbour-joining bootstrap values (1000 replicates) using a Jukes-Cantor model of nucleotide substitution; numbers below the branches are bootstrap proportions using parsimony (200 replicates). The branch to *Monostroma* is not proportional to its length of 0.765. Otherwise, the scale bar on the tree indicates the maximum likelihood distance. See Table 1.1 for detailed information on species, collection sites and abbreviations.

sequenced from southern British Columbia, were not the sporophytes of *Urospora, Ulothrix* or *Monostroma* (*Spongomorpha* is not present in the northeast Pacific). All 'Chlorochytrium' and 'Codiolum' isolates were placed within the clade including all of the *Acrosiphonia* isolates sequenced (Fig. 1.5). All other genera, including *Spongomorpha*, whose 'Codiolum' and 'Chlorochytrium' phases are reported to be endophytic in *Haemescharia hennedyi* (Harvey) Vinogradova and Yakoleva and *Polyides rotundus* (Huds.) Greville, respectively (Kornmann 1964) were distinguished as separate well-resolved clades, separated by relatively long branch lengths (Fig. 1.5) compared to those within the *Acrosiphonia* clade.

Based on characteristics described by Hudson (1974), only two morphological species of *Acrosiphonia*, *A. coalita* and *A. arcta*, are believed to be present along the British Columbia coast. The ITS sequence data revealed five genotypes present among *Acrosiphonia* and endophyte isolates from British Columbia. Low bootstrap support and limited resolution within the *Acrosiphonia* clade of the Codiolales phylogenetic tree do not, however, allow interpretation of (1) whether it is likely that the *Acrosiphonia* "species" sequenced represent separate biological entities or a single biological entity; or (2) whether the rate of nucleotide substitution in the ITS region is insufficient to resolve relationships at this scale of relatedness. Nonetheless, *A. arcta* and *A. coalita* do not appear to be conspecific based on the fact that consistent ITS sequence variation is correlated with morphological differences. Both *A. arcta* and *A. coalita* are present at Friday Harbor, Sooke and Bamfield; at each site their ITS sequences consistently differ at four or five nucleotide positions (Fig. 1.5). In the ITS trees the *A. arcta* isolates do not form a monophyletic clade, but this probably results from lack of information in the ITS sequence data, and possibly reflects problems with species delimitation.

Acrosiphonia specimens from Alaska were difficult to identify based on morphology. Yet, shared similarities (absence of hooked branchlets, filament cell diameter and habit) with UBC herbarium specimens identified as *Acrosiphonia hystrix* (Strömfelt) Jonaason, and ITS sequences that differed from all other *Acrosiphonia*, suggest consideration as a separate species. The 'Chlorochytrium' isolates from Sooke, Bamfield and Burrard Inlet with identical sequences to *A. sp.* (Alaska) would then represent its sporophyte (by implication, *Acrosiphonia* with the Alaskan ITS genotype should also be present at all three study sites). Likewise, a single *Acrosiphonia* sample obtained higher up in the intertidal zone at Sooke (on a massive boulder) is characterised by a different genotype. It also cannot be identified morphologically (and attempts to collect and sequence more isolates of this genotype were unsuccessful), but warrants

consideration as a separate species, since its sequence is almost identical to that of an isolate of *Acrosiphonia sonderi* (Fig. 1.5). *A. sonderi*, type locality Helgoland, has not been reported for the Pacific, and sexual reproduction has never been observed for this "species" (Kornmann 1962). This points to the need for resolution within the *Acrosiphonia* species complex. Further studies, perhaps taking a multiallelic population level approach, might clarify the breeding patterns of these algae, and also better link the endophytes with individual *Acrosiphonia* species.

Regardless of how many *Acrosiphonia* species are recognised, the ITS sequence data suggest that the morphologically distinct endophytes, 'Chlorochytrium' and 'Codiolum', can be produced by the same *Acrosiphonia* species, *i.e. A. arcta*'s sporophyte can colonise both blade and crust. 'Chlorochytrium' and 'Codiolum' isolates from Bamfield and from Sooke revealed 100% sequence identity to an *A. arcta* genotype (Fig. 1.4). This supports Kornmann's (1964) long-standing hypothesis that 'Chlorochytrium' and 'Codiolum' represent alternate phenotypes of the same sporophyte. However, no 'Chlorochytrium' / 'Codiolum' matches with *A. arcta* were found at Burrard Inlet, and only 'Codiolum' sequences were found to share a genotype with *A. coalita*.

Among 13 unambiguous sequences of 'Chlorochytrium' isolates, 9 were 100% identical to *A.* sp. from Alaska, three matched *A. arcta* sequences and one was 100% identical to the Sooke *A.* sp. None were found to share a genotype with *A. coalita*. This is consistent with previous culture studies where only 'Chlorochytrium' gave rise to *A. arcta* (Fig. 1.1), indicating that *Acrosiphonia coalita* may produce sporophytes which only colonise red algal crusts. More generally, the sporophytes of *Acrosiphonia* species may exhibit variable host specificity.

And yet, consideration of sequence ambiguity provides an alternative interpretation. The sequence ambiguity found in a number of 'Chlorochytrium' isolates, suggests that a heterogeneous group of endophytes may have been present in single DNA extractions (recall that DNA extracts consisted of patches of 'Petrocelis' and *Mazzaella splendens* colonised by > 30 cells). Theoretically the unicells in one DNA sample could have been derived from different sources, *i.e.* sporophytes from different *Acrosiphonia* species or from different genera could cohabit the same host [Miyaji and Kurogi (1976) reported obtaining three species of *Acrosiphonia* in culture from 'Chlorochytrium' endophytic in the same host plant from Japan] or some endophytes may represent independent species not associated with the life history of another alga. However, since all sequence ambiguity was found at the six variable sites, and fell

within the range of variation observed among *Acrosiphonia* sequences, it seems evident that all unicells were most closely related to *Acrosiphonia*. Verification of this assumption would require screening of multiple clones from each isolate. Regardless, in instances of unambiguous sequences where possibly only the dominant sequence was amplified, it can definitely be concluded that this predominant endophyte type was related to *Acrosiphonia*.

With regard to the implication that a mixture of endophytic sporophytes from different *Acrosiphonia* species may have comprised some DNA extracts, *i.e.* ambiguous sites suggested neither sequence was dominant, 'Chlorochytrium' (Sk2) may represent such a sample. This is the 'Chlorochytrium' isolate from Sooke which did not show 100% sequence identity with any *Acrosiphonia* sequences. Based on the sites which show heterogeneity, this isolate would consist of sporophytic endophytes from *A. coalita*, *A. arcta* and/or *A.* sp. (Alaska). Screening of multiple clones from this isolate could then substantiate whether 'Chlorochytrium' is in fact associated with *A. coalita*, contrary to culture study results. In California, where *A. arcta* is not known to occur, 'Chlorochytrium' cells were reported in *Mazzaella splendens* blades (Smith, 1944), suggesting *A. coalita*'s sporophyte may not be confined to crusts, at least not in that region. Ultimately, though, it seems that utilisation of a sequence region more variable than the ITS1 and ITS2, and DNA extracts from individuals (rather than > 30cells), would enable better understanding of host / endophyte relationships.

The fact that DNA sequences of 'Chlorochytrium' isolates share the genotype of an *Acrosiphonia* "species" from Alaska is particularly interesting, because no *Acrosiphonia* specimens collected from southern British Columbia in both 1997 and 1998 matched the Alaskan isolate sequences. Absence of the genotype in *Acrosiphonia* isolates sequenced may simply be due to small sample size or low numbers of the genotype present in *Acrosiphonia* populations. The high proportion of 'Chlorochytrium' isolates with 100% identity to *A.* sp. (Alaska), as well as the failure to detect 1) 'Codiolum' with an *A.* sp. match and 2) 'Chlorochytrium' at Burrard Inlet with the *Acrosiphonia* genotype found at Burrard Inlet, is also surprising. Perhaps the sporophyte of *Acrosiphonia* characterised by the Alaskan ITS genotype is host specific (colonising only red algal blades and so not associated with 'Codiolum'), and is more successful than the sporophyte of *A. arcta* in colonising *Mazzaella splendens*. On the other hand, the failure to detect 'Codiolum' sharing a genotype with *A.* sp. (Alaska) may simply be a function of low numbers of isolates sequenced. Again, a study taking a population genetics

approach or examining a more variable sequence region and more isolates, might shed light on these intriguing questions.

As already mentioned, *Acrosiphonia* ITS sequence variation was found to be highest at Sooke, where all five genotypes are present (two genotypes present at Bamfield and only one at Burrard Inlet). This is interesting because the three geographic sites represent environmentally different areas with regard to factors such as wave exposure and salinity. It is perhaps not surprising to find low sequence variation at Burrard Inlet because this is the most wave-sheltered and homogenous site. *Acrosiphonia* isolates from Sooke were collected from a number of different habitats, *i.e.* from different heights in the intertidal zone. Possibly, the degree of habitat heterogeneity present at a site may play a role (among other factors) in determining the degree of infrageneric variation present.

DNA amplification and sequencing have proven to be powerful tools in the identification of unicellular green algae endophytic in red algal hosts along southwestern British Columbia, Canada. The stalked unicell present in the crust 'Petrocelis franciscana' and the spherical unicell within the blade *Mazzaella splendens*, constitute the alternate phase of the free-living filamentous alga *Acrosiphonia*. Regardless of how many *Acrosiphonia* species are recognised, the ITS sequence data has shown that the morphologically different endophytes, 'Chlorochytrium' and 'Codiolum', can be produced by the same *Acrosiphonia* species, *A. arcta*. The sporophyte of *A. coalita*, on the other hand, has not been shown to be associated with 'Chlorochytrium', suggesting it is host specific, only colonising crusts. These results support previous culture studies (Hollenberg 1958, Fan 1959, Chihara 1969, Hudson 1974) and Kornmann's hypothesis that, at least in some cases (*e.g. A. arcta*), 'Chlorochytrium' and 'Codiolum' represent alternate phenotypes of the same sporophyte.

Chapter 2 will examine *Acrosiphonia*'s alternation of a filamentous gametophyte with a unicellular, endophytic sporophyte in nature.

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CHAPTER 2

LIFE HISTORY OF *ACROSIPHONIA* IN SOUTHWESTERN BRITISH COLUMBIA: A BASELINE STUDY

INTRODUCTION

Extensive studies, primarily in the 1960s and 1970s (Hollenberg 1958, Fan 1959, Jónsson 1959a 1959b 1962 1963 1966 1970, Kornmann 1961 1964 1970a 1972, Chihara 1969, Hudson 1974, Miyaji and Kurogi 1976, Miyaji 1984 1996) examined the *Acrosiphonia - Spongomorpha* complex in culture with the aim of elucidating the life cycle. However, no study to date has focused on the natural dynamics of *Acrosiphonia*, *i.e.* seasonal abundance and reproductive phenology of the filamentous gametophyte and endophytic sporophyte in nature. Lack of fundamental ecological studies may, in part, be due to the microscopic nature of the endophytic sporophytes of *Acrosiphonia*. This study establishes the relationship of *Acrosiphonia* and 'Chlorochytrium' and 'Codiolum' in the field, as well as the timing of life history events.

Acrosiphonia's alternation of heteromorphic generations (Fig. 0.1, Thesis Introduction) established through the culture studies noted and the molecular study in Chapter 1 is not unique among algae. Heteromorphic life histories occur in all three major groups of macroalgae (Rhodophyta, Chlorophyta, Phaeophyta). Examples include the green algal genera already discussed in Chapter 1 (*Urospora*, *Ulothrix* and *Monostroma*), as well as the red algae, *Mastocarpus*, *Porphyra* and *Schizymenia* (DeCew *et al.* 1992) and the brown algae, *Petalonia*, *Scytosiphon* and all kelps. Each of these algae have two separate ecologically distinct phases which are so dissimilar in morphology that they had been classified as separate species (with the exception of kelp gametophytes) and, in some cases, placed in separate families or orders. This is the case for *Acrosiphonia*, whose 'Chlorochytrium' and 'Codiolum' sporophytic phases were initially considered independent genera and placed, separate from *Acrosiphonia*, in the Order Chlorococcales. These algae also exhibit a seasonal or annual upright phase and a non-upright crustose, boring, endophytic or epilithic phase (some kelp sporophytes are perennial; not all genera display obligatory alternations of generations). Kelp gametophytes have recently been found as endophytes in foliose and filamentous red algae (Garbary *et al.* 1999a, b).

Description of* Acrosiphonia*'s phases in nature. Much taxonomic work has been carried out for both separation of *Acrosiphonia* and *Spongomorpha* (Agardh 1846, Wille 1900, Collins 1909, Kornmann 1965 1970b, Scagel 1966, Jónsson 1991) and the delineation of *Acrosiphonia* and *Spongomorpha* species (Setchell & Gardner 1920, Kornmann 1962, Jónsson 1962 1971, Scagel 1966, Hudson 1974). *Spongomorpha* is absent from the northeast Pacific, as are the following *Acrosiphonia* species: *A. grandis* and *A. sonderi* reported from Europe and *A. spiralis* Sakai and *A. heterocladia* Sakai reported from Japan. *Acrosiphonia hystrix* and *A. duriuscula* (Ruprecht) Collins , although present in the northeast Pacific, have not been reported south of Alaska. Scagel (1966) described five species of *Acrosiphonia* for northern Washington State and British Columbia. However, Hudson (1974) reported variability of morphological characters on which the species were based when plants were subjected to different environmental conditions in the laboratory. Furthermore, a number of plants collected in the Puget Sound region, Washington State, of different "species", were found to intergrade in all diagnostic characters. Nonetheless, allowing for broad range of variation, Hudson selected characters conservatively to establish two morphological species, *A. arcta* and *A. coalita* (she actually chose to accept *A. spinescens* in favour of *A. arcta*, because the true characteristics of *A. arcta* type specimens were unclear to her, but *A. arcta* has taxonomic priority). Her conclusions were based on 1) specimens collected from the Puget Sound Region, Washington State 2) descriptions of the type specimens of *A. coalita*, *A. mertensii* (Ruprecht) Setchell & Gardner, *A. saxatilis* (Ruprecht) Collins, *A. arcta* and *A. spinescens* and 3) specimens collected and identified by Collins, Setchell and Gardner, Scagel and Jónsson. For a more detailed account of the taxonomic work on *Acrosiphonia* refer to Hudson (1974). *Acrosiphonia coalita* appears to be present only in the northeast Pacific, whereas *A. arcta* has been recorded in both the northern and southern hemispheres, including the northeast Pacific, the Northwest Atlantic, the North Sea, Greenland, southern Chile and Antarctica. Seasonal abundance and reproductive phenology of *Acrosiphonia* are poorly understood.

Similarly, no studies have described 'Chlorochytrium' seasonal abundance and reproductive phenology in the northeast Pacific. 'Chlorochytrium inclusum' was first described by Kjellman (1883) in *Sarcophyllis arctica* Kjellman, an Arctic, foliose, red algal species. In its vegetative state 'Chlorochytrium' was observed to be almost spherical, 80-100 μm in diameter, attaining diameters of up to 275 μm when mature. Other researchers reported 'Chlorochytrium'

with maximum diameter of 75-100 μm from a number of foliose red algae from the Pacific northeast (Chihara 1969 from *Schizymenia*, Setchell & Gardner 1920 from *Weeksia*, *Constantinea* and *Mazzaella*), Europe (Jónsson 1959b 1962 1966, Kornmann 1961, 1964 from *Polyides* and *Dilsea*) and Japan (Miyaji and Kurogi, 1976 from *Farlowia*). The alternate phase of some of these has been identified in culture studies. For example, 'Chlorochytrium' from Europe gave rise to *Spongomorpha lanosa*, while 'Chlorochytrium' from Japan gave rise to *Acrosiphonia spiralis*, *A. heterocladia* and *A. duriuscula*. Two studies on Arctic and European 'C. inclusum' have found it to be fertile in winter (Kjellman 1883, Kornmann 1964).

Seasonal abundance and reproductive phenology of 'Codiolum' is also poorly understood, and is based on a handful of studies (none from the northeast Pacific). 'Codiolum petrocelidis' was first described by Kuckuck (1894) from specimens growing in the crust *Haemescharia hennedyi* in Helgoland, Germany. These cells were reported as having a vesicle portion 125-175 μm in length and 25-50 μm in diameter and a colourless stalk. A number of workers (Setchell & Gardner 1920, Jónsson 1958, Kornmann 1972) have noted polymorphism (with regard to vesicle and stalk size and shape) exhibited by 'Codiolum' cells. This phenomenon undoubtedly interferes with the ability to follow cell growth and development from specimens collected in the field. The filamentous plants that developed in culture from the zoospores of 'C. petrocelidis' (collected from *H. hennedyi* in Helgoland) were identified as *Spongomorpha lanosa* (Kornmann 1961, 1964). Other culture studies suggested that 'Codiolum' represents a phase in the life history of a number of *Acrosiphonia* species. Jónsson's (1959a, 1962) 'Codiolum' found within 'Petrocelis cruenta' J. Agardh [= tetrasporophytic phase *of Mastocarpus stellatus* (Stackhouse) Guiry] collected at Roscoff, France, were associated with *Acrosiphonia arcta*. In Japan Miyaji (1984) cultured zoospores of 'Codiolum', endophytic in 'Petrocelis' crusts of *Mastocarpus pacificus* (Kjellman) Perestenko, which developed into filamentous gametophytes identified as *A. saxatilis* [which may be synonymous with *A. arcta* according to Hudson (1974)]. On the Pacific Coast of North America 'Codiolum' reported in 'Petrocelis franciscana' gave rise to both *A. arcta* and *A. coalita* in culture (Hollenberg 1958, Fan 1959, Hudson 1974).

The only study to examine 'Codiolum' development in the field is that of Kornmann (1961). Based on cell sizes and fertility of specimens collected in the winter of 1963 and spring and summer of 1964, Kornmann suggested the youngest stages found within 'Petrocelis' in July pass the following winter in a vegetative state and become fertile the subsequent winter. In the northeast Pacific 'Codiolum' has been collected from 'Petrocelis franciscana' from February to

November (Setchell & Gardner 1920, Hollenberg 1958, Fan 1959, Hudson 1974, Dethier 1987), and Dethier (1987) noted dense colonisation in summer in Washington State.

Environmental requirements for gametophyte and sporophyte. Hanic (1965) established that 'Codiolum gregarium', the macroscopic (1-2 mm in length at maturity), free-living 'Codiolum' phase of *Urospora* is abundant and fertile in the winter; fertility being induced by cold temperatures. He observed that *Urospora* is present year round, but dies off substantially in the summer months. It is 'Codiolum gregarium' which presumably better survives high summer temperatures and desiccation (L. Hanic, pers. comm.). Experimental studies have not identified environmental requirements for the production and growth of *Acrosiphonia*'s sporophytes, 'Chlorochytrium' and 'Codiolum'. Nonetheless, Hudson (1974) and Miyaji (1996) have shown that *Acrosiphonia arcta* and *A. spiralis*, respectively, did not produce zoospores at temperatures ≥15 °C (but did at 5 °C and 10 °C). With regard to *Acrosiphonia*'s gametophyte, Hudson (1974) found that growth of plants in culture was inhibited at 15-20 °C, and long-day (16 hrs light:8 hrs dark photoregime) conditions were required for the germination of *Acrosiphonia* filaments. This implies seasonality of *Acrosiphonia*, and is supported by the fact plants have never been collected in the winter. Collections from the northeast Pacific (Hollenberg 1958, Fan 1959, Hudson 1974), Europe (Jónsson 1959a 1964 1986, Kornmann 1970a 1970b) and Japan (Miyaji 1996) were all carried out in spring and summer.

Urospora's suspected bet-hedging strategy, whereby filamentous and unicellular 'Codiolum' phases are adapted to a seasonally variable environment, may also apply to *Acrosiphonia*'s life history. *Acrosiphonia*'s endophytic sporophytes, 'Chlorochytrium' and 'Codiolum', are expected to demonstrate greater tolerance of extreme values of environmental factors such as temperature and photoperiod (keeping in mind that fertility of the endophytes may be triggered by cold temperatures in winter) than the filamentous gametophyte.

The purpose of the study detailed in this chapter is to establish the timing of events, illustrated in Figure 2.1, in *Acrosiphonia*'s life history in southwestern British Columbia, and to gain insights into the factors responsible for selection of this complex heteromorphic life history. More specifically, this study 1) identifies the filamentous gametophytes and endophytic sporophytes of *Acrosiphonia* in nature, 2) illustrates the seasonal abundance of *Acrosiphonia* (gametophyte) and its sporophytes, 'Codiolum' and 'Chlorochytrium', 3) determines

reproductive phenology of both phases and 4) examines endophyte cell size as an indication of age and growth in the field.

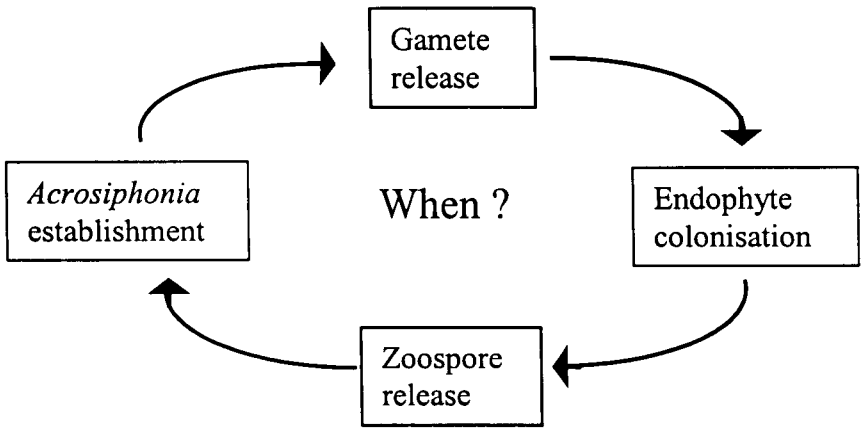

Figure 2.1. Establishing the timing of events of *Acrosiphonia*'s life history in nature.

MATERIALS AND METHODS

Field sampling. Field studies conducted over two years, 1996-1998, at Sooke, Bamfield and Burrard Inlet monitored seasonal abundance and reproductive phenology of *Acrosiphonia*, 'Chlorochytrium' and 'Codiolum'. Notable changes in morphology, colour and / or size of organisms were recorded. Due to difficulties with sampling all three sites within a 3-5 day low tide series, Bamfield was sampled much less frequently than Sooke and Burrard Inlet.

Acrosiphonia. Green filamentous algae were collected from the low to high intertidal zone at all three sites for identification to genus (*Acrosiphonia*) and to species, based on morphological / microscopic characters. Position in the intertidal zone and substratum type were noted. Sampling for percent cover and reproductive phenology of *Acrosiphonia* commenced with the appearance of plants in the spring of 1997.

Every 2-6 weeks (depending on whether *Acrosiphonia* was present) a 20 m transect line was placed parallel to the water line in the *Acrosiphonia* zone. The same area was sampled on

consecutive sampling dates and ranged from 0.1 to 2.0 m above zero tidal level (Canadian Chart Datum). A random number table was used to generate 30 sites for placement of a 20 cm x 20 cm quadrat along the transect. Quadrats were always centred on the transect line. Monofilament divided the quadrat into 400 squares. Where both *A. arcta* and *A. coalita* were recognisable, percent cover was noted for each species. When *Acrosiphonia* appeared in new areas, additional transects were established. This was the case at Sooke where transects needed to be placed in three different intertidal zones (as described in Thesis Introduction) to ensure representative sampling of the entire area colonised by *Acrosiphonia*. At Bamfield one additional transect was sampled in an adjacent area at the same tidal height.

To determine reproductive phenology for *Acrosiphonia arcta* and *A. coalita* at Sooke, Bamfield and Burrard Inlet, 10 thalli of each species were haphazardly collected from each *Acrosiphonia* zone on each sampling date, and brought back to the laboratory for microscopic examination. Microscopic characters used for distinguishing between the two species were useful in confirming the identity of individual plants, especially in juveniles. These thalli were then prepared as herbarium specimens for future reference, and some were later used for DNA extraction (Chapter 1).

'Chlorochytrium'. 'Chlorochytrium' cells were identified in *Mazzaella splendens*, the predominant foliose red alga at the three study sites. Seasonal abundance of 'Chlorochytrium' at Sooke, Bamfield and Burrard Inlet was based on density estimates of cells within *M. splendens* blades. A 12 m transect line for determining *M. splendens* density using the point-quarter method (see Chapter 3, Methods), was also used for the random collection of 32 blades per transect. Blades were collected, alternating between the largest and smallest (> 5 cm) blade per genet, from each quadrant of 8 points along the transect. Collection occurred approximately monthly, except on sampling dates where tides were not low enough to expose *M. splendens* or scarcity precluded collecting 30 blades.

Mazzaella splendens blades from Sooke, Bamfield and Burrard Inlet were either examined immediately or kept in the refrigerator for 2-3 days. Drying of blades was not feasible, since shrunken 'Chlorochytrium' cells did not adequately rehydrate, and were difficult to locate and identify; frozen blades often deteriorated considerably.

Although larger cells of 'Chlorochytrium' (≥ 120 μm) could be seen in the field by holding thin blades of *Mazzaella splendens* that were not epiphytised up to the light, magnification was

usually required to detect the bright green endophytic cells. This often meant scraping off patches of bryozoans and / or diatom films from the *M. splendens* blades, especially late in the summer and fall. Furthermore, both sides of the blade required inspection under the dissecting microscope, since the endophyte was found to reside in both the upper and lower cortex region.

For blades with < 100 cells (or where single cells were scattered), individual cells could be counted, but densely colonised patches of endophytes needed to be estimated. Estimates were carried out by counting groups of approximately 100 cells (groups were based on the area occupied by 100 cells). Estimates were checked by actually counting five groups of 100 estimated cells from a blade where ≥ 1000 cells and ≥ 10,000 cells were estimated. This procedure was replicated five times. Absolute numbers of 'Chlorochytrium' cells were recorded per blade, as were frequency and range of cell size and location of endophyte patches. To calculate 'Chlorochytrium' densities cm^{-2}, *M. splendens* blades were photocopied and the surface area determined as a percentage of the weight of a sheet of paper of known area. 'Chlorochytrium' cells were dissected from several blades after each sampling trip to check for fertile cells, and to record variation in morphology. Cross-sectioned *Mazzaella* tissue enabled cells to be examined within their host.

'Codiolum'. 'Codiolum' cells were present in 'Petrocelis franciscana', a conspicuous red algal crust at all three study sites. Seasonal abundance of 'Codiolum' was determined by counting numbers of cells present in patches of 'Petrocelis' roughly every month. Thirty small patches of approximately 5 x 5 mm were randomly scraped by razor blade from 'Petrocelis' growing on boulders or consolidated rock within the low to high intertidal zone (from 0.1 to 5.1 m above zero tidal level, Canadian Chart Datum). About 10 patches were collected from each zone. 'Petrocelis' patches with 'Codiolum' could be kept in the refrigerator for a few days prior to examination. 'Codiolum' cells were detected by squashing the 5 x 5mm 'Petrocelis' patch onto a microscope slide. Density, 'Codiolum' cell size, polymorphism among cells and reproductive state were recorded. In the summer and fall when densities at Sooke and Burrard Inlet exceeded 10,000 cells cm^{-2}, it was necessary to estimate numbers present in the field of view under the 10x objective lens. This method of estimation was established by actually counting the cells visible in a field of view for 10 different fields of view for five individual crust patches, *i.e.* the average cell count per field of view was used for the estimation.

Data Analysis. *Acrosiphonia*, 'Chlorochytrium' and 'Codiolum' data sets are comprised of percent cover and density values (sample size ranged from 30 to 90 for *Acrosiphonia*; approximately 30 for 'Chlorochytrium' and 'Codiolum') for 15 - 23 sampling dates. Statistical analyses were performed using SPSS® 9.0 for Windows (1999). The Levene test showed that the assumption of homogeneous variances was violated. Although transformations reduced the Levene statistic, heterogeneity was not reduced to nonsignificant levels. Consequently, the data sets were also checked for normality, since the processes that produce non-normal distributions may also result in variance heterogeneity (Day & Quinn, 1989). The data satisfied the Kolmogorov-Smirnov Test for normality. Since standard non-parametric tests are inherently less powerful than parametric tests (Zar, 1996), and should not be used as a simple means to avoid the problem of unequal variances (Day & Quinn, 1989), ANOVAs (which are generally robust to variance heterogeneity, Zar 1996) were used in SPSS® 9.0 for Windows (1999). One-way ANOVAs and the Games-Howell *post hoc* test were performed on square-root transformed data to test for significant differences in *Acrosiphonia*, 'Chlorochytrium' and 'Codiolum' abundance over time. The Games-Howell test is more powerful than other *post hoc* tests for unequal variances (Games *et al.*, 1983), and is recommended when number of treatments is small and sample size ≥ 7 (Day & Quinn, 1989). It also does not require equal sample sizes, which is important for Bamfield 'Codiolum' data where sample size was variable (7-30) due to the occasional misidentification of 'Petrocelis' in the field.

RESULTS

Acrosiphonia *identification*. Two morphological species, *Acrosiphonia arcta* and *A. coalita* (Figs. 2.2, 2.3), comprise the majority of *Acrosiphonia* plants in southwestern British Columbia. Species identification was based on a number of morphological / microscopic characters studied by Hudson (1974) for plants in the Puget Sound region and commonly used by other workers (Kjellman 1893, Collins 1909, Setchell and Gardner 1920, Scagel 1966). Those criteria I deemed dependable for distinguishing between species are thallus morphology, filament diameter, hooked branchlet type and number of fertile cells in a branch (Table 2.1). The DNA sequences of the ITS regions of specimens identified as *A. arcta* and *A. coalita* showed

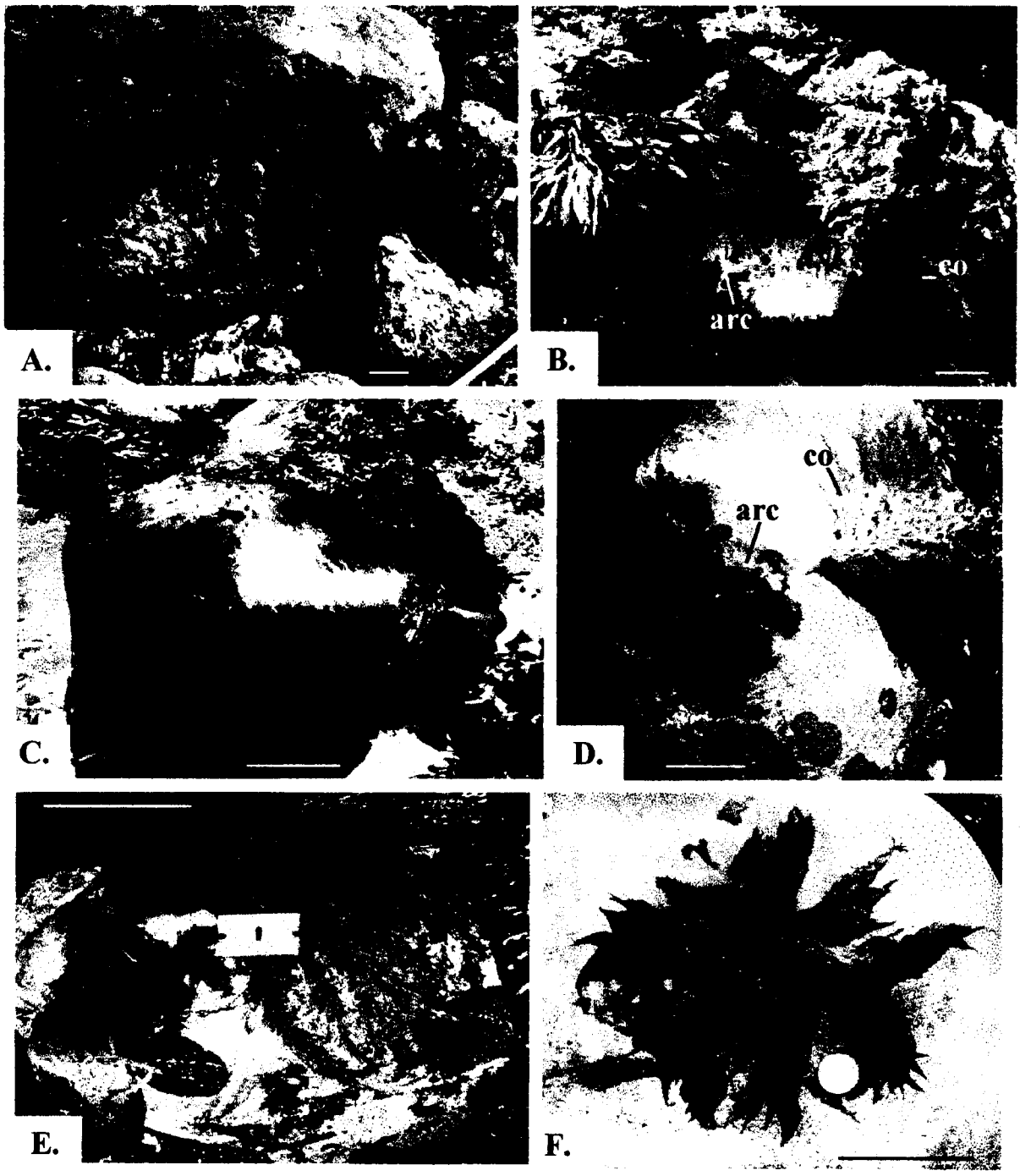

Figure 2.2 A. *Acrosiphonia arcta* at Burrard Inlet. **B**. *Acrosiphonia arcta* and *A. coalita* at Bamfield. **C. E**. *A. coalita* at Sooke. **F.** *A. arcta* at Sooke. **D**. *A. arcta* and *A. coalita* at Sooke. Scale bar is 5 cm. arc = *A. arcta* co = *A. coalita.*

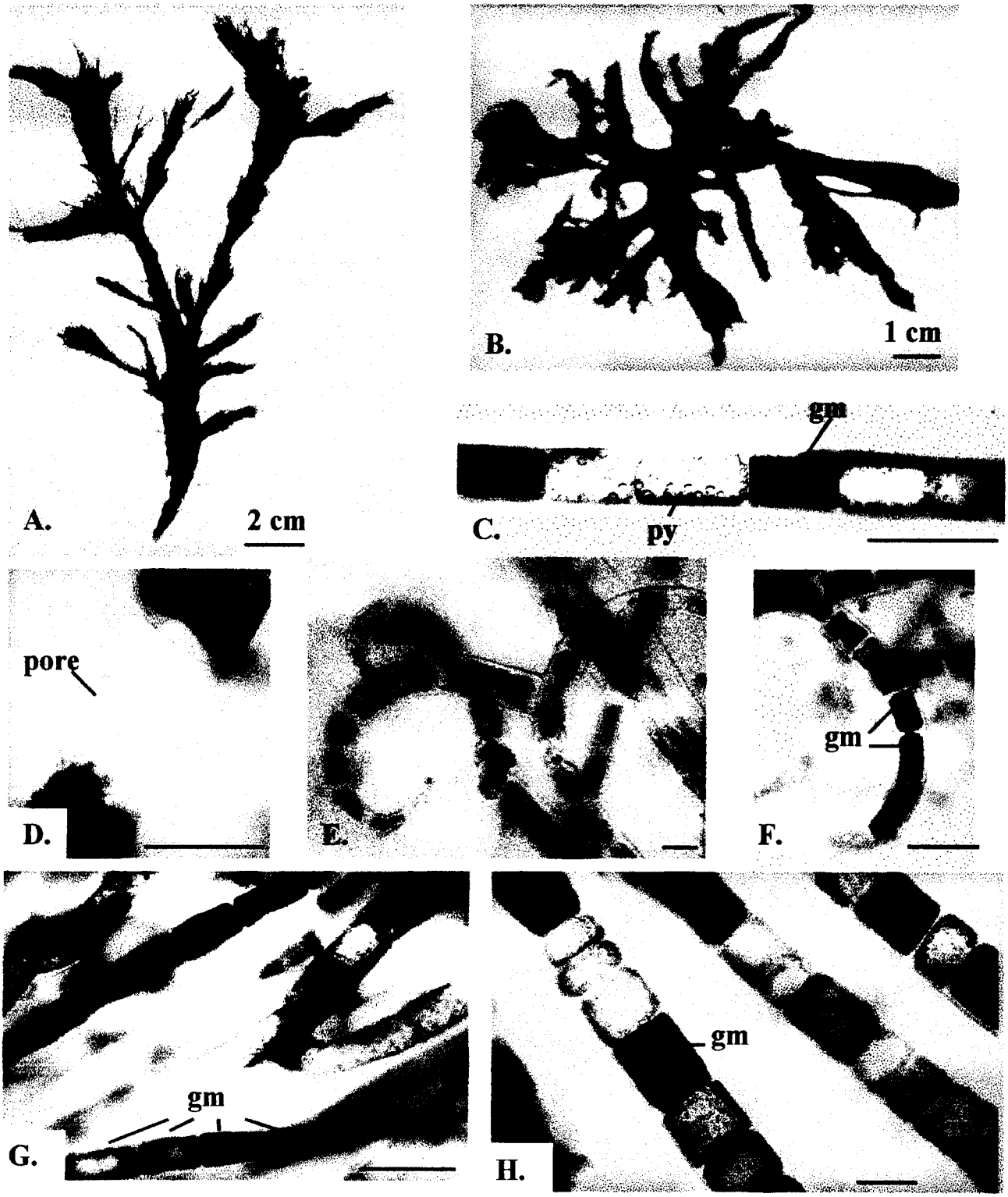

Figure 2.3 A. *Acrosiphonia coalita* habit. **B.** *Acrosiphonia arcta* habit. **C.** Fertile cells. **D.** Empty gametangium with pore for gamete release. **E.** Compound hook of *A. coalita.* **F.** Simple hook. **G.** Many cells fertile in a series, *A. arcta.* **H.** Single cells fertile in *A. coalita* branches. Scale bar is 200 µm. Py = pyrenoids; gm = gametangium.

consistent base pair differences, thus supporting their status as distinct molecular / morphological species (see Chapter 1 Fig. 1.4). Juveniles were difficult to distinguish both in the field and laboratory, because the branching pattern of *A. arcta* was generally not evident until plants were greater than 2 cm in height, and compound hooks (a hooked branchlet upon another hooked branchlet) tended not to be developed on very young *A. coalita*.

Table 2.1 Distinguishing characters for *Acrosiphonia arcta* and *Acrosiphonia coalita* in southwestern British Columbia.

	A. arcta	*A. coalita*
Thallus length	3-6-(10) cm	12-20-(35) cm
Habit	very bushy; much branched; forming hemispherical to globose tufts	tends to form long rope-like branches
Texture	soft	coarse
Hooked branches	simple; rarely compound	simple and compound
Filament diameter	60-100-(120) μm	120-230 μm
# cells fertile in series	3 - 5 - (entire branch)	1 - 2 (less common)
Reproductive phenology	April (less common) - May	March - April (less common)

The *Acrosiphonia* specimens from Alaska, whose ITS DNA sequences were 100 percent identical to a number of 'Chlorochytrium' isolates (see Chapter 1, Fig. 1.4), shared similar characters (absence of hooked branchlets and filament diameter of 160-200 μm) with UBC herbarium specimen A83088 obtained from Prince William Sound, Alaska and identified as *A.*

hystrix. Although the *A.* sp. (Alaska) / 'Chlorochytrium' DNA matches found in the molecular study suggest the presence of *A.* sp. (Alaska) in southwestern British Columbia, no *Acrosiphonia* plants with this combination of characters were found at Burrard Inlet, Sooke or Bamfield. Interestingly, *A. hystrix* is not reported to occur south of Alaska (Setchell and Gardner, 1920), and was in fact not included in the five *Acrosiphonia* species originally identified by Scagel (1966) for British Columbia.

One of the *Acrosiphonia* specimens collected at Sooke, which was very closely related to *A. sonderi* based on ITS sequences (see Chapter 1, Fig.1.5), was not morphologically distinguishable as a separate species, nor are descriptions by Kornmann (1962) adequate for identification.

Acrosiphonia *distribution and seasonal abundance*. At Burrard Inlet only *Acrosiphonia arcta* was present. This was supported by ITS DNA sequences (see Chapter 1 Fig.1.4). Plants were found on boulders and as epiphytes on *Fucus* in the low and mid-intertidal zone (Fig. 2.2A). Smaller plants grew on the base of the Stanley Park seawall (5.1 m above zero tidal level (Canadian Chart Datum) where freshwater runoff provided a moist habitat. Distribution was patchy at all tidal heights. Bright green *Acrosiphonia* juveniles first appeared sparsely distributed in early March 1997 (sampling day 100 from start, Fig. 2.4A). Although growth rate was not measured, the average size and abundance of plants present on consecutive sampling dates suggests rapid growth. Abundance peaked in April at 12.7 percent cover, with a small proportion of the thalli fertile at this time. In *Acrosiphonia* vegetative intercalary cells located either in side branches or in the main filament of the plant differentiate into gametangia. These gametangia change from dark green to brown as they reach maturity (Figs. 2.3C, F-H), and evidence of gamete release is seen by empty gametangia (Fig. 2.3D). Here I refer to fertile thalli as those where brown gametangia were visible. In April some plants were already covered with diatoms and darker green; others were bleached. By late May / June most plants were heavily epiphytised and bleached, and fifty percent of plants sampled were fertile. On several occasions littorinid snails were observed on *Acrosiphonia* plants. In late June (sampling day 209 from start, Fig. 2.4A) *Acrosiphonia* was absent from the intertidal zone and seawall, having been replaced by *Enteromorpha* and *Ulva* as the predominant algae. In 1998 *Acrosiphonia* peak percent cover was twice as high as in 1997.

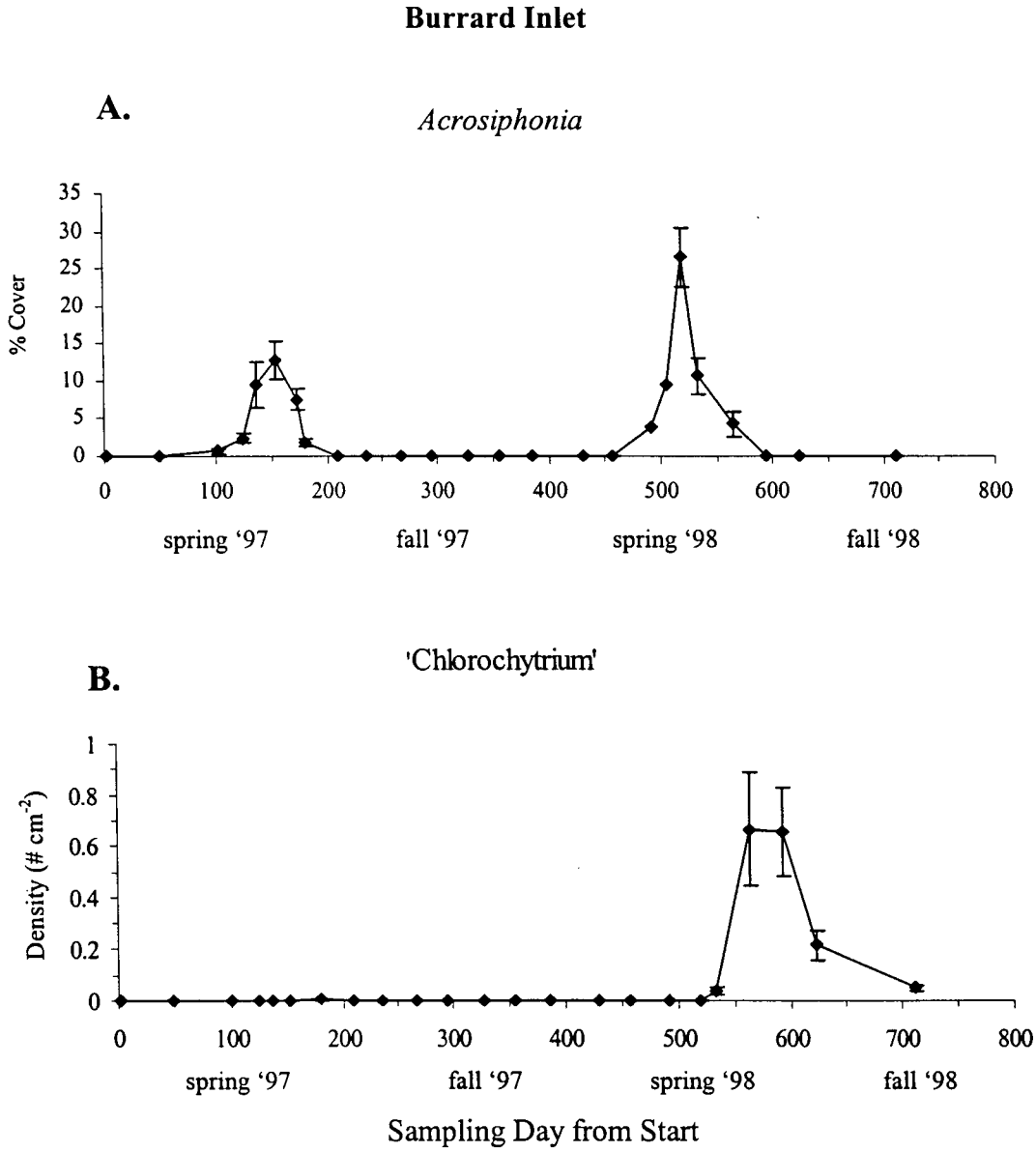

Figure 2.4 **A.** *Acrosiphonia* percent cover at Burrard Inlet over two years. Data are means ± S.E. from 30 quadrats placed along transect lines. The S.E. are missing for two data points, due to missing data (theft of field notes). **B.** 'Chlorochytrium' densities at Burrard Inlet. Data are means ± S.E. from cells counted in 30 *Mazzaella splendens* blades.

At Sooke and Bamfield both *Acrosiphonia arcta* and *A. coalita* were present, and showed patchy distribution (Fig. 2.2B-D). Examination of total *Acrosiphonia* percent cover at Sooke (from all three transects) revealed a pattern of seasonal abundance similar to Burrard Inlet (Figs. 2.4A, 2.5A). *Acrosiphonia* plants at Sooke, however, persisted much longer than at Burrard Inlet. Juvenile plants were present early March (sampling days 117 and 480 from start), percent cover peaked in April (approximately 9 percent) and by July / September *Acrosiphonia* was no longer present. In 1997 a number of healthy thalli were collected at Sooke in mid August, whereas Burrard Inlet plants had disappeared two months earlier. This difference was much less pronounced in 1998. By late July only a few badly deteriorated remnants were found at Sooke. Also observed at Sooke was the occurrence of a second pulse of *Acrosiphonia* establishment. In both sampling years by mid May mature *Acrosiphonia* percent cover was drastically reduced and replaced by *Ulva* and *Microcladia*, but juvenile *Acrosiphonia* of 2-3 percent cover was present. A second percent cover peak of *Acrosiphonia* adults was observed in July 1997 and to a much lesser extent in June 1998.

Overall, *Acrosiphonia arcta* and *A. coalita* showed the same pattern of seasonal abundance. The two species did, however, differ in seasonal abundance and dominance among the three different intertidal heights. Nonetheless, lack of clear patterns over the two year study, compounded by the difficulty of distinguishing among species in the field, makes it virtually impossible to separate *Acrosiphonia* natural dynamics for each species. It was noted, though, that *A. coalita* and *A. arcta* initially occupied different intertidal zones: *A. coalita* was found on boulders and sand and epiphytic on eelgrass in the low intertidal zone; *A. arcta* was found on the same substrata higher in the intertidal zone. Within one month the two species were observed to coexist throughout the intertidal zone (Fig. 2.2D). Yet *A. arcta* always remained the dominant species in the high intertidal zone, even though it became more abundant in the low intertidal zone later in the summer. Reproductive phenology was also different for *A. arcta* and *A. coalita*. Twenty percent of *A. coalita* plants were fertile in early March, with 100 percent reproductive plants sampled in mid April (and variable numbers through to July / August). Fertile *A. arcta* plants (approximately 30 percent), on the other hand, were not found until the end of April (variable percent reproductive plants were recorded through to July / August).

Acrosiphonia at Sooke was also observed on a large boulder (about 2 m in height), located in the low intertidal zone. This boulder showed distinct zonation: the middle zone

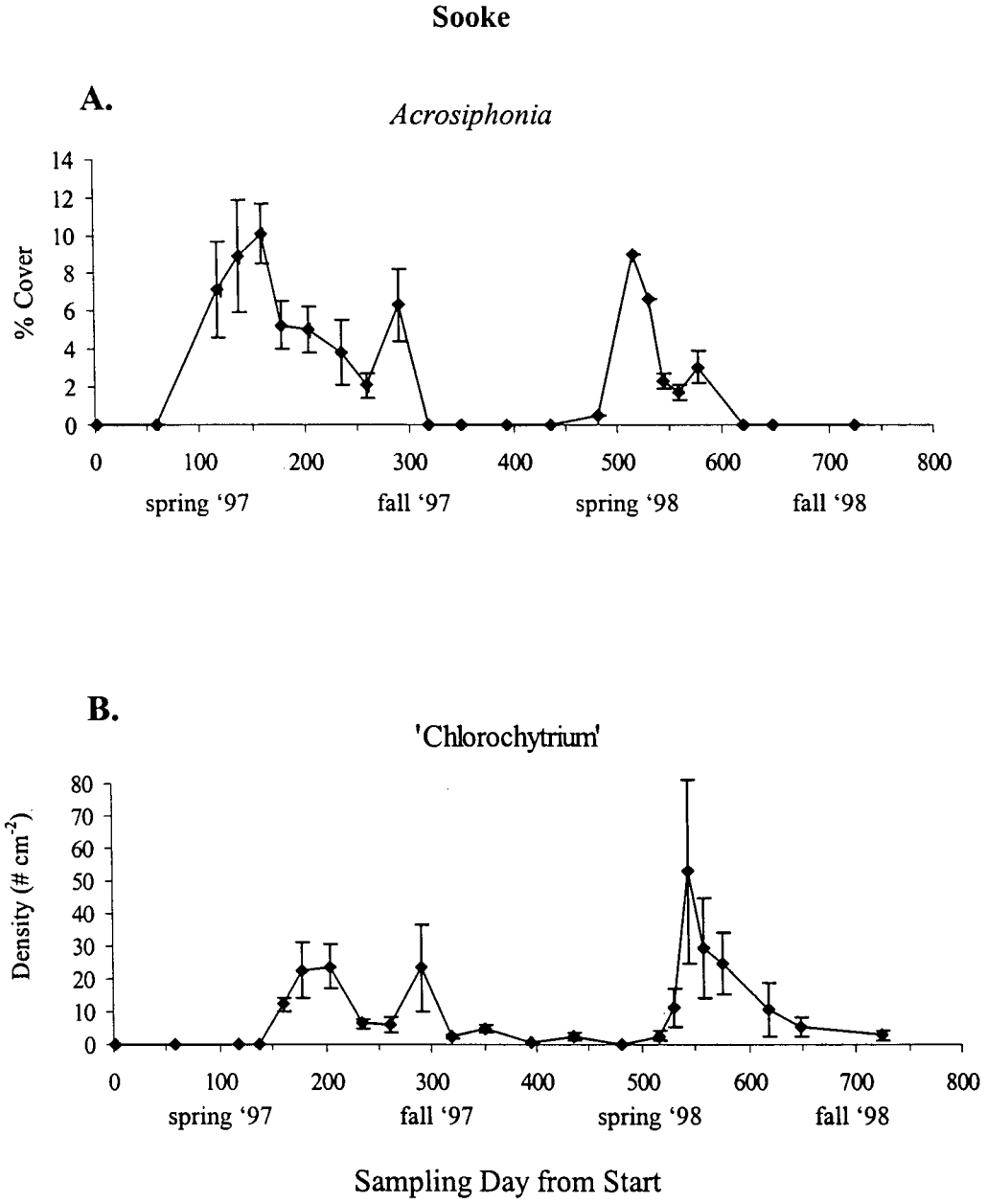

Figure 2.5 A. *Acrosiphonia* percent cover at Sooke over two years. Data are means ± S.E. from 30 quadrats placed along transect lines. The S.E. are missing for three data points, due to missing data (theft of field notes). **B.** 'Chlorochytrium' densities at Sooke. Data are means ± S.E. from cells counted in 30 *Mazzaella splendens* blades.

containing *Acrosiphonia*; the upper dominated by mussels and barnacles and the lower by *Mazzaella splendens* and kelps such as *Alaria*, *Costaria* and *Hedophyllum*. The plants resembled *A. arcta* morphologically, but did not become established until mid May, and also seemed to remain bright green with few epiphytes longer than other *A. arcta*. The ITS DNA sequences of several isolates indicated the majority of the plants on the boulder were *A. arcta*. One specimen, however, yielded a unique DNA sequence (see Chapter 1, Fig.1.4).

Although fewer data are available for Bamfield, it is evident that seasonal abundance of *Acrosiphonia* (Fig. 2.6A) follows the pattern elucidated for Sooke and Burrard Inlet. Sampling in 1997 failed to detect recruitment of *Acrosiphonia*, but the following year revealed establishment at the end of March (sampling day 515 from start), some 3-4 weeks later than at Sooke. *A. arcta* and *A. coalita* were found growing together on consolidated rock (Fig. 2.2B). Their distribution was much patchier than at Sooke and Burrard Inlet with peak percent cover only reaching 4.1 percent in 1997. In 1998, however, as was seen at Burrard Inlet, *Acrosiphonia* increased in abundance, with peak abundance at 8.4 percent. Peak abundance may have occurred later than at Burrard Inlet and Sooke. Since no sampling was carried out between March 26 and May 26 in both years (for logistical reasons), it is difficult to ascertain whether higher abundance may have been observed at an earlier date. Seasonal dominance of one species over another was variable; as at Sooke no clear pattern was illustrated. Persistence of healthy plants was observed well into July (no sampling was conducted in August) for both 1997 and 1998, unlike at Sooke where 1998 plants disappeared earlier. *A. arcta* and *A. coalita* became reproductive later than at Sooke (*A. coalita* becoming mature first). Also, similarly to the large boulder at Sooke, a rock wall at Bamfield showed later establishment and longer presence of plants. Rather than *A. arcta*, though, it was primarily *A. coalita* which occupied this rock wall. As *Acrosiphonia* plants disappeared from the intertidal zone, *Cladophora* visibly dominated.

Biological or statistical significance? Figures 2.4A-2.6A portray the seasonality of *Acrosiphonia* at Burrard Inlet, Sooke and Bamfield: early March to June / July (August). This seasonality is statistically significant ($p < .05$, Table 2.2). However, statistically significant differences among the data points are not easily detectable by the Games-Howell *post hoc* test. At all sites, though, for both 1997 and 1998, one or more spring / summer peak percent covers are significantly higher ($p < 0.05$) than percent covers during the period of *Acrosiphonia* establishment or disappearance. Smaller differences in percent cover such as increased

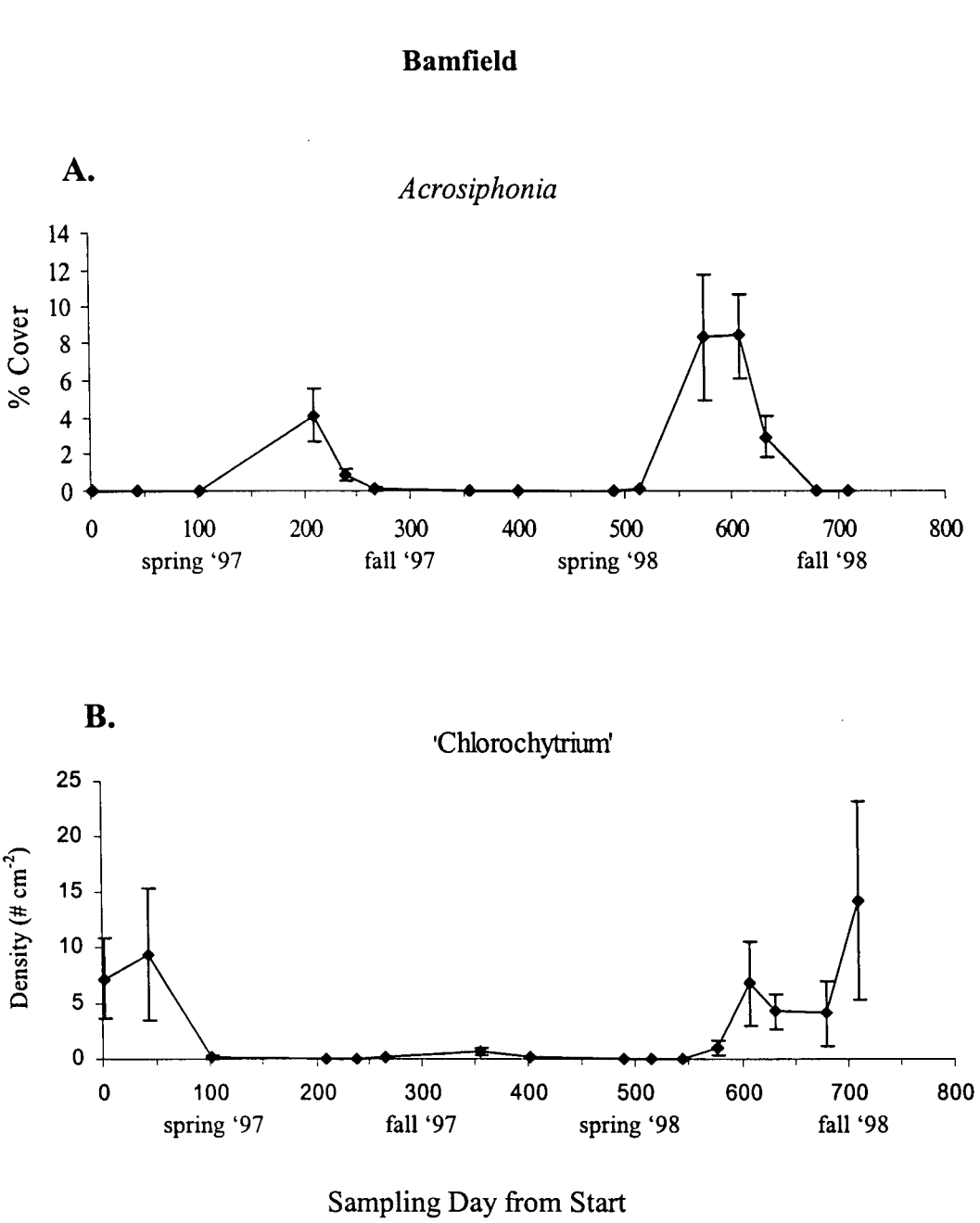

Figure 2.6 A. *Acrosiphonia* percent cover at Bamfield over two years. Data are means ± S.E. from 30 quadrats placed along one or two transect lines. **B.** 'Chlorochytrium' densities at Bamfield. Data are means ± S.E. from cells counted in 30 *Mazzaella splendens* blades.

abundance of *Acrosiphonia* from one year to another (Figs. 2.4A, 2.6A) or a second pulse of *Acrosiphonia* establishment at Sooke (Fig. 2.5A) were, however, not confirmed by the *post hoc* test. These phenomena are visible in the graphs and were very noticeable in the field, suggesting that the extreme patchiness of *Acrosiphonia*, and hence high variance in the data, may have resulted in failure to detect such differences. In any case, *Acrosiphonia*'s seasonality has clearly been established.

Table 2.2 *Acrosiphonia* percent cover. Results of one-way ANOVA on data shown in Figures 2.4A-2.6A and Figures 2.11A-2.13A. The significant F value indicates a statistically significant difference among two or more sampling dates.

Percent cover	SS	df	MS	F	p
Burrard Inlet	352.638	9	39.182	19.963	< 0.0001
Sooke	214.751	11	19.523	9.403	< 0.0001
Bamfield	76.030	6	12.672	8.909	< 0.0001

'Chlorochytrium' morphology and location in host. 'Chlorochytrium' cells were found embedded among the cells of *Mazzaella splendens* from Burrard Inlet, Sooke and Bamfield. In transverse section they were consistently located in the cortical layer of the blade (Fig. 2.7B). Cell morphology ranged from spherical to ovoid to unusually elongated (Fig. 2.7 C,D,E), evidently due to the surrounding host tissue differentially impeding growth. These unusually elongate cells were found in very thin tetrasporophytic blades collected from Sooke in January, February and November 1998. Spherical cells comprised the majority of cells and were grouped into the following six size classes: 40-80 μm, 80-120 μm, 120-160 μm, 160-200 μm, 200-240 μm and > 240 μm diameters. The largest cells observed were 300 μm. The unusually elongate cells were 10-20 percent longer than the diameter of the largest spherical cells observed. A net-like chloroplast and numerous pyrenoids were visible in vegetative cells (Fig. 2.7). Based on a small number of observations in blades collected from Sooke late February 1998 and November

1998 blades (also from Sooke) maintained in a seawater tank for observation, fertile cells tended to (1) form protuberances which extended toward the surface of the host (Fig. 2.7F), (2) darken at the apex, (3) change from bright green to olive coloured, and (4) became homogeneous and bumpy with chloroplasts and pyrenoids no longer distinguishable. A single 'Chlorochytrium' cell, 160 μm in diameter, from material collected late February was observed releasing > 800 zoospores (Fig. 2.7G). The zoospores were approximately 5 μm in diameter, possessing red eyespots and four equal length flagella.

Other than the fact that large 'Chlorochytrium' cells tended to be concentrated at the base of numerous *M. splendens* blades collected from late August to January, no distinct pattern of distribution on blades was detectable. Cells were either scattered over the entire blade surface, grouped in patches along blade margins or at the apex or base of the blade or some combination of patches and scattering of individual cells.

'Chlorochytrium' seasonal abundance. 'Chlorochytrium' seasonality (Figs. 2.4B-2.6B) is statistically significant (Table 2.3). At Sooke 'Chlorochytrium' abundance peaked in May (sampling days 177 and 545 from start, Fig. 2.5B), approximately one month after establishment of *Acrosiphonia*. 'Chlorochytrium' densities from September to late March are significantly lower ($p < 0.05$) than densities in April / May 1997 (sampling days 177 / 204 from start) and June 1998 (sampling day 576), and coincide with *Acrosiphonia* absence. No other significant differences were detected when the *post hoc* test was performed on square root transformed data.

At Burrard Inlet and Bamfield peak densities in 1998 of 0.70 cells cm^{-2} or 81.62 cells per blade and 14 cells cm^{-2} or 2060 cells per blade, respectively, were lower than at Sooke (53 cells cm^{-2} or 5573 cells per blade). Very few 'Chlorochytrium' cells were found in *Mazzaella splendens* at Burrard Inlet in 1997 (Fig. 2.4B). The following year, however, a significantly higher number of cells ($p < 0.05$) were present from June to August (sampling days 563-623), six weeks after *Acrosiphonia* establishment. Abundance of 'Chlorochytrium' at Bamfield was also extremely low in 1997 (Fig. 2.6B). The three sampling dates October 27/96 (day 1), July 21/98 (day 632) and October 6/98 (day 710) show significantly higher densities (4th root transformations of data, $p < .05$) than all other sampling days. It is interesting to note that 'Chlorochytrium' peak density at Bamfield (at least for 1998) occurred much later than at Burrard Inlet or Sooke.

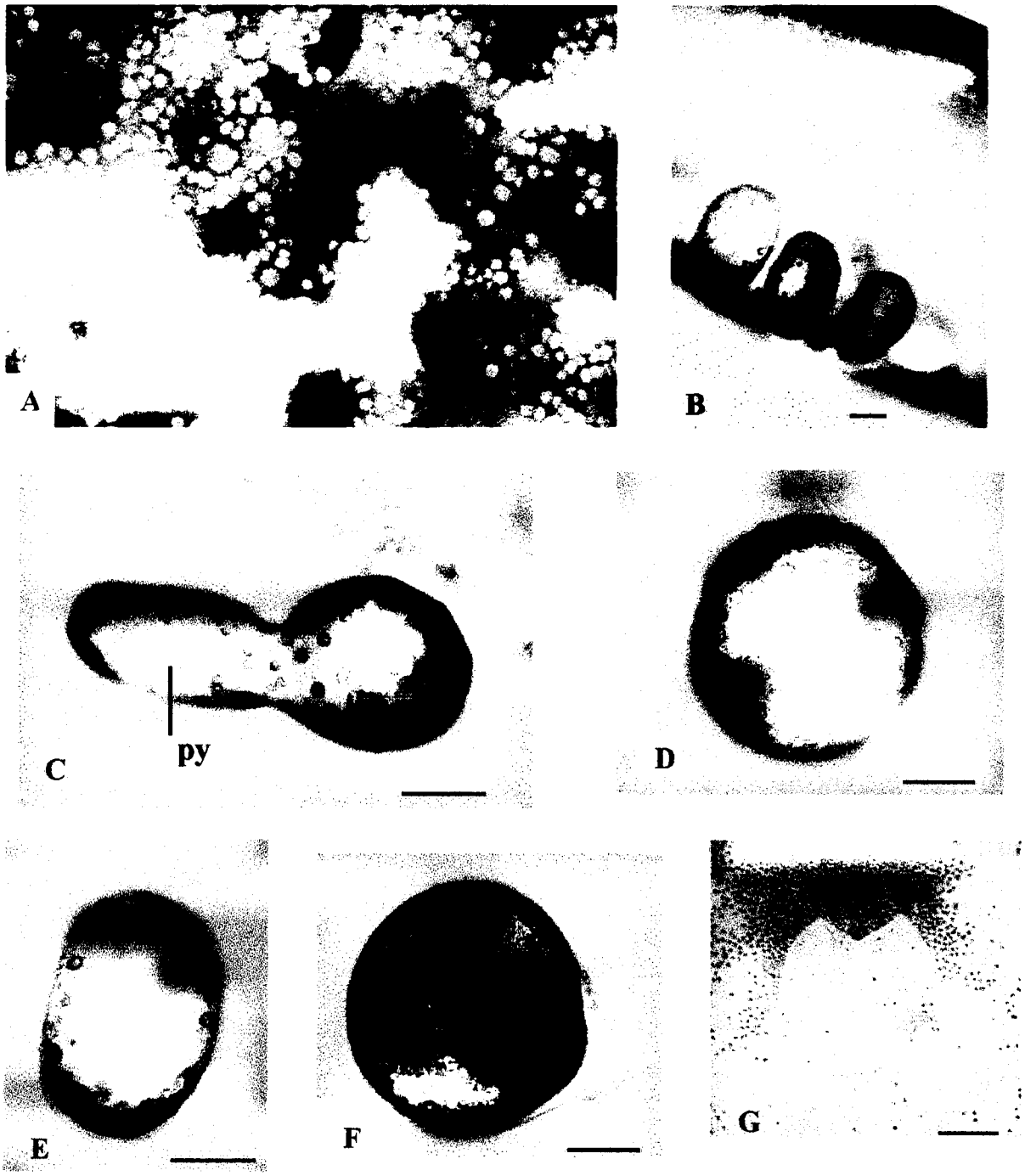

Figure 2.7 A. Surface view of 'Chlorochytrium inclusum' cells (80-160 µm) in *Mazzaella splendens.* **B.** Transverse section showing 'Chlorochytrium' in cortex region of *M. splendens.* **C. D. E.** Vegetative 'Chlorochytrium' cells. **F.** 'Chlorochytrium'cell near maturity. **G.** 'Chlorochytrium' cell releasing > 800 zoospores. Scale bar is 50 µm. py = pyrenoids.

Table 2.3 'Chlorochytrium' density. Results of one-way ANOVA on data shown in Figures 2.4B-2.6B. The significant F value indicates a statistically significant difference among two or more sampling dates.

Density	SS	df	MS	F	p
Burrard Inlet	19.890	10	1.989	9.501	< 0.0001
Sooke	633.455	19	33.340	5.534	< 0.0001
Bamfield *	1.731	15	0.115	7.263	< 0.0001

* 4th root transformations were carried out

When absolute numbers, rather than densities of 'Chlorochytrium', were used to generate data points, the same seasonal abundance patterns resulted for all three study sites.

'Chlorochytrium' size. Average and maximum 'Chlorochytrium' cell sizes recorded for each sampling date were used to generate a 'Chlorochytrium' life history schematic (Fig. 2.8). The schematic is based on Sooke data since it is the most complete (frequent sampling and occurrence of high 'Chlorochytrium' densities). Smallest cells (average 40 μm) were observed in greatest abundance in early spring. Throughout the spring and summer average cell size gradually increased to 200 μm, evidently due to growth of cells. Average cell size decreased in late summer with the observation of a large number of small cells, and then increased again in the fall and winter as cells grew. The following spring only small cells were present with the largest cells 120 μm. During all other seasons maximum cell size was > 120 μm, and remained > 280 μm from June (sampling day 239 from start) to February (sampling day 480).

Schematics for Burrard Inlet and Bamfield would differ from the Sooke schematic, because high densities of the smallest cells were not observed in late summer. The average cell size curve would therefore not decrease in late summer (Fig. 2.8), but instead remain fairly level until spring, at which point the curve would again coincide with the Sooke schematic.

An interesting phenomenon not evident in the schematic is the presence of small cells still observed in December 1997 and January 1998 at Sooke and Bamfield, long after *Acrosiphonia* has disappeared.

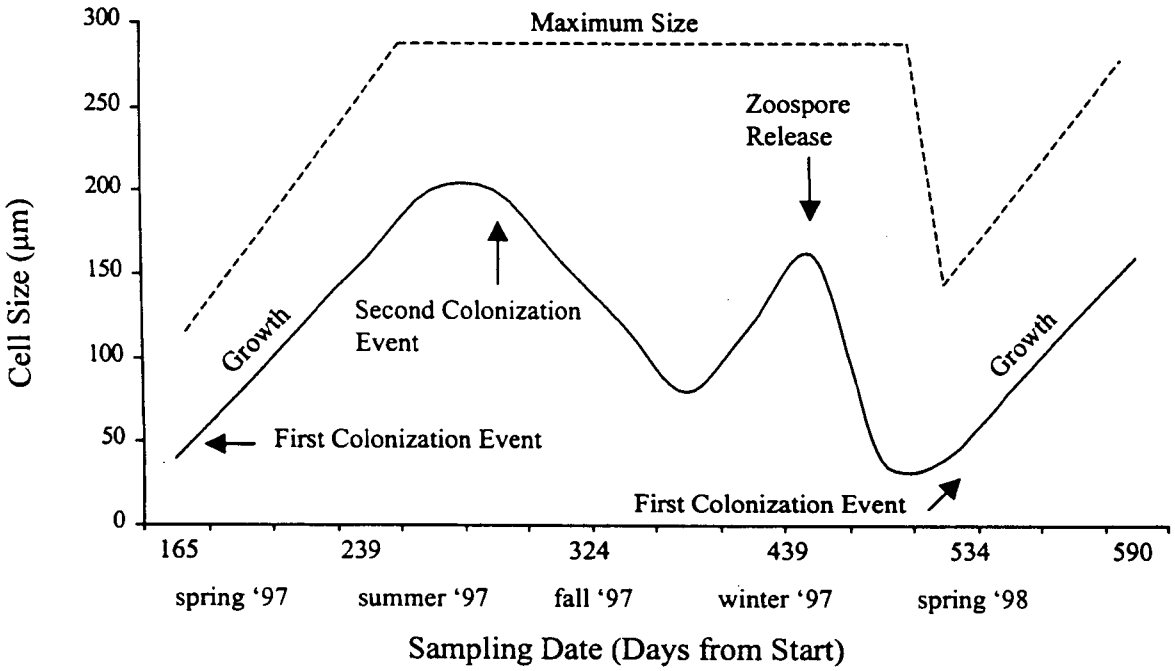

Figure. 2.8. 'Chlorochytrium' life history schematic based on Sooke cell size observations over two years. The solid line represents average cell size and the dotted line maximum cell size.

'Codiolum' morphology and location in host. 'Codiolum' cells were found attached to and completely embedded in the filamentous system of 'Petrocelis franciscana' (Fig. 2.9). They are generally distinguished from 'Chlorochytrium' by their differentiation into an ovoid vesicle and colourless stalk. However, extreme polymorphism exhibited by individual cells included 'Codiolum' cells where the stalk was lacking. In general the ovoid vesicle was slender (120-160 µm x 30 µm, 50-80 µm x 15 µm) or fat (100 µm x 60 µm, 80 µm x 40 µm, 50 µm x 30 µm); the stalk relatively long (70-100 µm), short (20-50 µm) or non-existent (Figs. 2.9, 2.10). Infrequently 'Codiolum' cells were seen where a secondary stalk was developing from the primary stalk (Fig. 2.10). Vegetative cells varied from yellow-green to bright green. The presence of oil droplets obscured any internal structures, *e.g.* chloroplast and pyrenoids. Although no fertile cells were observed discharging zoospores, mature 'Codiolum' were

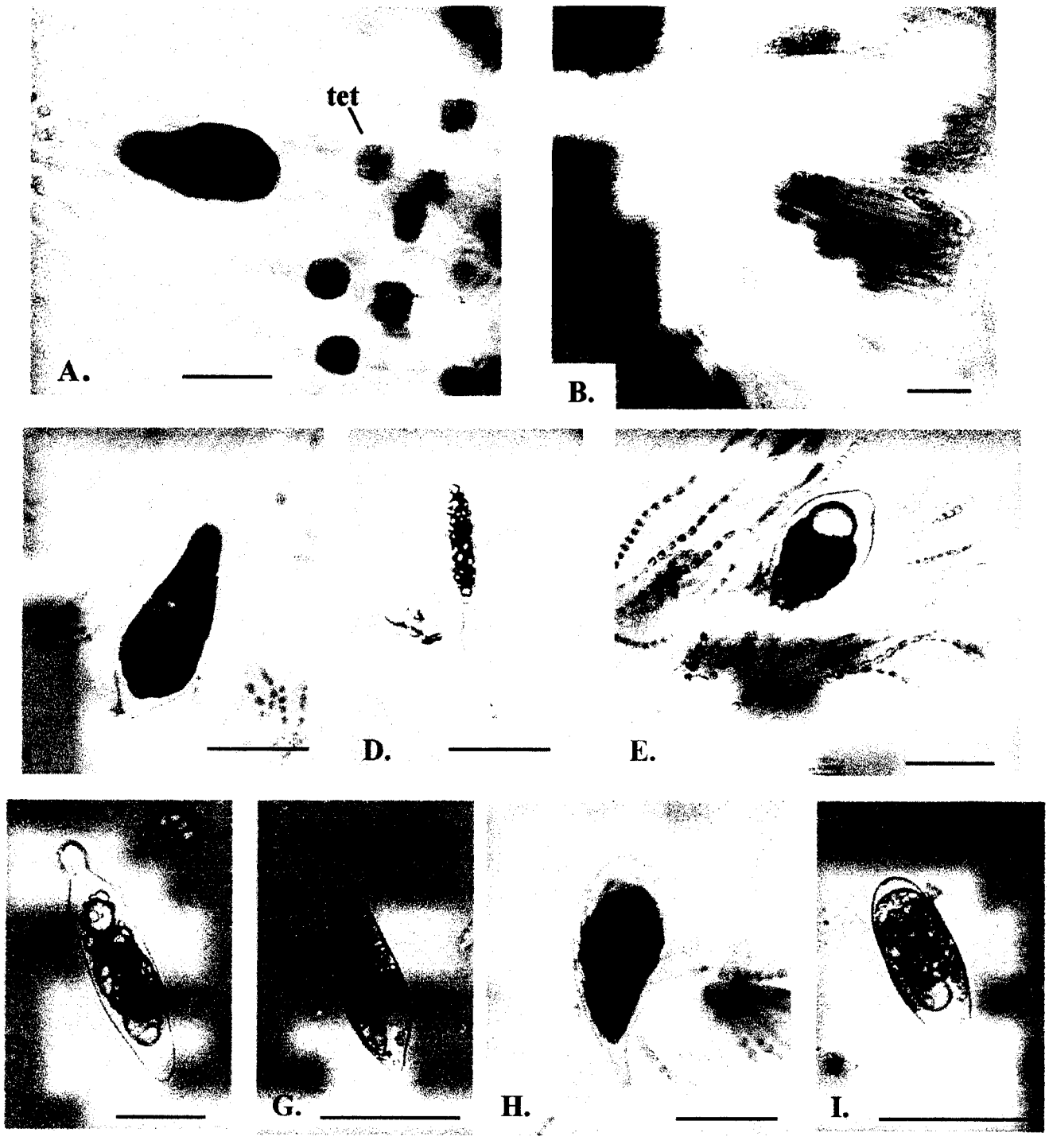

Figure 2.9 Polymorphism of 'Codiolum petrocelidis'. **A. B. C. E.** show cells among 'Petrocelis' filaments. Note both fertile 'Codiolum' and fertile 'Petrocelis' in **A**. Cells with long stalks are shown in **D** and **E**. Juvenile 'Codiolum' are represented by **G** and **I**. Large fertile cells are shown in **A. C. H.** Scale bar is 50 μm. Tet = tetrasporangium.

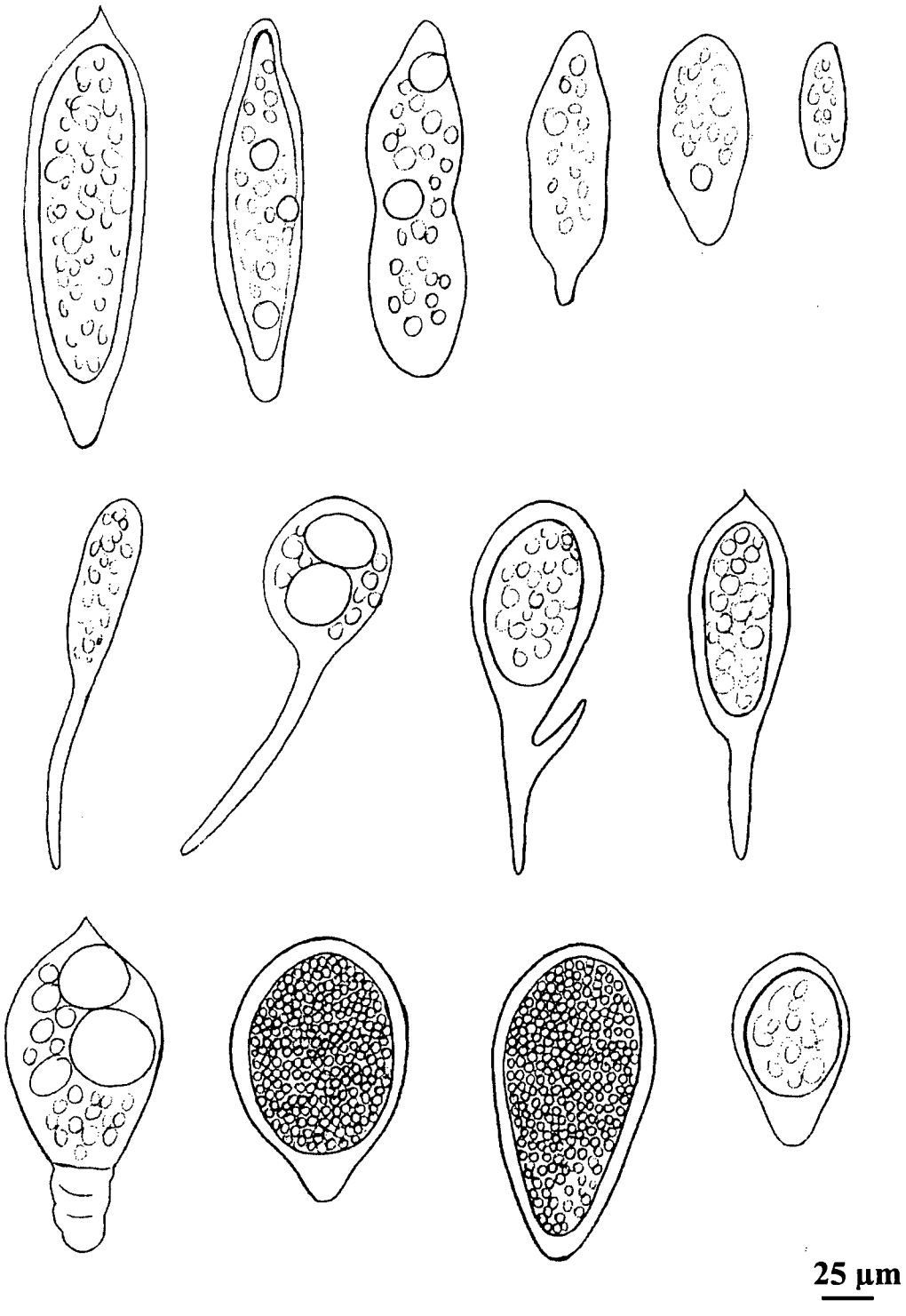

Figure 2.10 Polymorphism of 'Codiolum petrocelidis'. Note the large, fertile cells filled with zoospores in the third row. The last cell in the first row is believed to represent the youngest cells.

identified by darkening of the cell and division of the entire contents of the cell into spores approximately 5 μm in length. Fertile cell vesicles were generally ≥ 80 μm in length and 40 μm in width (Figs. 2.9, 2.10). Reproductive phenology of 'Codiolum' coincided with that of 'Petrocelis', from late fall to spring (Fig. 2.9A).

'Codiolum' seasonal abundance. 'Codiolum' density curves for Burrard Inlet (Fig. 2.11B) and Sooke (Fig. 2.12B) show clear patterns of seasonality supported by ANOVAs (Table 2.4) and *post hoc* tests (Figs. 2.11B, 2.12B). Unlike 'Chlorochytrium' abundance, 'Codiolum' densities at Sooke and Burrard Inlet peaked late June in 1997 (sampling days 234 and 209 from start, respectively) and in July of 1998 (days 619 and 593, respectively). This is approximately one to two months later than 'Chlorochytrium' peaked and two to three months after *Acrosiphonia* establishment. At Sooke peak 'Codiolum' densities were 17,900 cells cm^{-2} of 'Petrocelis' in 1997 and 22,400 cells cm^{-2} of 'Petrocelis' in 1998; there was no significant difference ($p > 0.05$) between the two years. At Burrard Inlet, however, 'Codiolum' peak density of 16,500 cells cm^{-2} of 'Petrocelis' in 1997 (comparable to Sooke peak densities) was significantly lower than the 1998 peak density of 99,100 cells cm^{-2}.

At Bamfield the pattern of 'Codiolum' seasonality is not clearly elucidated (Fig. 2.13B), and few significant differences in densities were detected. This may in part be due to less sampling and smaller sample sizes for some data points ('Petrocelis' at Bamfield was often difficult to distinguish from other algal crusts, because they tended to exist together, overlapping with one another.) Yet, it is evident that much lower densities of 'Codiolum' colonised 'Petrocelis' at Bamfield than at Sooke and Burrard Inlet. Even peak density in October 1998 (last sampling day), which is significantly higher than any other sampling day for Bamfield, is almost 10 times lower than peak densities at the other study sites. Furthermore, peak density in 1997 does not exceed 255 cells cm^{-2}, and, due to high variance, the mean is not statistically different from zero. The October 1998 peak density occurs at least four months after *Acrosiphonia* establishment and three months later than 'Codiolum' peak densities at Sooke and Burrard Inlet. Endophyte density curves suggest 'Codiolum' peak densities were maintained much longer than 'Chlorochytrium' peak densities (Figs. 2.5B, 2.11B, 2.12B).

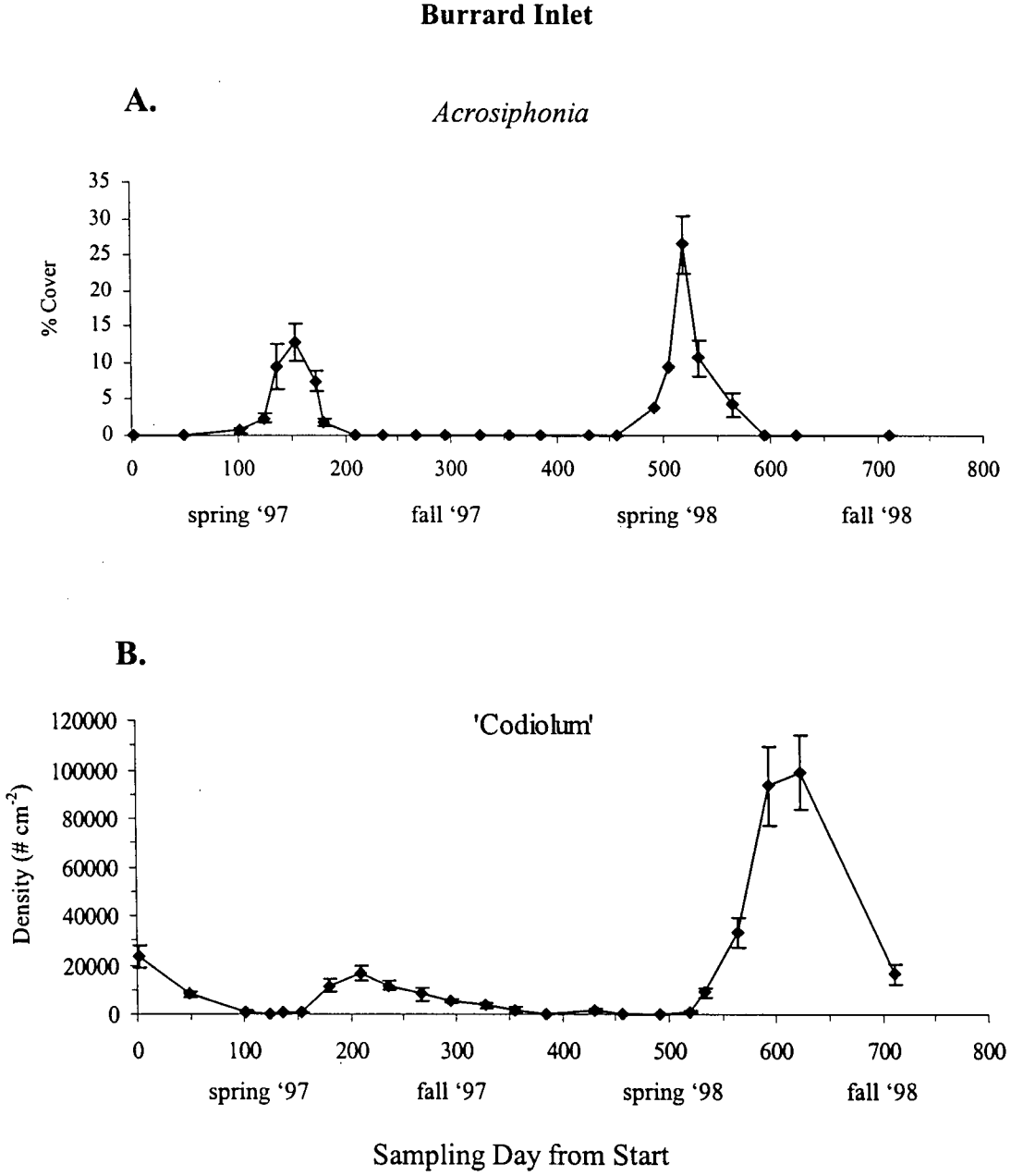

Figure 2.11 A. *Acrosiphonia* percent cover at Burrard Inlet over two years. Data are means ± S.E. from 30 quadrats placed along transect lines. The S.E. are missing for three data points, due to missing data (theft of field notes). **B.** 'Codiolum' densities at Burrard Inlet. Data are means ± S.E. from cells counted in 30 'Petrocelis' patches.

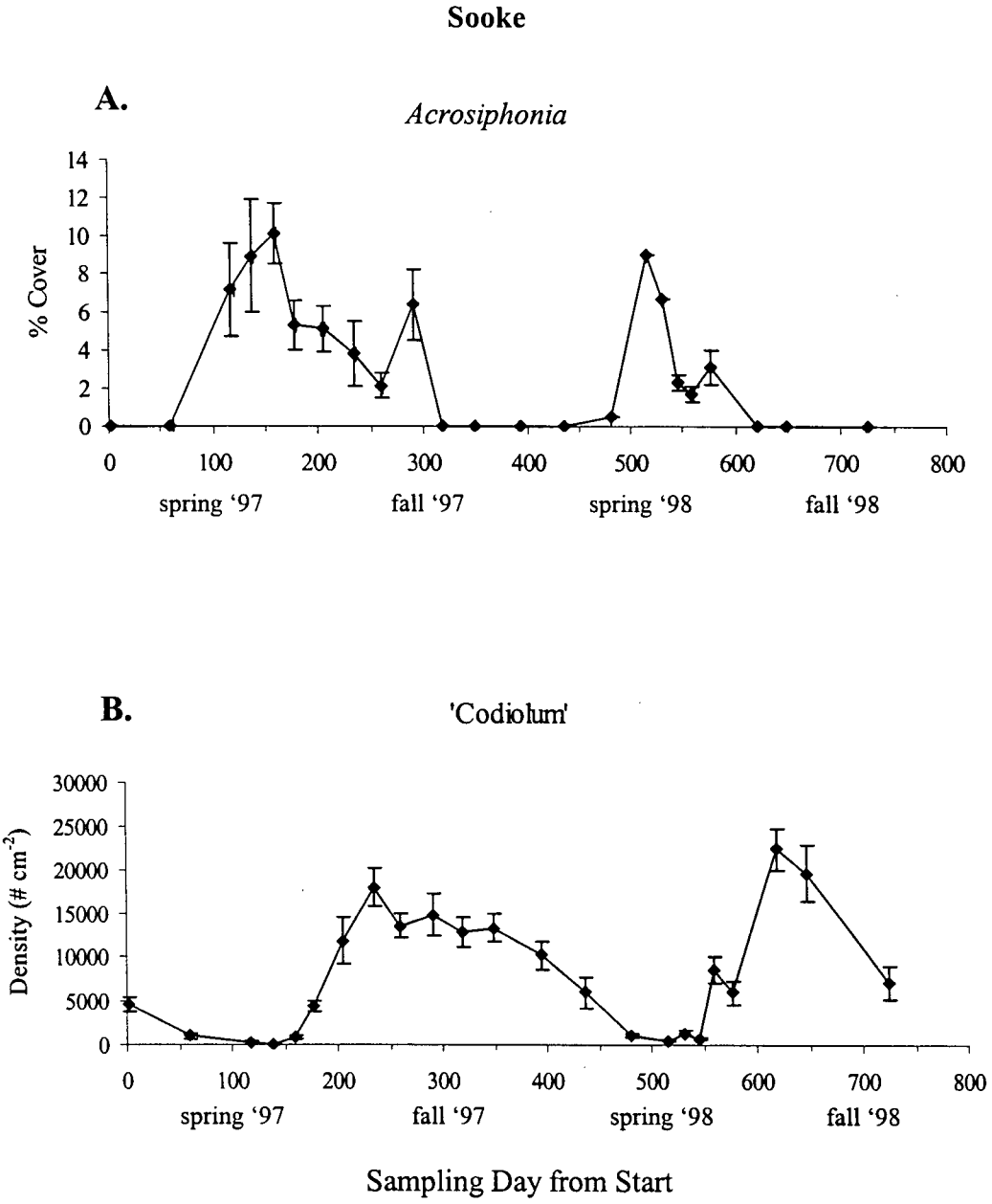

Figure 2.12 A. *Acrosiphonia* percent cover at Sooke over two years. Data are means ± S.E. from 30 quadrats placed along transect lines. The S.E. are missing for three data points, due to missing data (theft of field notes). **B.** 'Codiolum' densities at Sooke. Data are means ± S.E. from cells counted in 30 'Petrocelis' patches.

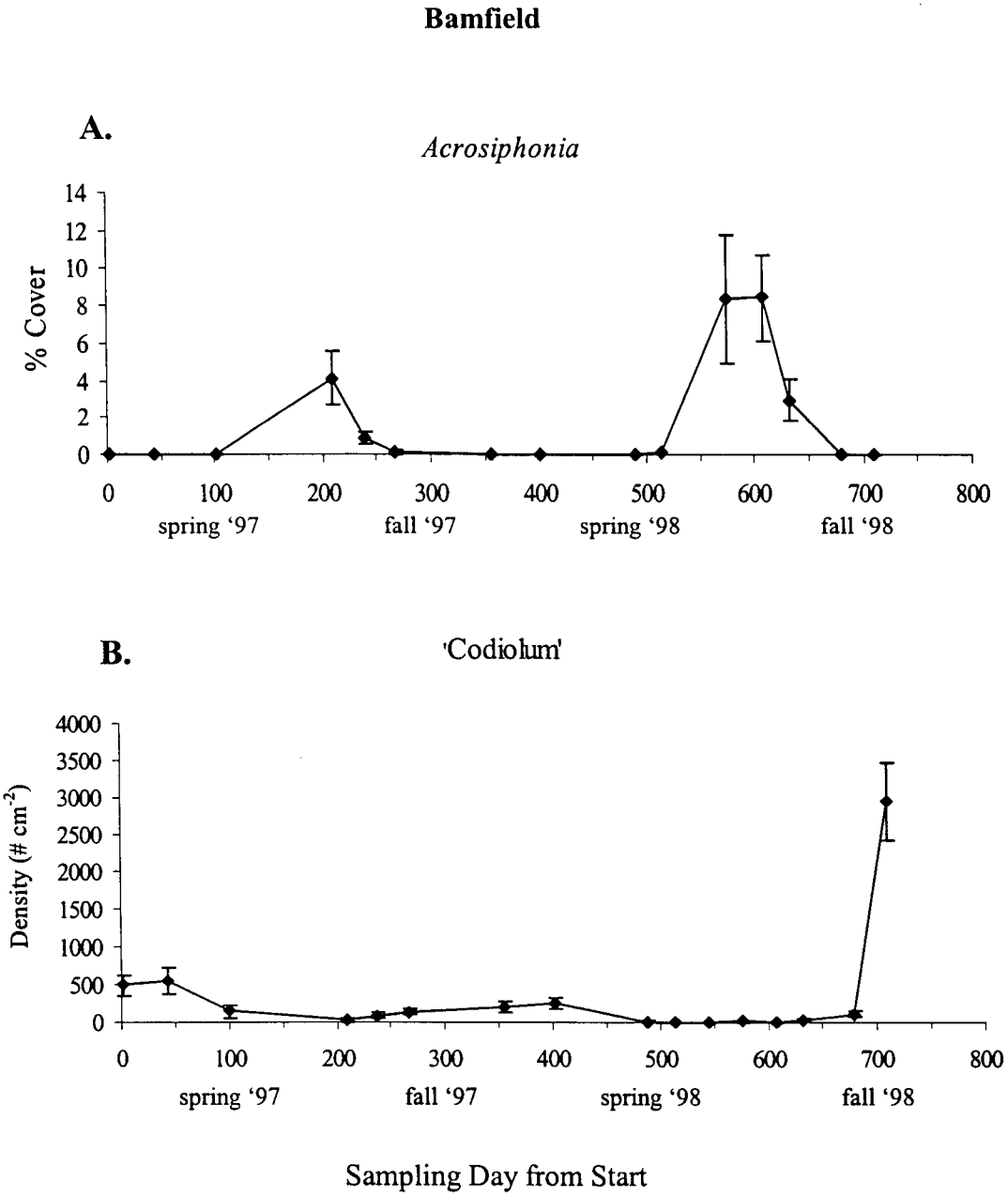

Figure 2.13 **A.** *Acrosiphonia* percent cover at Bamfield over two years. Data are means ± S.E. from 30 quadrats placed along one or two transect lines. **B.** 'Codiolum' densities at Bamfield. Data are means ± S.E. from cells counted in 30 'Petrocelis' patches.

Table 2.4 'Codiolum' density. Results of one-way ANOVA on data shown in figures 2.11B-2.13B. The significant F value indicates a statistically significant difference among two or more sampling dates.

Density	SS	df	MS	F	p
Burrard Inlet	3706916.3	22	168496.194	44.246	< 0.0001
Sooke	1145984.4	22	52090.202	44.846	< 0.0001
Bamfield	53047.159	15	3536.477	34.687	< 0.0001

Sample sizes, means, standard deviations and standard errors for untransformed *Acrosiphonia*, 'Chlorochytrium' and 'Codiolum' data are provided in Appendices A, B and C.

'Codiolum' size. Due to the extreme polymorphism exhibited by 'Codiolum' cells, it was not possible to monitor cell size changes the same way as for 'Chlorochytrium' cells. Instead, cell size for each sampling date was based on vesicle size only and recorded as either large (≥ 80 μm in length) or small (< 80 μm in length). The 'Codiolum' life history schematic in Figure 2.14 was generated from data from all three study sites, and shows that large cells (many of them fertile) were consistently most abundant during fall and winter, whereas only small cells were found in 'Petrocelis' patches in the spring. Summer sampling revealed variable numbers of large and small cells, but no fertile cells. Surprisingly, as was the case for 'Chlorochytrium' from Sooke and Bamfield, many small 'Codiolum' cells were observed in the winter of 1997 at Sooke and Burrard Inlet, long after *Acrosiphonia*'s disappearance.

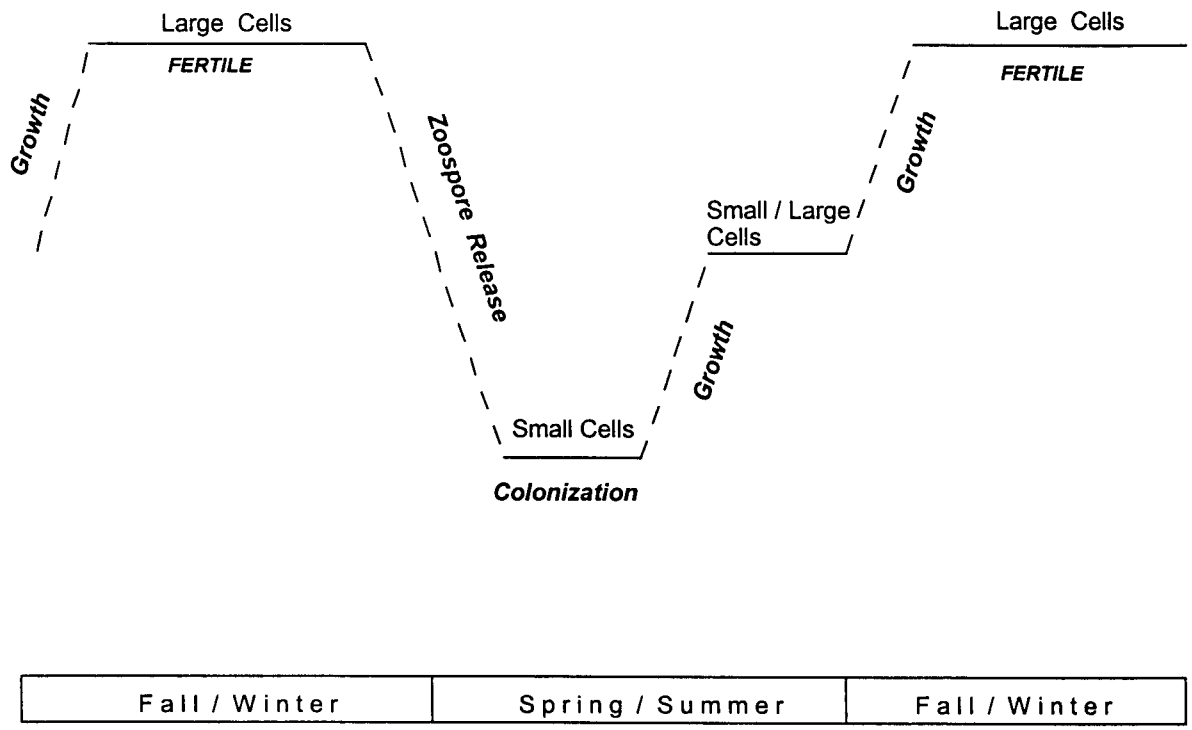

Figure 2.14 'Codiolum' life history schematic based on observations of cell size and reproductive state of cells over two years at Burrard Inlet, Sooke and Bamfield.

DISCUSSION

The relationship between *Acrosiphonia*'s gametophyte and sporophyte in nature is illustrated in Figure 2.15. Filamentous *Acrosiphonia* plants are relatively short-lived, abundant during spring and summer (Figs. 2.4A, 2.6A). Growth is rapid and fertile cells develop almost immediately after *Acrosiphonia* is established. Observation of empty gametangia indicates release of gametes throughout the spring and summer. The unicellular endophytic sporophytes, 'Chlorochytrium' and 'Codiolum', colonise *Mazzaella splendens* and 'Petrocelis', respectively one to three months after *Acrosiphonia* establishment (Figs. 2.4B-2.6B, 2.11B-2.13B). Colonisation inferred from cell size may occur primarily as two major events (in spring and summer, Fig.2.8) corresponding to two *Acrosiphonia* pulses or continuously over the spring and summer. Growth of 'Chlorochytrium' and 'Codiolum' appears to be rapid during this time. Endophytes mature primarily in winter, and zoospore release must occur in winter and / or spring: large fertile

'Codiolum' cells were consistently found in fall / winter, but were no longer present the following spring (Fig. 2.14); several fertile 'Chlorochytrium' cells were observed in February, and maximum 'Chlorochytrium' cell size decreased substantially from winter to spring (Fig. 2.8). Germination of zoospores in spring and summer gives rise to the filamentous gametophytic plants and completes *Acrosiphonia*'s life cycle.

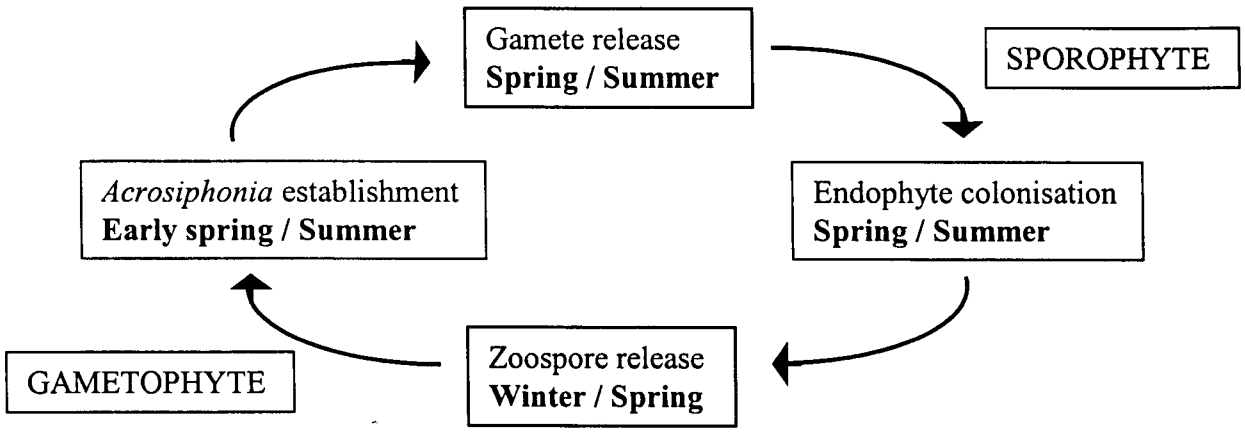

Figure 2.15 Timing of events of *Acrosiphonia*'s life history in nature.

Acrosiphonia *identification in the field*. Distinguishing among *Acrosiphonia* species in the field is problematic. As already mentioned, it is proposed that the two morphological species *A. arcta* and *A. coalita* (which consistently show 4 or 5 bp differences in their ITS DNA sequences, Chapter 1) comprise the majority of plants at the three study sites. However, as also previously noted, juvenile plants are difficult to identify in the field. Furthermore, some mature *A. arcta* plants were found without any hooked branchlets. Others were found with short branchlets beginning to form on simple hooked branchlets and so resembled compound hooks, a diagnostic character of *A. coalita*. Hudson (1974) concluded that the presence or absence of hooked branchlets is a character which should not be given a great deal of weight in distinguishing among plants with otherwise similar appearance. She suggested that the presence

or absence of hooks on plants growing in apparently the same conditions may be due to 1) the plants being genetically the same but growth conditions actually sufficiently different to produce a morphological difference or 2) formation of hooks being sporadic. Furthermore, light intensity and day length were found to affect such characters as cell diameter, length to width ratios and amount of branching (Kornmann 1965, Hudson 1974). The Alaskan *Acrosiphonia* specimens, found to lack hooked branchlets and have large cell diameters, may then not be distinct from *A. arcta* (with small cell diameter). Indeed, Setchell & Gardner (1920) claimed *A. hystrix*, resembling the Alaskan specimens, to be a high northern species of the *arcta* group, differing primarily from *A. arcta* in the greater diameter of filaments.

The *Acrosiphonia* specimen similar to *A. sonderi* based on ITS sequences (see Chapter 1), was indistinguishable morphologically, again suggesting the diagnostic morphological characters on which species are defined are too variable and responsive to environmental conditions to adequately diagnose *Acrosiphonia* species. The ITS DNA sequences supported the recognition of *A. arcta* and *A. coalita* as species, but too few informative sites were present in the ITS region to resolve any further infrageneric relationships within *Acrosiphonia*. I will reiterate that there is great need for a molecular study utilising a more variable region to resolve the taxonomic confusion plaguing the genus *Acrosiphonia*. Furthermore, clarification of the number of *Acrosiphonia* species and identification of their sporophytes at a given site, along with documentation of seasonal abundance and reproductive phenology, may make a more detailed account of *Acrosiphonia*'s life history in the field possible.

Acrosiphonia *seasonality*. Seasonal abundance of *Acrosiphonia* differed among *A. coalita* and *A. arcta* and among the three study sites, but seasonality was consistent with collections reported in the literature. Hudson (1974) obtained fertile *A. arcta* and *A. coalita* plants from the Puget Sound region, Washington State throughout the spring and summer of 1970-73, and Fan (1959) found *A. coalita* at Moss Beach, California from April to October in 1957. In Europe Kornmann (1970a, 1970b) collected *A. arcta* and *A. grandis* (not reported from North America) from Helgoland, Germany in the spring and summer of 1969 and 1970, and Jónsson (1959a, 1964, 1986) obtained *A. arcta* from Brittany, France in the spring of 1958, 1964 and 1986. It is not unusual that *A. coalita* plants were found in October in California, because specimens were also found during this study in early October 1997 from Seppings Island (near Bamfield), Barkley Sound.

***Factors affecting* Acrosiphonia *seasonal abundance and distribution*.** Temperature and photoperiod are important factors in the germination and growth of *Acrosiphonia*. Hudson (1974) found that in culture upright filaments of *Acrosiphonia* sprouted from rhizoid pieces under long-day (16:8 photoregime) conditions, but not under short-day (8:16) conditions at the same temperatures. Also, at 15 °C *A. arcta* plants grew less well than at 5 °C and 10 °C under the same photoregime, and sporophytes ('Chlorochytrium' or 'Codiolum') died before division into zoospores could occur. Miyaji (1996) found this to also be true for *A. spiralis* plants collected in Japan. Vegetative growth of *A. coalita*, on the other hand, was only inhibited at 20 °C. Furthermore, when both *A. arcta* and *A. coalita* plants were grown under low light intensity (below 9.2 $\mu mol\ m^{-2}\ s^{-1}$), motile gametes were not produced. These studies suggest longer days in the spring trigger germination of *Acrosiphonia*, increased light intensity plays a role in the production of viable gametes and the effects of high summer temperatures contribute to the mortality of *Acrosiphonia*.

It also seems reasonable to conclude that afternoon low tides in summer, resulting in additional effects of heat and desiccation, are responsible for the earlier disappearance of *Acrosiphonia* plants at Burrard Inlet than at Sooke and Bamfield (Figs. 2.4A-2.6A) where low tides occur in the morning. In 1999, after sampling had ceased, *Acrosiphonia* was seen at Burrard Inlet (although at a new site) several weeks later than its disappearance in 1997 and 1998. This may be attributed, at least in part, to the lack of sunny days in June and July 1999 (pers. observ.), and so would imply local weather conditions also affect *Acrosiphonia*'s seasonal abundance. It may then not be surprising that *Acrosiphonia* disappeared at Sooke almost a full month earlier in 1998 than the previous year (Fig. 2.5A), since considerably less rainfall than usual was recorded (Environment Canada, Climate Data Services) the summer of 1998 (an El Niño year). Yet, that same year *Acrosiphonia* at Bamfield did persist as in the previous year, and, as mentioned, specimens were collected from Seppings Island (near Bamfield) in early October. This, and the fact variable abundance of *Acrosiphonia* occurred from one year to the next among study sites, *e.g.* increased abundance at Burrard Inlet and Bamfield in 1998, but no difference recorded for Sooke between 1997 and 1998 (Figs. 2.4A - 2.6A) suggests micro-habitat factors such as salinity, nutrient levels and herbivore species and abundance also play a significant role in *Acrosiphonia*'s seasonal abundance.

The greater abundance of *Acrosiphonia* observed at Burrard Inlet and Bamfield in 1998 may be explained by 1) increased survivorship of endophytes (but not necessarily high densities,

since low numbers of both 'Codiolum' and 'Chlorochytrium' were recorded for Bamfield in 1997) 2) increased dispersal of endophytes and drift reproductive *Acrosiphonia* thalli and 3) increased survivorship of overwintering rhizoids of *Acrosiphonia*. As suggested by Scagel (1966), it is still unproven whether *Acrosiphonia* gametophytes can overwinter by means of rhizoids and, if they do, how important this is to the maintenance of *Acrosiphonia*'s population. Purely vegetative propagation by rhizoids was shown under short-day conditions in culture (Hudson, 1974), yet the following observations lead me to speculate that overwintering via rhizoids may not be very important in *Acrosiphonia*'s life history at my study sites: 1) establishment of *Acrosiphonia* in new areas from year to year at all three study sites, 2) 0 percent cover of *Acrosiphonia* found in preliminary 20 cm x 20 cm marked areas formerly noted with 75 - 80 percent cover at Bamfield and 3) the inability to detect rhizoids after disappearance of the upright plant.

Dispersal patterns of gametes, reproductive spores or propagules of marine algae have been little studied (Anderson & North 1966, Amsler & Searles 1980, Reed *et al.*1988, Kendrick & Walker 1991, 1995). *Acrosiphonia arcta* plants (though lacking hooked branchlets) were found on Kitsilano Beach, Vancouver, B.C. in May 1997, despite the fact that *Mazzaella splendens* and 'Petrocelis' were absent. In fact, the only foliose algae present were *Laminaria saccharina* (L.) Lamouroux and *Ulva*, neither of which were found to be colonised by 'Chlorochytrium', and no crustose algae were found. This finding supports the idea that dispersal of unicellular sporophytes, zoospores or reproductive *Acrosiphonia* thalli may be long range, *e.g.* from Burrard Inlet (approximately 6 km). This also implies the survival and overwintering of free-living sporophytes, perhaps in rock crevices. A number of researchers (Jónsson 1959a 1959b 1962 1966, Kornmann 1961 1964, Chihara 1969, Hudson 1974, Miyaji 1996) demonstrated the ability of 'Chlorochytrium' and 'Codiolum' to survive free-living in culture. The epilithic existence of *Acrosiphonia*'s sporophyte in the field could be tested experimentally by placing sterilised plates in the intertidal zone among fertile *Acrosiphonia* in the summer, and examining these for settlement and growth of the unicellular sporophyte later in the year. Furthermore, unlike at my study sites where hosts are abundant for the survival of *Acrosiphonia*'s sporophyte, vegetative propagation of *Acrosiphonia* by overwintering rhizoids may be important in maintenance of populations in habitats (such as at Kitsilano Beach, Vancouver) devoid of hosts for 'Chlorochytrium' and 'Codiolum'.

Although not statistically significant, both *Acrosiphonia arcta* and *A. coalita* showed a second pulse at Sooke in both sampling years, *i.e.* after *Acrosiphonia* abundance dropped off, percent cover peaked a second time. This was much less pronounced in 1998 (an El Niño year), perhaps due to lower than normal precipitation (Environment Canada, Climate Data Services 1998) resulting in increased desiccation and hastening mortality of plants. Possible explanations for two *Acrosiphonia* pulses at Sooke include: 1) delayed germination of zoospores of another 'species' of *Acrosiphonia*, 2) differential maturity of sporophytes resulting in differential release of zoospores, 3) parthenogenesis of unfused gametes germinating directly into gametophytes, 4) zoospores developing within *Acrosiphonia* filaments and germinating directly into gametophytes and 5) vegetative propagation from rhizoids of old plants. I was unable to determine whether a second pulse occurred at Bamfield and at other locations where *Acrosiphonia* seasonality extends beyond July. Parthenogenesis (Jónsson 1963, Miyaji 1996) and recycling of filamentous plants by zoospores (Kornmann, 1962) has been observed in *A. arcta*, *A. sonderi* and *A. spiralis* in culture. However, sexual reproduction was by far the dominant mode of propagation, and whether parthenogenesis and asexual reproduction occur in nature remains to be demonstrated.

Acrosiphonia arcta *and* A. coalita *differences in the field*. The two *Acrosiphonia* species, *A. arcta* and *A. coalita*, occupy the same habitat, but showed variable patterns of abundance and reproductive phenology at Sooke and Bamfield. *Acrosiphonia coalita* was absent from Burrard Inlet. Hudson's culture work (1974) showed different effects of environmental factors such as temperature on the two species: *A. coalita* seems to have a higher tolerance for high temperatures. Hudson (1974)noted that *A. coalita* persisted longer than *A. arcta* in the intertidal zone of the Puget Sound region, Washington State, and that *A. arcta* had not been reported south of Washington State. Although *A. arcta* did not consistently disappear from the intertidal zone earlier than *A. coalita* at Sooke and Bamfield, *A. arcta*'s abundance decreased at Sooke in the high intertidal zone and increased in the low intertidal zone as the summer progressed. However, *A. arcta* was always the dominant species higher in the intertidal zone, suggesting that in southwestern British Columbia temperature tolerance alone does not determine species distribution and abundance.

The absence of *A. coalita* from Burrard Inlet may be due to a lack of unidentified micro-habitat requirements. On the other hand, *A. coalita* may simply have never become established. One way to test for this is to plant *A. coalita* in the intertidal zone at Burrard Inlet. Identification

of micro-habitat requirements may best be undertaken in the laboratory with more extensive culture studies.

Contrary to observations in the field, in culture *A. arcta* became fertile before *A. coalita* (Hudson, 1974). At both Sooke and Bamfield fertile *A. coalita* plants were found almost immediately after establishment of plants, whereas fertile *A. arcta* (also at Burrard Inlet) were not detected until the following sampling date, about one month later. Since sampling only occurred monthly, it is possible that *A. arcta* may actually have become reproductive less than one month after *A. coalita*. Furthermore, the percentage of reproductive plants was higher for *A. coalita* throughout the sampling period. More detailed studies are necessary to pin-point differential reproductive phenology.

Endophyte colonisation. Colonisation of the sporophytic endophytes was deduced by examining both cell density and cell size over time. An important assumption is that the smallest cells represent the youngest cells. Polymorphism of 'Codiolum' cells complicates this assumption, and so cells were categorised on the basis of fertility and vesicle length (greater or less than 80 μm) regardless of stalk length. Based on cell density increase (and the monthly sampling regime), the onset of 'Chlorochytrium' colonisation occurred in late April about one month after *Acrosiphonia* establishment (Figs. 2.4B-2.6B), and one month earlier than for 'Codiolum' (Figs. 2.11B-2.13B) at all three study sites. This is somewhat counter-intuitive, since *A. coalita* became fertile before *A. arcta*, and its sporophyte appears to be 'Codiolum' based on culture and molecular work (see Chapter 1).

The onset of endophyte colonisation was also implied by the occurrence of the greatest abundance of smallest cells (Figs. 2.8 and 2.14). Kornmann's (1964) findings (within *Haemescharia hennedyi* from Germany) agree with the present study in that the greatest abundance of young 'Codiolum' cells occurred in late spring, four to six weeks after reproductive *Spongomorpha lanosa* plants were detected. The presence of smallest cells from spring to early fall in the present study implies colonisation is continuous and synchronised with *Acrosiphonia* gamete release. Dethier's (1987) finding of high densities of 'Codiolum' within 'Petrocelis' from Washington State in summer supports spring / summer colonisation. The time required and the mechanism for zygote / endophyte penetration of *Mazzaella splendens* and 'Petrocelis' remains poorly understood. More detailed culture and microscopy studies are

needed to shed light on these events, and to better understand the time-lag between *Acrosiphonia* gamete release and endophyte establishment.

It was possible to detect two major colonisation events by endophytes at Sooke (Fig. 2.8), which coincide with the two *Acrosiphonia* pulses observed at Sooke (Fig. 2.5A). However, due to an inability to obtain average 'Codiolum' cell size (categories were simply large or small) and the difficulty in distinguishing relative densities of newly colonised cells from older cells because of polymorphism, it is unlikely that two events for 'Codiolum' colonisation at Sooke could have been detected. An added complication is the fact that no experimental data are available for endophyte growth rates.

Colonisation of 'Chlorochytrium', and especially 'Codiolum', at Bamfield occurred considerably later than at Burrard Inlet or Sooke. This may in part be explained by the fact that *Acrosiphonia* percent cover peaked later and plants persisted longer.

Endophyte growth, fertility and duration in host. Growth of 'Chlorochytrium' and 'Codiolum' is believed to be rapid, judging by the range of cell sizes observed from one sampling date to the next. Note the steepness of the "maximum size" curve and "growth" portion of the 'Chlorochytrium' life history schematic curve (Fig. 2.8). Most 'Chlorochytrium' cells first observed in abundance at Sooke in early spring were 40 μm in diameter, but cells up to 120 μm were also already present. Less than two months later cells of 200 – 240 μm were commonly found. Setchell & Gardner (1920), Scagel (1966), Chihara (1969) and Hudson (1974) described 'Chlorochytrium inclusum' cells from the northeast Pacific of size range 75 – 100 μm. Many of the collections were, however, made early in the spring. 'Chlorochytrium inclusum', endophytic in *Farlowia* (Rhodophyta) from Japan, and associated with three *Acrosiphonia* species unknown to southwestern British Columbia, was also reported to be ≤ 100 μm in diameter (Miyaji and Kurogi, 1976). Kjellman's (1883) description of 'C. inclusum' within the arctic alga *Sarcophyllis arctica* Kjellman (Rhodophyta) is the only case I have found where 'Chlorochytrium' cells are noted to reach 275 μm, in agreement with the size attained by 'Chlorochytrium' cells in *Mazzaella splendens* in this study.

Chihara (1969) detected larger 'Chlorochytrium' cells closer to the base of the foliose host, whereas Kjellman (1883) noted individuals in the central part of the blade to be largest. Hudson (1974) observed the greatest concentration of cells toward the base. None of these observations were substantiated in my study, but might be explained by the much smaller sample

size of blades examined by the other researchers and the fact they collected blades primarily in spring and summer. In late August to January a large number of *M. splendens* blades from Sooke and Bamfield were found with large 'Chlorochytrium' cells (160 – 300 μm) concentrated at the base of the blade. A possible explanation is that 1) the oldest (largest) cells are positioned closer to the base (the oldest part) of the blade and 2) newly colonised cells are sparsely scattered over the entire blade (the disappearance of *Acrosiphonia* implies gradual cessation of zygote settlement), and are growing slower than in the summer due to decreased light in the fall and winter months.

'Chlorochytrium' fertility and zoospore release occur in winter / early spring (Fig. 2.8) coinciding with *Mazzaella splendens* reproductive phenology (90 – 100 percent *M. splendens* blades collected from all three sites from October to February were reproductive). Kjellman (1883) found 'Chlorochytrium' fertile within *Sarcophyllis arctica* in winter, but did not observe zoospore release (unlike this study, however, he noted that 'Chlorochytrium' was most abundant in winter). Kornmann (1964) also collected fertile 'Chlorochytrium' (from *Polyides*, a foliose red seaweed, in Helgoland, Germany) in the winter, just months before *Spongomorpha lanosa* establishment. 'Chlorochytrium' of 80-100 μm in diameter, obtained from *Schizymenia* (a bladed red seaweed) in Washington State, became fertile in culture and gave rise to 32, 64 or more zoospores 10 μm long (Chihara, 1969). Unlike Chihara's fertile 'Chlorochytrium', the few fertile cells detected in material collected from Sooke were much larger, 160-200 μm , and more than 800 zoospores, 5 μm long, were released from one mature sporophyte.

The absence of large cells in spring, after zoospore release, leads me to believe 'Chlorochytrium' cells spend less than one year in their host. However, it is unknown whether cells colonising *M. splendens* late in the summer remain small during the winter, *e.g.* 120 μm, to become reproductive the following winter, as was proposed by Kornmann (1964) for 'Codiolum' in Helgoland, Germany. Chapter 3 will investigate this possibility.

Juvenile cells of 'Codiolum', first detected in 'Petrocelis' in spring were 40 – 50 μm x 10 – 20 μm, apparently without stalk, and densely entangled among 'Petrocelis' filaments. It was difficult to detect stalks of newly colonised 'Codiolum' cells, but this does not mean the cells lacked them. Culture studies (Fan 1959, Jónsson 1970, Kornmann 1972, Miyaji 1984) demonstrated that germlings of 'Codiolum', only 10 – 15 μm in length, were capable of becoming differentiated into vesicle and stalk within 5 – 15 days. A number of researchers (Setchell & Gardner 1920, Jónsson 1958, Kornmann 1972) have reported polymorphism among

'Codiolum' cells. This polymorphism was evident in specimens collected from all three study sites throughout the year, except in spring when juvenile cells were more uniform and distinct from older cells. The secondary stalk observed on some specimens (Fig. 2.10) was also described by other investigators (Printz 1926, Kornmann 1961, Hanic 1965) and referred to as a lateral stipe appendage.

Fertile 'Codiolum' cells observed in the present study were generally ≥ 80 μm in vesicle length and tended to have a proportionately large diameter (Figs. 2.9, 2.10). They were detected in 'Petrocelis' samples collected from fall through early spring, coinciding with the reproductive phenology of their host (tetrasporangia were present in 'Petrocelis' from October / November to February / March). Hanic (1965) found fertile 'C. petrocelidis' in tetrasporic 'Petrocelis franciscana' collected from Sooke in December 1963, and Kornmann (1961, 1964) reported fertile 'Codiolum' associated with *Spongomorpha lanosa* in tetrasporophytic *Haemescharia hennedyi*, a crustose red alga, from Helgoland in December 1963 and February 1964. As with 'Chlorochytrium', zoospore release of 'Codiolum' generally occurs in the winter (Fig. 2.14), and the detection of only small juvenile cells the following spring suggests 'Codiolum' cells spend less than one year in their host. This is in contrast with Kornmann's (1961, 1964) finding: a mixture of 'Codiolum' cell sizes were found together in *Haemescharia hennedyi* at the time of 'Codiolum' colonisation, indicating some cells may spend about 18 months in their host, not releasing zoospores until the second winter. It seems unlikely to me that the very small cells observed in my study in greatest abundance in the spring and summer were comprised of both newly colonised cells and cells which had colonised the crust the previous spring or summer. Duration of 'Codiolum' in 'Petrocelis' will be examined further in the next chapter.

A simplified sporophyte life history schematic, illustrated in Figure 2.16, integrates the dynamics of 'Chlorochytrium' and 'Codiolum' in *Acrosiphonia*'s life history. Large, fertile cells are present in fall and winter; following zoospore release endophyte colonisation and growth take place throughout the spring and summer. 'Chlorochytrium' colonisation occurs before 'Codiolum' colonisation.

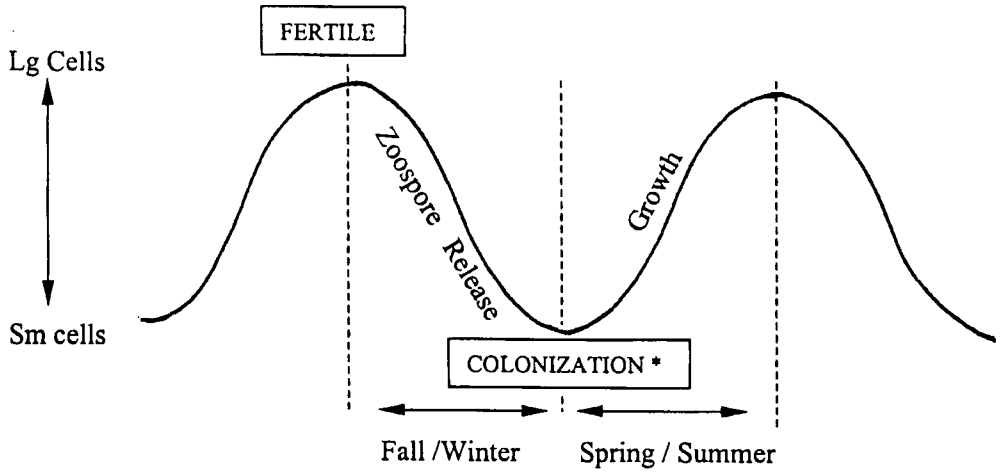

Figure 2.16 Generalised life history schematic of *Acrosiphonia*'s sporophyte.
* 'Chlorochytrium' colonisation occurs earlier than 'Codiolum' colonisation.

Many 'smallest' cells of both 'Chlorochytrium' and 'Codiolum' were observed in January 1998 at the study sites where relatively high densities of endophytes were present. Since *Acrosiphonia* plants are long gone by this time, what is the origin of these putative juvenile 'Chlorochytrium' and 'Codiolum'? Perhaps they represent zygotes / sporophytes which colonised their host late in the season, and grow slowly during the fall and winter. They may grow quickly in the spring and still become reproductive for spring zoospore release, or remain vegetative until the winter or following spring, in which case Kornmann's hypothesis would be supported. Yet, this scenario does not explain the fact that a large number of such small cells was not detected in the fall of 1997. Furthermore, in the spring, prior to *Acrosiphonia* establishment, some juvenile *Mazzaella splendens* blades and 'Petrocelis' patches of new growth were found to be colonised by endophytes. One possible explanation, although never observed in culture, is that 'Chlorochytrium' and 'Codiolum' cells reproduce themselves, *i.e.* zoospores are released within the host or zoospores of free-living 'Chlorochytrium' and 'Codiolum' colonise *M. splendens* or 'Petrocelis'. An alternative explanation is that these small cells, although morphologically resembling 'Chlorochytrium' and 'Codiolum', are not in fact the sporophytes of *Acrosiphonia*. However, this seems rather unlikely, since PCR and DNA sequencing did not

detect any bp ambiguities which would indicate the presence of cells not associated with *Acrosiphonia* (see Chapter 1).

Endophyte abundance variability. Higher abundance of *Acrosiphonia* in 1998 than in 1997 at Burrard Inlet and Bamfield is correlated with significantly higher endophyte densities in 1998 for both study sites (Figs. 2.4, 2.6, 2.11, 2.13). The significantly lower abundance of 'Chlorochytrium' and 'Codiolum' at Bamfield in 1997 (Figs. 2.6, 2.13), however, can not be explained in terms of lower 1997 *Acrosiphonia* abundance.

Bamfield's much lower endophyte colonisation than Sooke's, over the entire sampling period, may be due to cells having more difficulty in settling on and penetrating their hosts at wave-exposed sites. At Burrard Inlet the discrepancy between low numbers of 'Chlorochytrium' in 1997 and much higher densities in 1998 [(Fig. 2.4B) more than would be expected simply from a doubling of *Acrosiphonia* percent cover] may result from zygotes from *Acrosiphonia* sp. (Alaska) exhibiting higher colonisation success than *A. arcta*. The four 1998 'Chlorochytrium' DNA sequences from Burrard Inlet isolates were 100 percent identical to the Alaskan *Acrosiphonia* isolates (Chapter 1).

Endophyte abundance in 1997 at Bamfield showed no correlation with 1998 *Acrosiphonia* abundance, *i.e.* low endophyte densities did not result in low *Acrosiphonia* percent cover. Endophyte survivorship (discussed in Chapter 3), however, may have been high. Furthermore, some *Acrosiphonia* populations may not be solely dependent on endophyte abundance and survivorship, *i.e.* vegetative propagation or dispersal as discussed earlier may affect the distribution and abundance of *Acrosiphonia*.

Endophyte densities, particularly 'Chlorochytrium' densities, unexpectedly decreased after onset of colonisation (Figs. 2.5B, 2.6B, 2.11B, 2.12B). Two possible explanations are 1) *Mazzaella splendens* blades increase in size as the summer progresses causing endophyte densities to decrease correspondingly, and 2) excessive crowding of 'Chlorochytrium' cells results in the mortality of some cells. The first explanation can be ruled out, because seasonal abundance patterns generated from absolute numbers of 'Chlorochytrium' and from densities of 'Chlorochytrium' were similar, *i.e.* 'Chlorochytrium' abundance actually decreased in summer. I therefore propose that the decrease in 'Chlorochytrium' cells may be due to mortality of cells caused by crowding of neighbouring cells. During the summer, especially at Sooke, 'Chlorochytrium' cells were often seen touching one another (Fig. 2.7A). High densities of

'Codiolum' were also observed (Fig. 2.9B), but more space seems to be available among the filaments of 'Petrocelis' than among the cells of the cortex region of *M. splendens*. Greater knowledge of *M. splendens* growth rate, and isolation of other factors which may affect endophyte survivorship are fundamental to a better understanding of 'Chlorochytrium' and 'Codiolum' survival.

***Selection of heteromorphy in* Acrosiphonia**. Speculation on selective mechanisms for maintenance of heteromorphy in haplodiploid life cycles (Stebbins & Hill 1980, Valero *et al.* 1992, Klinger 1993) has resulted in two hypotheses. The first proposes that the two morphologically distinct phases differ with respect to their tolerance of environmental conditions (Conway *et al.* 1976). The second suggests that the phases represent mutually exclusive adaptations to fluctuations in grazing pressure, *i.e.* the upright phase is adapted to counter increased competition (growth and reproduction) when grazing pressure is low, and the non-upright phase is adapted to survive high grazing pressure by being grazer resistant or exploiting micro-habitats which offer protection from herbivory (Lubchenko & Cubit 1980, Slocum 1980, Littler & Littler 1980).

This study, carried out in the field at three environmentally different sites, has clearly shown that the morphologically different gametophytic and sporophytic phases of *Acrosiphonia* occur in different environmental conditions. The filamentous gametophyte is seasonally abundant [March - June / July (October)]. Based on culture results, *Acrosiphonia*'s seasonality may be dependent on abiotic factors such as photoperiod, light intensity, desiccation and temperature. The endophytes, 'Chlorochytrium' and 'Codiolum' show high tolerance to environmental extremes. They survive high summer temperatures (which appear to ultimately kill the filamentous *Acrosiphonia* plants), and spend the winter (possibly too adverse for *Acrosiphonia*, which seems to require high light intensity for gamete production and long-day conditions for optimal growth) within *Mazzaella splendens* and 'Petrocelis franciscana'. The host may offer protection from the physical environment, as well as from herbivores and competition from other algal species.

With regard to *Acrosiphonia*'s life history representing an adaptation to variable grazing pressure, *i.e.* during high herbivore pressure the filamentous gametophyte has disappeared, while the endophytes are protected within their hosts, no empirical data are available on differential susceptibility of *Acrosiphonia*, *M. splendens* and 'Petrocelis' to grazing. Laboratory feeding

experiments and / or herbivore exclusion experiments may help to determine whether 1) herbivory is a factor in the seasonal distribution and abundance of *Acrosiphonia* and 2) grazing pressure is an evolutionary force in the maintenance of *Acrosiphonia*'s heteromorphic life history.

This study also demonstrates *Acrosiphonia*'s success (and adaptability) in different environments. Present at three study sites, characterised by differences such as wave exposure, salinity and timing of summer low tides, *Acrosiphonia* plants showed variable seasonality (*e.g.* persisted over a longer period in more wave-exposed environments) and abundance (dependent on factors such as local weather, micro-habitat factors and survivorship of endophytes). In addition, variable abundance of the two morphological species, *A. arcta* and *A. coalita*, at different tidal heights, and *A. coalita*'s absence at Burrard Inlet, suggests adaptations at the species level, and *Acrosiphonia*'s appearance in new areas from year to year implies its ability to successfully colonise new habitats / sites. 'Chlorochytrium' DNA sequences which shared a genotype with *Acrosiphonia* sp. (Alaska), and the unique *Acrosiphonia* sequence (specimen collected from the large boulder at Sooke, see Chapter 1), hint at colonisation of *Acrosiphonia* "species" other than *A. arcta* and *A. coalita*, *e.g. A. sp.* (Alaska) and /or *A. sp.* (Sk5), and / or adaptability of *Acrosiphonia* to new habitats.

The role the endophytic sporophytes play in *Acrosiphonia*'s unique life history is still poorly understood, and many fundamental questions remain unanswered. For example, it is unknown whether the sporophyte phase is actually required for the maintenance of *Acrosiphonia* populations in southwestern British Columbia. Perhaps *Acrosiphonia* can successfully recycle through rhizoid overwintering of gametophytic plants or by gametophytic plants giving rise to haploid zoospores (only shown in culture) which survive the winter. If so, the relative contribution of sexual reproduction (and the role of sporophytes) may be less significant compared to asexual reproduction and / or vegetative propagation in *Acrosiphonia*'s life history. In some algae, *e.g.* the brown alga, *Analipus japonicus* (Harvey) Wynne, and the coralline alga, *Lithothrix aspergillum* Gray, loss of sexual reproduction may actually occur at the northern range of the species (Nelson 1980, DeWreede & Vandermeulen 1988). Also of interest is the question of whether the unicellular sporophytes can survive epilithically or planktonically. Given the evolution and maintenance of this alternation of heteromorphic generations, what is the adaptive significance of sporophytes evolving an endophytic condition? To experimentally test in nature whether the sporophyte phase (endophytic or free-living) is required for the maintenance of

Acrosiphonia populations, patches in the intertidal zone could be cleared / sterilised in the fall. The appearance of juvenile *Acrosiphonia* plants in the spring would suggest they are derived from zoospores of the unicellular sporophyte (provided all putative rhizoids were removed and disregarding the possibility of asexual reproduction in the field). Of course, should no juvenile *Acrosiphonia* appear, this would not mean the sporophyte phase is insignificant in *Acrosiphonia*'s life history, since patchy distribution of *Acrosiphonia* in the present study suggests patchy zoospore dispersal.

The presence of 'Chlorochytrium' and 'Codiolum' at all three study sites, although variable in abundance from year to year, suggests to me the endophytes are successful at colonisation. At Bamfield much lower endophyte densities imply lower success - it may be more difficult to colonise blades or crusts in a more wave-exposed environment - such that asexual or vegetative means of reproduction may contribute (more) to maintenance of *Acrosiphonia*. Consequently, and considering the lack of evidence to support alternate life histories for *A. arcta* and *A. coalita* in the field [*e.g.* Kornmann (1962) has shown sexual reproduction in cultured *A. sonderi* to be absent, and the 'Codiolum' phase of *A. grandis* in culture to develop directly into the filamentous gametophyte (1970a)], success of this heteromorphic life history seems apparent.

Heteromorphy can be seen as a bet-hedging strategy, whereby different phases are adapted to a seasonally variable environment or variable grazing pressure. With regard to *Acrosiphonia*, not only have two phases evolved, but molecular data (Chapter 1) have now shown that the sporophytic phase of at least *A. arcta* can colonise two alternate hosts (crustose and foliose red algae). The availability of these hosts is another important factor in *Acrosiphonia*'s life history, and will be explored in the following chapter.

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CHAPTER 3

HOST AVAILABILITY FOR COLONISATION AND SURVIVAL OF 'CHLOROCHYTRIUM' AND 'CODIOLUM'

INTRODUCTION

Two red algal hosts commonly provide a habitat for the unicellular sporophytes 'Chlorochytrium' and 'Codiolum' in British Columbia. One is the foliose alga, *Mazzaella splendens*, conspicuous on rocky shores in the low intertidal of the northeast Pacific; the other the crustose alga, 'Petrocelis franciscana', predominant throughout the intertidal of the southern British Columbia coast. In general, the host organism provides the specific ecological niche required by the endophyte. Benefits to the endophyte may include providing colonisable substrate, ensuring its exposure to light and nutrients and escape from harsh environmental conditions and potential herbivores. *Mazzaella splendens* and 'P. franciscana' appear to be ideal hosts: they are present in high abundance presumably year round [pers. observation and Paine *et al.*'s (1979) study on the longevity of 'Petrocelis'], providing relatively high surface area for maximum colonisation, exposure to light and nutrients and survival. But are they in fact ideal hosts? What is known about variation in host abundance from year to year or month to month? And can variable host abundance play a role in endophyte survival?

Since many algae exhibit marked seasonal changes in abundance, it is important to establish whether host seasonal patterns exist. Some work has documented seasonal changes in *Mazzaella splendens* density (Dyck and DeWreede 1995, F. Shaughnessy pers. comm.) along the coast of southwestern British Columbia, but little is known about the ecology of 'Petrocelis'. Paine *et al.*'s (1979) extensive study examined growth patterns and longevity of 'Petrocelis' found on a sandstone wall in Washington, but no studies have closely examined 'Petrocelis' seasonal abundance on boulder strewn shores. My study quantifies monthly abundance of *M. splendens* (over two years) and of 'Petrocelis' (over 12 and 18 month periods at different sites) in order to elucidate any seasonal patterns, and considers possible factors in producing such patterns. Finally, 'Chlorochytrium' and 'Codiolum' survival are assessed in relation to availability of *M. splendens* and 'Petrocelis', and I speculate on the length of time the endophytes remain in their hosts and why this strategy may have evolved.

MATERIALS AND METHODS

Host availability for 'Chlorochytrium' and 'Codiolum' was examined at Burrard Inlet, Sooke and Bamfield. As mentioned in Chapter 2, Bamfield was sampled less intensively. Two investigations using seawater tanks were carried out to examine 'Chlorochytrium' survival in dislodged *Mazzaella splendens* blades.

'Petrocelis' surface area. 'Petrocelis' crusts were sampled at Sooke from May 1997 to November 1998 and at Burrard Inlet from August 1997 to August 1998. Sampling occurred approximately monthly within the low to high intertidal (between 0.2 to 5.1 m above the Canadian Chart Datum). Thirty granite boulders were haphazardly chosen (10 each from the low, mid and high intertidal) from the total number occupied by 'Petrocelis' crust. Each boulder was marked with West System® epoxy (Gougeon Brothers Inc., Bay City, MI) after an area had been cleared and dried with a propane flame torch. Within each epoxy patch (variable in size but on average 5 cm in diameter) a numbered Dymo® I.D.™ ribbon was embedded. In this way it was possible to identify each of the 30 'Petrocelis' crusts. Weathering by water motion, however, resulted in ribbon numbers not being legible over time, so that crusts were then only identifiable by the unique shape of the epoxy marker. Repeated sampling of known crusts was chosen rather than random sampling as with *M. splendens* blades, because I wished to determine the persistence and performance of specific crusts, *i.e.* are changes in patch size consistent for all crusts? To facilitate locating boulders, especially when low tides occurred after dark, ¼ inch rebar marked with flagging tape was pounded into the ground at each corner of the sampling area at Sooke. The total area measured approximately 20m x 20m. At Burrard Inlet the area sampled was bounded by the seawall and measured about 10m x 20m.

Sampling at Burrard Inlet began later than at Sooke, because dense overgrowth of boulders by juvenile barnacles, mussels and colonial diatoms made it difficult to locate 30 boulders with 'Petrocelis' patches from May to August 1997.

On each sampling day all 'Petrocelis' crusts were photographed with 100 ASA Kodak film (Eastman Kodak Co., Rochester, NY); flash photography was used at night . A five cm ruler was placed next to the crust for scale. Care was taken to photograph perpendicular to the crust and to select crusts with as little parallax as possible. It was not always possible to locate and photograph all 30 crusts, since boulders became overgrown with other algae and sessile invertebrates (especially *Ulva* and *Fucus* and juvenile barnacles and mussels in spring and

summer) making it difficult to locate the epoxy markers. In addition, with time epoxy markers deteriorated to the point where by November 1998 (after 20 months of monitoring) only 23 markers could still be found at Sooke. This was particularly true at Burrard Inlet, where overgrowth by juvenile barnacles contributed to the loss of epoxy markers: only one year after boulders had been marked, ten epoxy markers could no longer be located.

The photographic series also permitted examination of limpet abundance, barnacle and mussel settlement and the establishment of new crusts. Twelve *Tectura scutum* limpets were collected from Sooke in February 1997 for gut analysis as possible evidence for herbivory.

'Petrocelis' crusts could not be adequately monitored at the Bamfield site, because rocky land masses rather than boulders comprise the shore, and the 'Petrocelis' usually overlaps with both *Ralfsia* and *Hildenbrandia*, making it difficult to mark and identify individual 'Petrocelis' patches. Instead, the condition of the crusts was monitored, noting evidence of new growth (thickness and colour) and the presence of green colouration and unhealthy-looking tissue. The presence or absence of limpets on 'Petrocelis' was also recorded.

Mazzaella splendens *density*. Sampling of *Mazzaella splendens* occurred over a two year period from October / November 1996 to October / November 1998, and was monthly except during those periods where tides were not low enough to expose *Mazzaella* or blades were very scarce. Both the condition, *i.e.* health and degree of epiphytisation, and density of *M. splendens* was monitored. At each site a 12 m transect line was randomly placed, parallel to the sea, in an area where *M. splendens* was present. The same area, between 0.2 and 1.0 m above zero tidal level (Canadian Chart Datum), was sampled each time. A random number table was used to choose eight points along the length of the transect. These points were permanently marked with flagging tape, and served as points for the point-quarter distance method of density estimation (Krebs, 1999).

***'Chlorochytrium' survival in drift* Mazzaella splendens *blades*.** Drift *Mazzaella splendens* blades collected at Bamfield were found to be colonised by 'Chlorochytrium', prompting the question of 'Chlorochytrium' survival in dislodged blades. The following two investigations were conducted (1) Twelve *M. splendens* blades colonised by 'Chlorochytrium' (average size 120-160 μm) were collected from the low intertidal at Sooke in November 1998. The blades were left in a laboratory seawater tank with constant flow at 11°C under 12 μmol m^{-2}

s^{-1} of cool white light and examined every 7-10 days for two months to determine 'Chlorochytrium' condition, *i.e.* average size, health and whether fertile. Observations were not made beyond two months due to disintegration of algal blades. (2) *Mazzaella splendens* blades were collected from the low intertidal at Bamfield in December 1998. Those blades colonised by 'Chlorochytrium' were placed in an outdoor tank (approximately 1.5 m x 1.5 m; steady flow, water temperature from 6-8°C and natural light). Plexiglas plates (approximately 20 cm x 20 cm) were positioned on the bottom of the tank to establish whether zoospores would be released and settle on the plates. A neighbouring tank lacking *M. splendens* blades served as a control tank. The tanks were checked one and two months later.

Data analysis. All 'Petrocelis' crust photographs were scanned and enhanced (painted black or white to contrast with the background) in Adobe Photoshop® 5.02 for Windows (1998). The imaging program SigmaScan Pro® 5.0 for Windows (1999) was then used to calculate the surface areas of crusts for each sampling date. Images were calibrated with the 5 cm ruler present in each image. For each time period an average value was calculated for 'Petrocelis' crust surface area. Seasonal differences in mean crust surface area were determined by locating maximum (highest surface area) and minimum (lowest surface area) points from the graphed data. These points corresponded to summer / fall (maxima) and winter (minima). Significance of differences between pairs of maximum / minimum points was tested using an independent samples *t* test. Transformations of some of the data did not reduce heterogeneity of variances to nonsignificant levels [Levene Test, SPSS® 9.0 for Windows (1999)], but the Kolmogorov-Smirnov Test showed normality. Furthermore, the *t* test is generally robust to variance heterogeneity (Zar, 1996), and the independent samples *t* test in SPSS® 9.0 for Windows (1999) includes an option for unequal variances which I used. Seasonality of 'Petrocelis' abundance and 'Codiolum' density were compared.

Mean densities of *Mazzaella splendens* were plotted against time, and significance of seasonal differences determined by checking for overlap of 95% confidence intervals. *M. splendens* seasonality was compared to seasonal densities of 'Chlorochytrium' to assess availability as a host.

RESULTS

'Petrocelis' seasonal abundance. Marked seasonal fluctuations in 'Petrocelis' abundance were evident both at Sooke and Burrard Inlet. 'Petrocelis' abundance peaked in summer / early fall, and was at its lowest in the winter at both sites. This seasonal change was visible both from the photographic time series and from plotting mean surface areas against time (zero values for surface area were omitted from calculations). From October 1997 to January / February 1998 mean crust surface area decreased by 43 % at Burrard Inlet (Fig. 3.1A) and by 58 % at Sooke (Fig. 3.2A); during the 6 month period from January / February 1998 to July / August 1998 crust surface area increased by 45 % at Burrard Inlet and 61 % at Sooke. These represent significant differences (all p's $\leq .05$, Table 3.1).

Burrard Inlet crusts. 'Petrocelis' crusts at Burrard Inlet at the start of observation ranged from 1.95 to 20.49 cm^2 with a mean surface area of 6.29 (1 S.E. = 1.15) cm^2. Crust distribution was much patchier than at Sooke, and in general 'Petrocelis' abundance was notably lower. Within one year 39% of the crusts for which epoxy markers could still be found had disappeared. The mean net change in surface area in one year was - 20%, reflecting this high mortality (Fig. 3.3A). This may in part be attributed to dense settlement of juvenile barnacles on crusts (9/18 crusts in August 1998 were partially or totally covered by barnacles) as shown in Figure 3.4A. It is not clear if crust tissues can persist beneath barnacle settlement, because barnacles were not removed from crusts. Among the 61% of crusts which survived during the observation period, mean net change in surface area in one year was + 30%. Change in surface area of individual crusts could not be examined, due to the theft of field notes which identified the individuals.

Sooke crusts. Crust patches at Sooke were larger, from 2.58 to 46.4 cm^2 with a mean surface area of 14.31 (1 S.E. = 1.75) cm^2. At Bamfield 'Petrocelis' crust size was difficult to interpret due to overlapping with other crusts. Nonetheless, on average, patches were much larger than at Sooke, reaching sizes greater than 100 cm^2. After 18 months, of those crusts still identifiable at Sooke, 55% showed a net increase, 17% a net decrease and 28% had disappeared. This translates to a mean net change in surface area of - 24%, again reflecting the mortality of individual crust patches (Fig 3.3B) as was the case for Burrard Inlet. However, among the crust individuals which did not go extinct, the mean net change in surface area is +21%. Although a considerable amount of variation exists among the individual crust patches (variances are high),

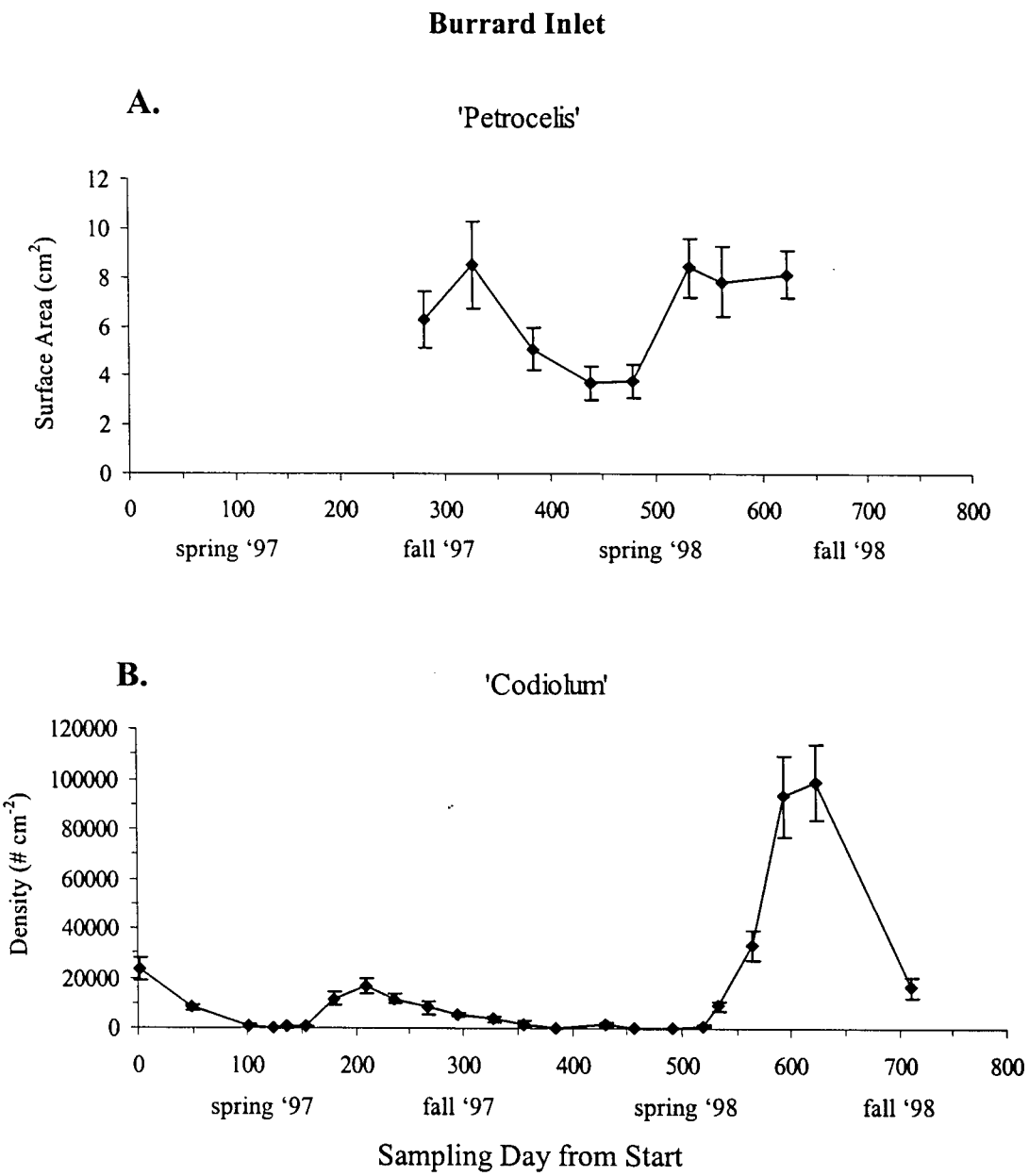

Figure 3.1 A. Surface area of 'Petrocelis' crust at Burrard Inlet over 1 year. Data are means ± S.E. from 26 crusts. Surface area values of zero were omitted from the calculation of the mean. **B.** ' Codiolum' densities at Burrard Inlet. Data are means ± S.E. from cells counted in 30 'Petrocelis' patches.

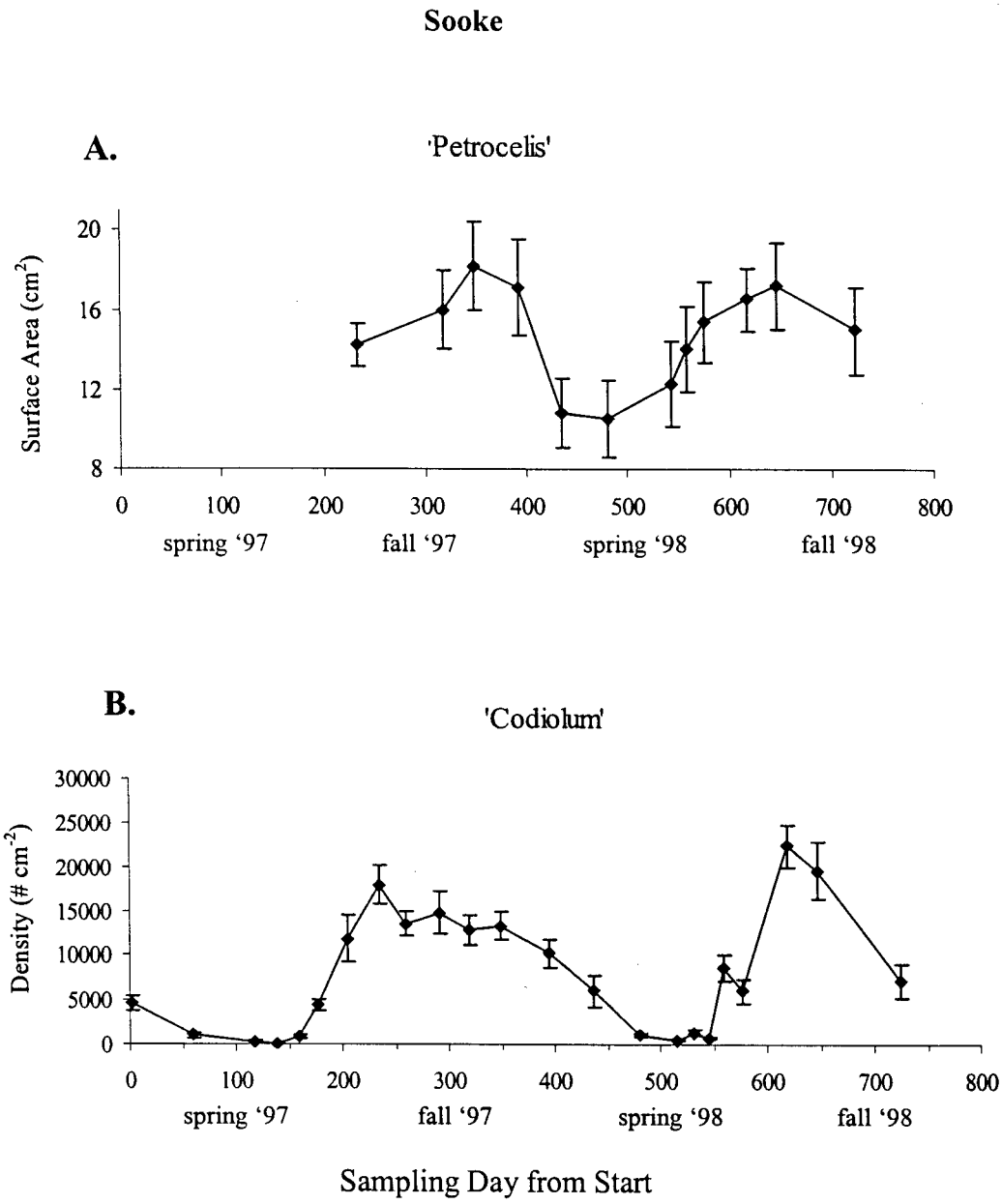

Figure 3.2 A. Surface area of 'Petrocelis' crust at Sooke over 18 months. Data are means ± S.E. from 30 crusts. Surface area values of zero were omitted from the calculation of the mean. **B.** ' Codiolum' densities at Sooke. Data are means ± S.E. from cells counted in 30 'Petrocelis' patches.

Table 3.1 Seasonal differences of 'Petrocelis' crust surface area. Results of independent samples *t*-test performed on pairs of maximum and minimum data points shown in Figures 3.1A and 3.2A. The significant *t* value indicates a statistically significant difference among maximum and minimum surface area values. Note that pairs of points correspond to (1) (summer) fall and winter and (2) winter and summer.

Burrard Inlet	n	Mean	S.D.	S.E.	*t*	p
Oct. 1997	26	8.5240	8.9499	1.7552	2.555	0.016
Feb. 1998	17	3.7070	2.8374	1.8853		
Feb. 1998	17	3.7070	2.8374	0.6882	3.873	0.001
Aug. 1998	11	8.1798	3.2049	0.9663		
Sooke	N	Mean	S.D.	S.E.	*t*	p
Oct. 1997	28	18.1921	11.7437	2.2193	2.619	0.012
Jan. 1998	26	10.9112	8.5354	1.6739		
Jan. 1998	26	10.9112	8.5354	1.6739	2.351	0.024
July 1998	14	16.3614	6.0001	1.6036		

on average 'Petrocelis' crust surface area decreases significantly in winter and increases significantly in summer (Fig. 3.2A). No definitive relationship between crust size and change in surface area was noted, except that the smallest individuals (2.5-3.5 cm^2) tended to undergo little fluctuation in surface area, instead exhibiting slow and steady growth in size.

Crust dynamics and invertebrate observations. On average crusts both at Sooke and at Burrard Inlet grew during the summer and early fall of 1997 as illustrated in the time series in Figure 3.5. New growth of 'Petrocelis' was also apparent at Bamfield in the summer. In the field older parts of the crust were observed to be lighter in colour and thinner. Limpets were rarely present in crust photographs, nor observed in any abundance in the field during the summer at all three sites.

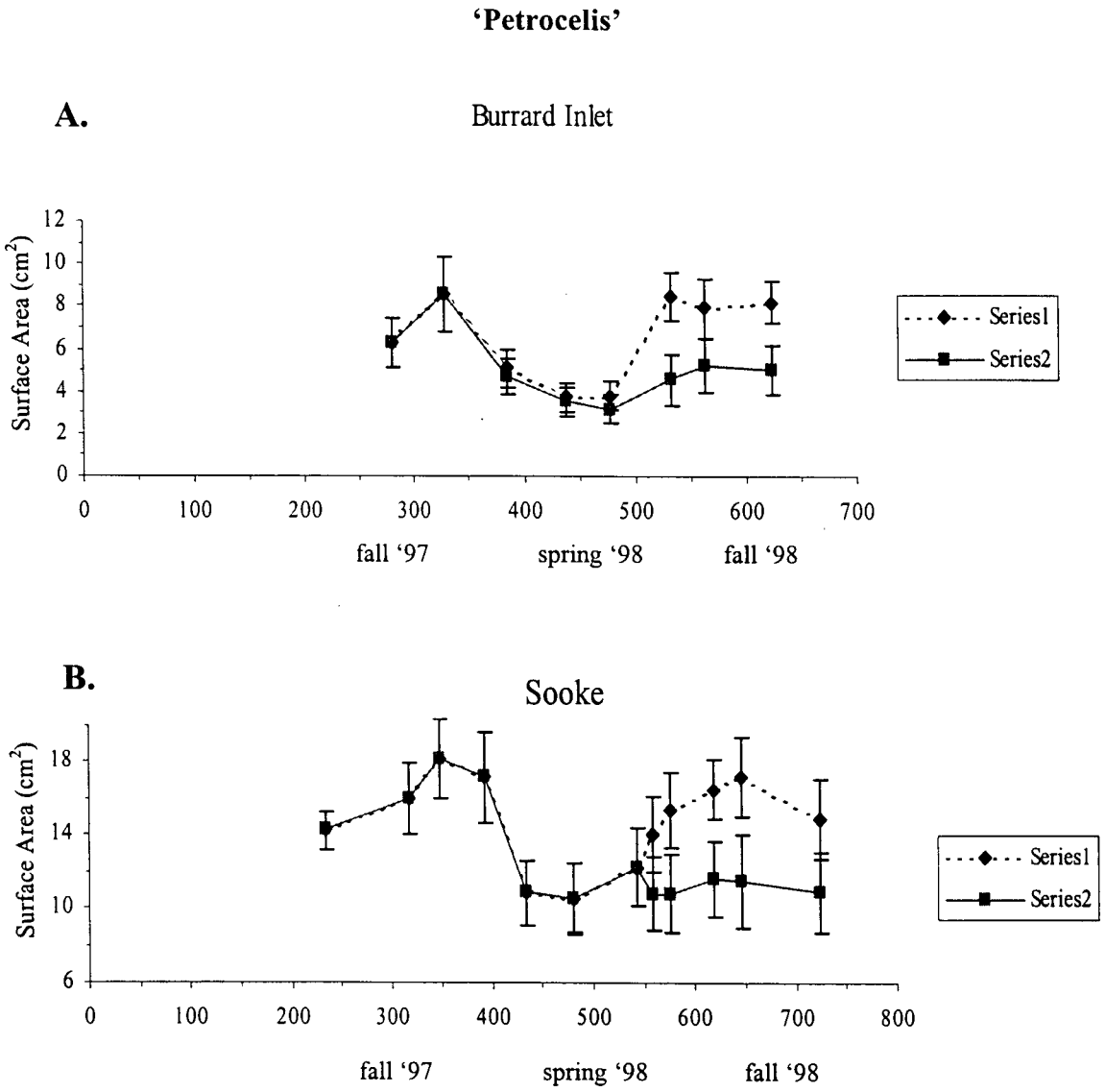

Figure 3.3 Surface area of 'Petrocelis' crust patches at Burrard Inlet (**A**) and Sooke (**B**). Series 1 illustrates change in surface area of crusts where values of zero were omitted. Series 2 includes zero surface area values, *i.e.* crusts which disappeared during the observation period, to illustrate crust survivorship. Data are means ± S.E. from 26 (**A**) and 30 (**B**) crusts.

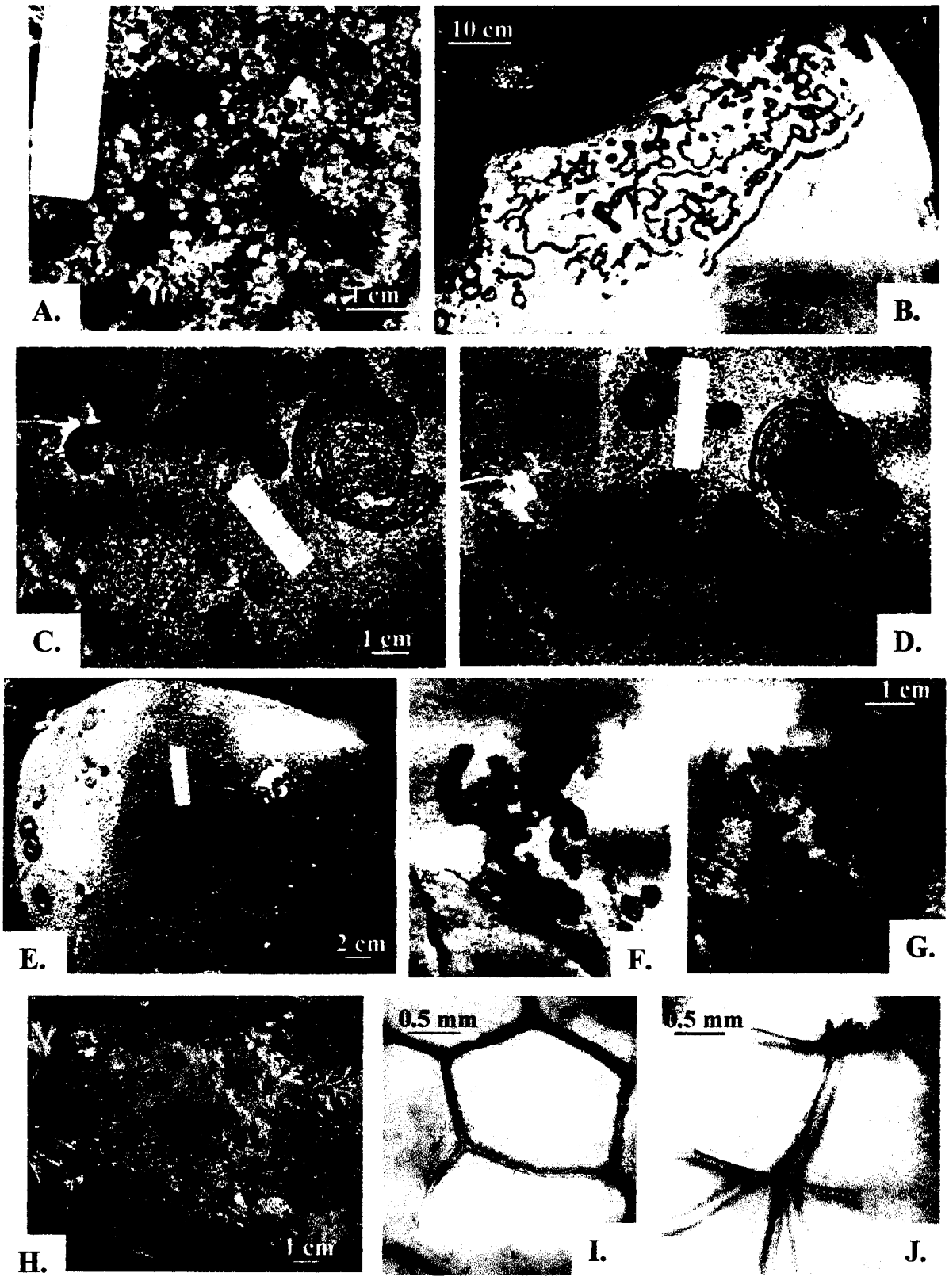

Figure 3.4 A. Barnacle settlement on 'Petrocelis' at Burrard Inlet. **B**. Intricate crust pattern at Sooke. **C. D**. Limpet abundance and notable tissue loss from Oct. to Nov. **E.** Fragmentation of single crust? **F. G. H**. Green, mucilaginous tissue. **I. J.** "Vein" pattern on *Mazzaella splendens.*

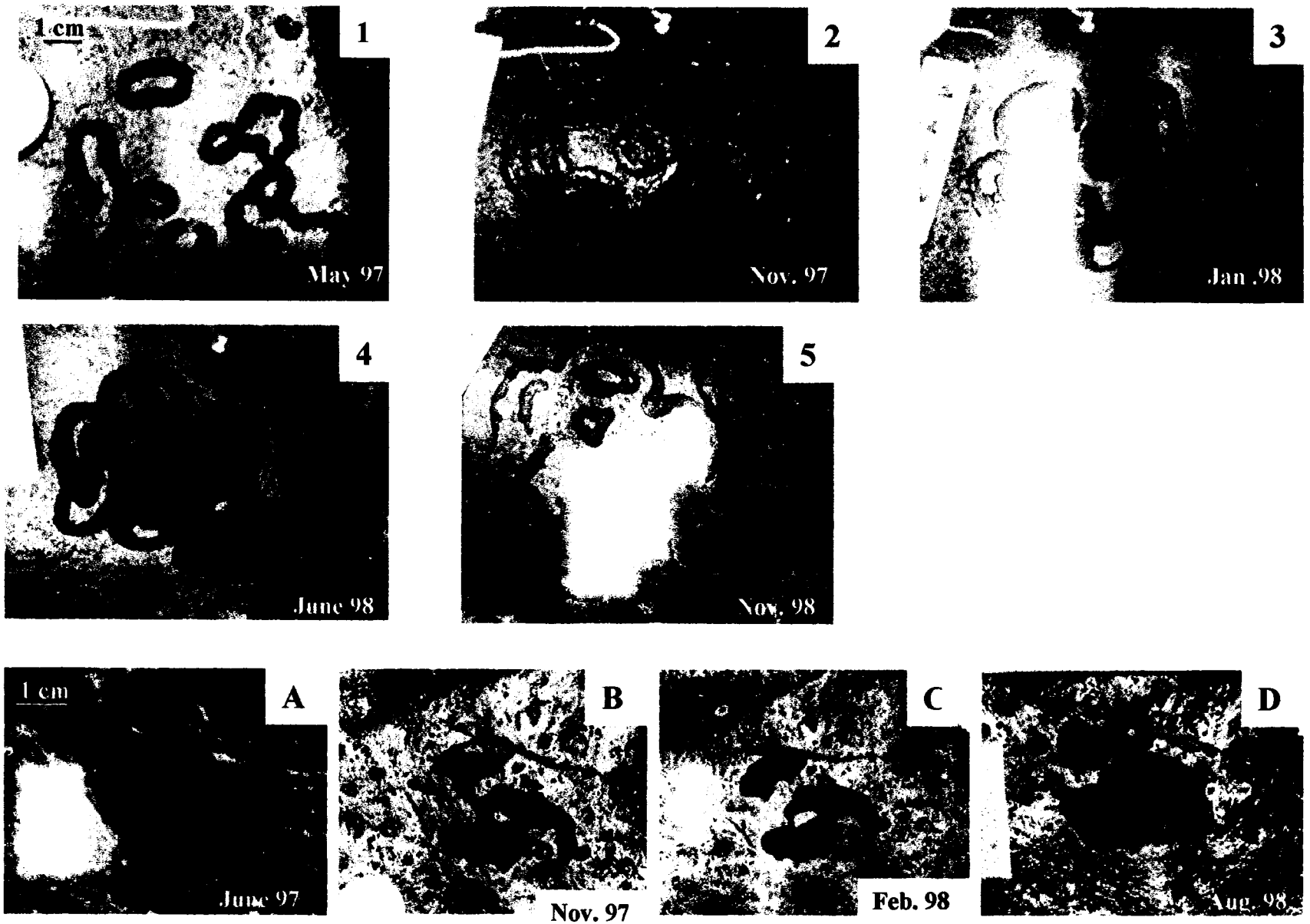

Figure 3.5 Time series of two 'Petrocelis' crusts at Sooke.

Following this growth period, in the winter, 80% of individuals at Sooke decreased in crust surface area or experienced patch fragmentation. This was also the pattern for the average of 30 crusts at Sooke and Burrard Inlet. In general, it took patches a minimum of 6 - 8 months to begin to disintegrate, but loss of crust tissue could easily be seen from one month to another. At Sooke primarily central tissue seemed to be lost, with perimeter tissue more persistent. This was not as evident at Burrard Inlet where crusts were smaller and barnacle overgrowth made it difficult to assess growth patterns. The limpets *Tectura scutum* and *Tectura persona* Rathke were found in abundance at Sooke and Burrard Inlet, and often observed on 'Petrocelis' crust, from October to January and during this time photographs often revealed loss of crust tissue from one month to the next (Fig. 3.4C,D). Densities of 160 limpets m^{-2} were estimated from Sooke crust photographs taken in October and November 1997. Crust patches with intricate patterns such as illustrated in Figure 3.4B were not uncommon at Sooke. Gut analyses of *Tectura scutum* limpets revealed a multitude of diatoms, filamentous red and green algal fragments, foraminiferans and possibly crust cellular components. No distinctive 'Petrocelis' filaments could be identified. Limpets, as well as the chiton, *Katharina tunicata* were commonly seen on the rocky shores at Bamfield during the winter.

Some fragmented crust patches began to regenerate lost tissue (most had not fully regenerated by the end of the monitoring period) in the spring and summer. Other fragmented patches continued to lose tissue, decaying until eventually none of the original patch remained. New growth of crust tissues was also observed. On a few occasions at Sooke it was difficult to assess whether the original marked crust had actually been a remnant of an older larger, continuous patch, parts of which still existed on a different area of the boulder (Fig. 3.4E). At Burrard Inlet difficulty in determining crust mortality stemmed from dense overgrowth by barnacles obscuring crust patches. Of those crusts at Sooke which underwent another growth period in the spring and summer of 1998 (64%), a few produced intriguing time series patterns as a result of variable loss and regrowth of tissues. Often internal spaces (loss of central tissue of a crust) became filled in with new tissue and subsequent loss of tissues resulted in a negative-image effect [Fig. 3.5 (**4**,**5**)].

Health of crusts. Green-coloured, mucilaginous surface tissue (Fig. 3.4F,G,H) was noted for a number of crusts in July and August of 1998 at all three sites. Most individuals appeared healthy-looking again by fall 1998, but a few seemed to continue to decay. This was

less evident at Bamfield, because individuals could not be identified. The phenomenon had also been recorded (to an even greater extent with many crusts disintegrating) in the winter of 1996 prior to the photographic time series. Smallest individuals, however, were never observed as greenish or mucilaginous.

At Bamfield olive-green crust existed intermingled with the darker red/brown 'Petrocelis'. Unlike the green mucilaginous crust this crust appeared healthy-looking, and upon examination, revealed the same tissue structure as 'Petrocelis franciscana'. It has been suggested (R. Scrosati, pers. comm.) that this "healthy" crust may be the tetrasporophytic phase of *Mastocarpus jardinii* West; in culture it strongly resembles 'Petrocelis franciscana' (West *et al.* 1978).

Crust recruitment. During the sampling period only three crust recruits were detected, two of these at Burrard Inlet in July 1998, the other at Sooke in June 1998. One occupied the area on a boulder where a known crust had disappeared, and the other two were observed in the vicinity of a known crust which was still present. From the photograph of the Sooke crust in question, it seems the new crust may be a resurrected crust, *i.e.* it may have been present at an earlier time and have been part of a larger continuous crust, since many fragments were still present on the boulder (Fig. 3.4E). In the spring and summer of 1997 prior to sampling, several new crust patches were noted on the base of the seawall at Burrard Inlet.

Figures 3.1 and 3.2 show that 'Petrocelis' seasonal abundance, *i.e.* highest and lowest mean surface area, corresponds to highest and lowest 'Codiolum' densities in these crusts at Sooke and at Burrard Inlet.

Mazzaella splendens *seasonal abundance and condition of blades.* Seasonal changes in density of *M. splendens* were similar for Burrard Inlet, Sooke and Bamfield sites (Figs 3.6, 3.7, 3.8). The highest densities occurred in the summer months, corresponding to the highest 'Chlorochytrium' densities in these blades for Sooke, Burrard Inlet (summer 1998 only) and Bamfield (summer 1998 only). This pattern was less evident for Bamfield. During the winter months *M. splendens* densities were lowest (and significantly lower than summer densities based on 95% confidence intervals not overlapping). In fact at Sooke and Bamfield, densities of $< 0.1/m^2$ and absence of blades in the intertidal were recorded; at Burrard Inlet blades appeared to persist year round, although markedly decreased in number. 'Chlorochytrium' densities were

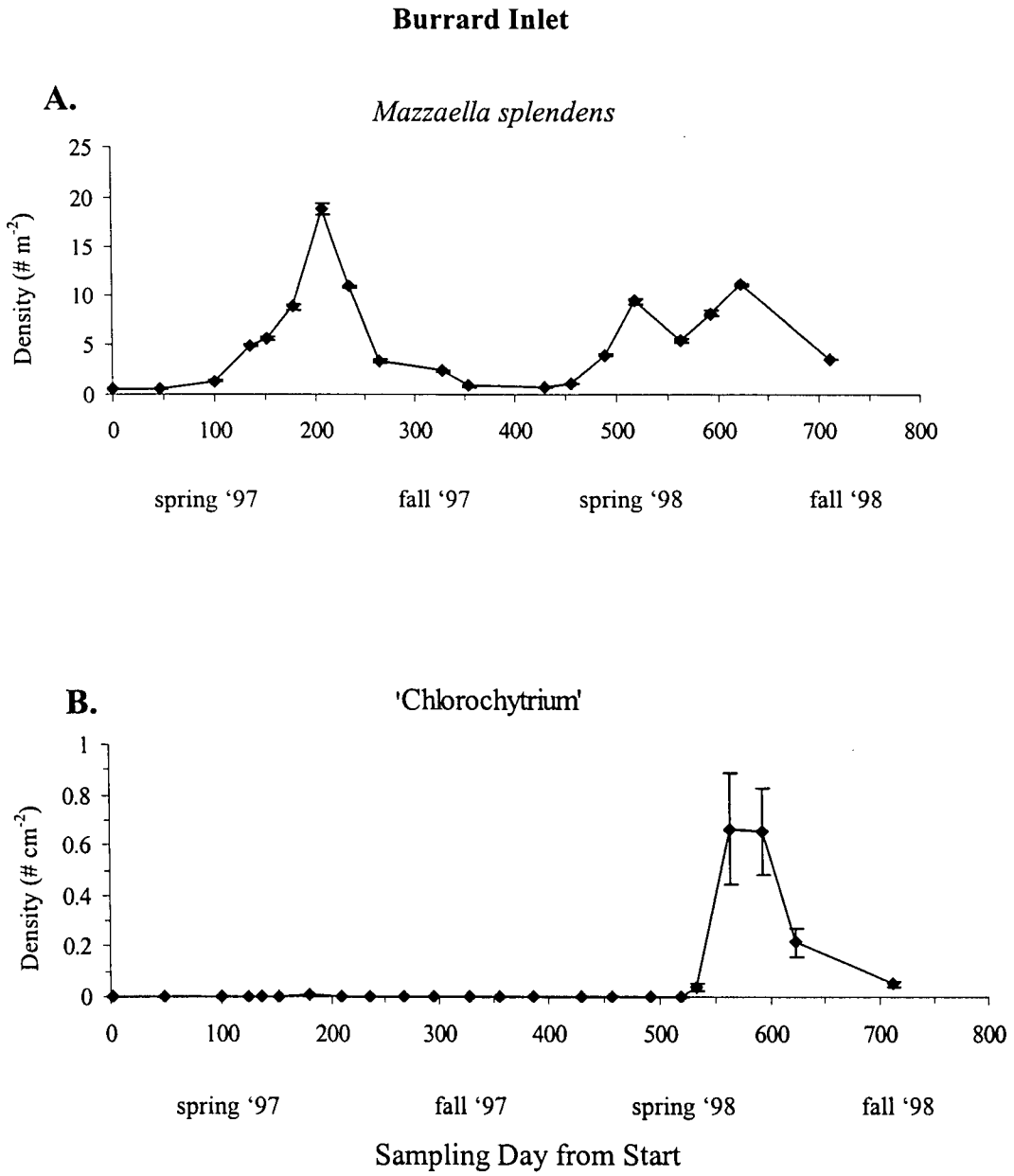

Figure 3.6 A. *Mazzaella splendens* density ± S.E at Burrard Inlet over two years. The point-quarter method of density estimation was based on locating 8 random points along a transect. **B.** 'Chlorochytrium' densities at Burrard Inlet. Data are means ± S.E. from cells counted in 30 *M. splendens* blades.

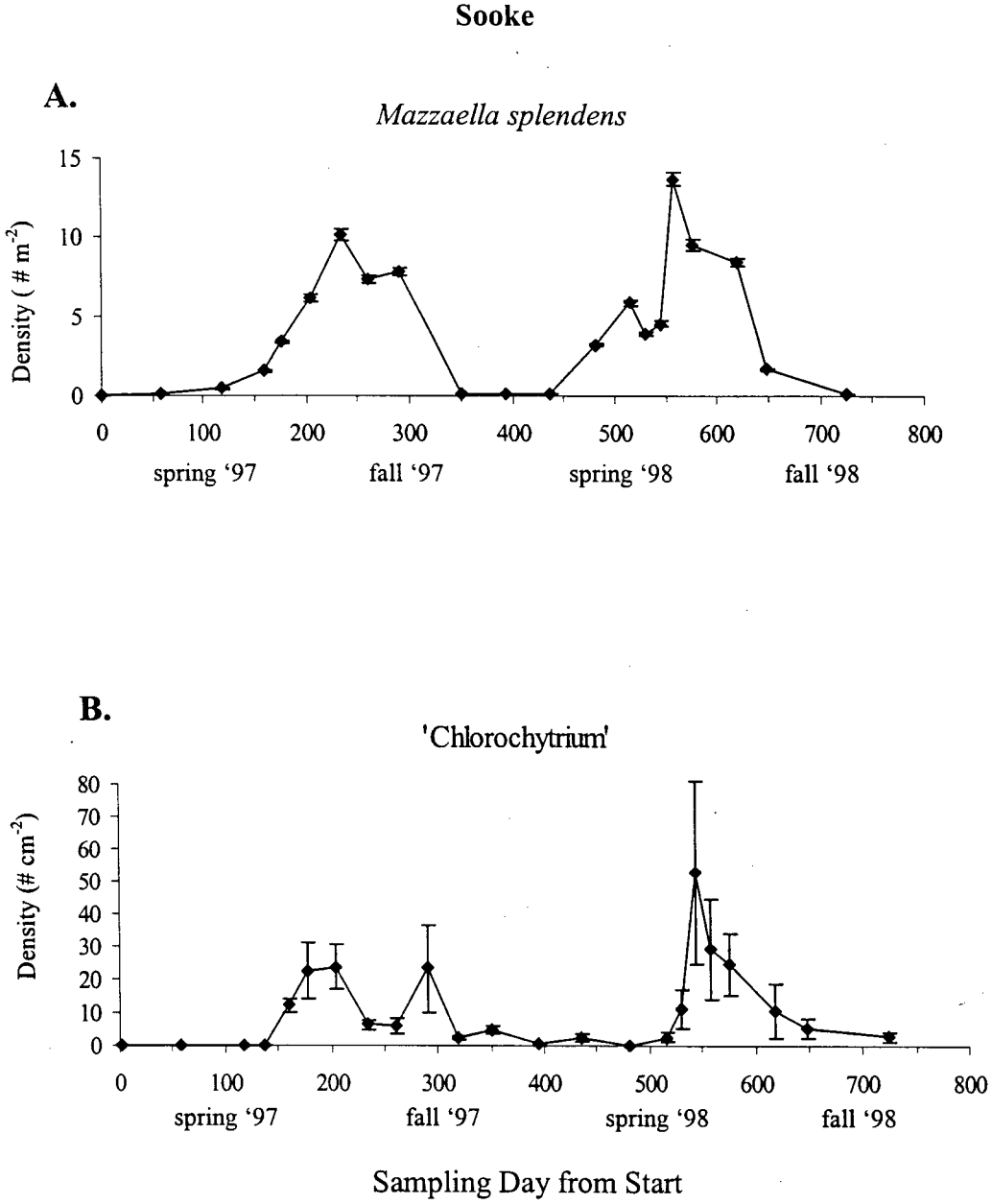

Figure 3.7 A. *Mazzaella splendens* density ± S.E. at Sooke over two years. The point-quarter method of density estimation was based on locating 8 random points along a transect. **B.** 'Chlorochytrium' densities at Sooke. Data are means ± S.E. from cells counted in 30 *Mazzaella splendens* blades.

Bamfield

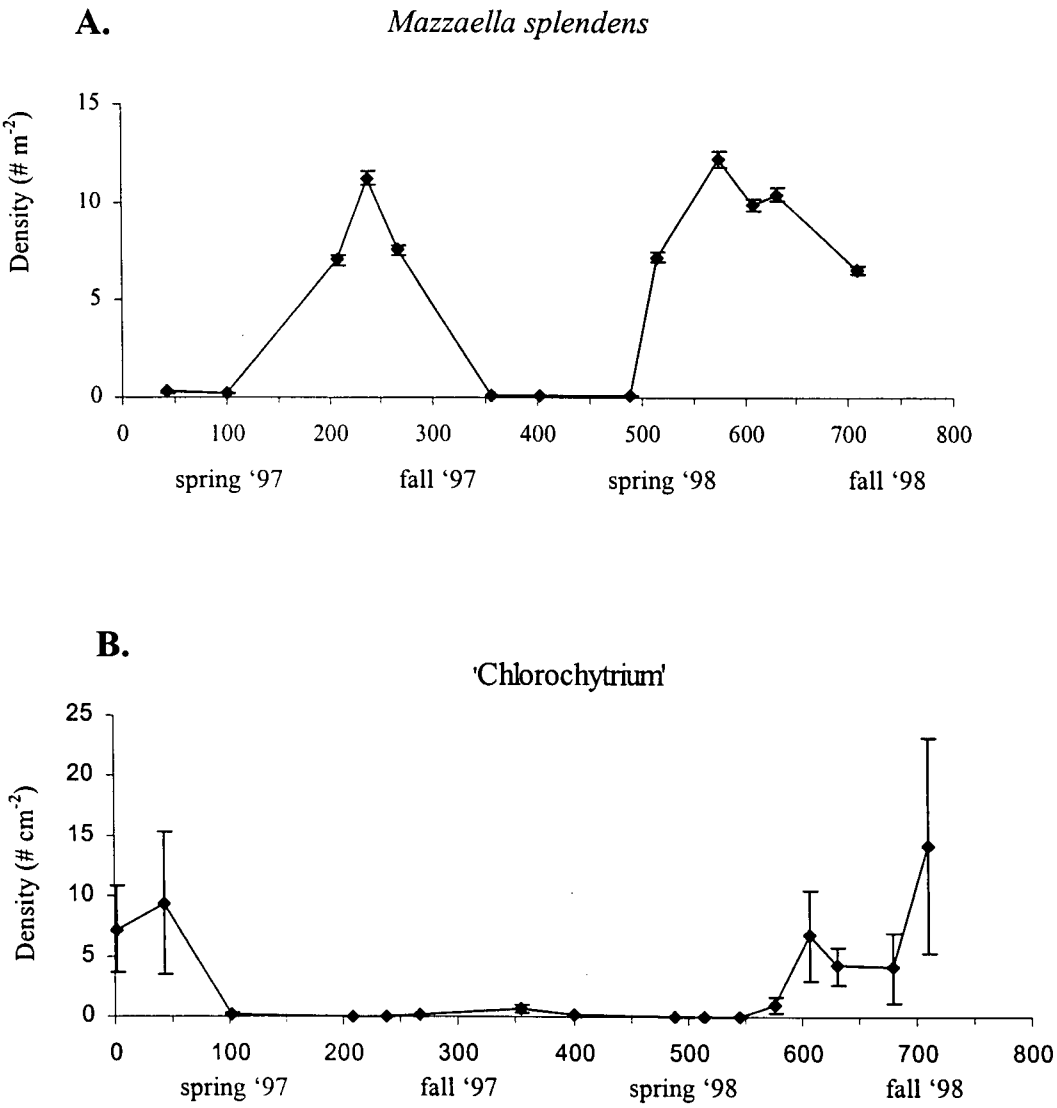

Figure 3.8 A. *Mazzaella splendens* density ± S.E. at Bamfield over two years. The point-quarter method of density estimation was based on locating 8 random points along a transect. **B.** 'Chlorochytrium' densities at Bamfield. Data are means ± S.E. from cells counted in 30 *Mazzaella splendens* blades.

lowest late fall to early spring.

A total of 1840 *Mazzaella splendens* blades were microscopically examined over two years, and a number of observations regarding the seasonal condition of thalli was recorded (see Table 3.2 for a detailed account).

Table 3.2 Seasonal condition of *Mazzaella splendens* thalli.

Site	Blade condition	Epiphytes/-zoans	Littorinids	Other
Burrard Inlet	bleached blades (May '96/'97/'98, July/Aug. '98) tattered / "chewed" blades (Aug - Jan)	*Microcladia* *Enteromorpha* bryozoans diatoms juvenile mussels	+	"veins"[1] 30-70 % Apr - Nov '98
Sooke	bleached blades (Apr./Aug.'98) tattered / "chewed" blades (Aug-Jan) few blades Dec-Jan	*Enteromorpha* bryozoans diatoms	-	"veins"[1] 20-30 % Apr - Nov '98
Bamfield	bleached blades (May/Jun '98) tattered/ "chewed" blades (Aug-Jan) few blades Dec-Jan	*Microcladia* (≥50 % Jun-Oct) *Enteromorpha* bryozoans diatoms *Porphyra*	+	"veins"[1] 10-30 % June-Oct '98 *Endodictyon* 1996

[1] see Fig. 3.4 I, J

***'Chlorochytrium' survival in drift* Mazzaella splendens *blades*.** After 7-10 days 50% of the 'Chlorochytrium' cells examined (about 50 cells) from the 12 "drift" *M. splendens* blades from Sooke had darkened at the apex and become olive-coloured with a more uniform texture. Some had developed a protuberance at the apex; pyrenoids were no longer visible and a few cells showed red pigment suggesting the presence of eyespots, as was observed in fertile cells releasing zoospores. None released zoospores. After one month 'Chlorochytrium' cells were still healthy-looking and most had acquired the fertile-looking condition; only two *M. splendens* blades remained healthy-looking. By mid-January (two months in laboratory seawater tank) host blades were in very poor shape, but average 'Chlorochytrium' size had increased to 180-200 μm (from 120-160 μm), cells looked fertile and healthy and zoospores could be seen within cells.

The *Mazzaella splendens* blades colonised by 'Chlorochytrium' which had been placed in an outdoor tank at Bamfield in December 1998 were found dead a month later. When the tank was checked in early February green filaments (1-2 cm long) growing in the tank were identified as *Acrosiphonia arcta*. No green filaments were present in the control tank.

DISCUSSION

***'Petrocelis' dynamics*.** Conspicuous seasonal changes in 'Petrocelis' abundance occur in habitats such as those examined at Sooke and Burrard Inlet, contrary to work conducted by Paine *et al.* (1979) at the entrance to the Strait of Juan de Fuca , near Neah Bay, Washington State, and Dethier (1994) in the San Juan Islands, Washington State. Since 'Petrocelis' has the capacity for rapid growth and regeneration (as shown in the photographic time series, Fig. 3.5), it is necessary to monitor abundance over short time intervals, *i.e.* if individual crusts are only sampled once or twice a year, it may appear to the observer as though crust patches are static, when indeed they may not be. Mean crust surface area at Sooke and Burrard Inlet peaked in summer / early fall 1997 and summer 1998 with the lowest surface area occurring in winter 1997. On average 'Petrocelis' crusts experienced as much as -58% surface area change over the winter and +61% in summer / fall; hence surface area is clearly not static. The net change of +21/30% over 12/18 months for patches which survived the observation period reflects 'Petrocelis' ability for rapid regeneration and vegetative growth. Dethier (1994) found 'Petrocelis' to have the fastest growth

rate (some individuals doubling in size over two years) among crusts sampled in the San Juan Islands, but she did not detect seasonal changes in 'Petrocelis' abundance, perhaps due to infrequent sampling (twice yearly).

Paine *et al.* (1979) followed the fate of individual crusts at 1 to 6 month intervals over 6-7 years. Unlike my study, crusts examined by Paine and co-workers (1979) in general grew much more slowly (4% yr^{-1} increase in surface area) and on average experienced a period of variable growth (about 3 years), a period of size stability (3-4 years) and a terminal period of patch fragmentation. In the present study 'Petrocelis' patches at Sooke and Burrard Inlet fragmented within 6-8 months. Major differences between the two studies which may account for differences in crust performance are habitat type and crust patch size: the average size of patches was much greater ($\geq$ 100 cm^2) at the San Juan Islands site where the habitat consisted of a sandstone wall (occupied by few macroalgae) with 'Petrocelis' found 2.3-2.4 m above the zero tidal datum. At Sooke and Burrard Inlet 'Petrocelis' grew on granite boulders covered by a high diversity of macroalgae (absent in the winter), and was present throughout the intertidal. Paine *et al.* (1979) did, however, note that patches from closely neighbouring sites tended to behave differently, with some patches showing a seasonal change in percent cover. Furthermore, they were surprised by the microtopographic variability in individual crust performance (some increasing in size while others fragmented). This is consistent with results from Sooke: although the average of 30 individuals shows the pattern in Figure 3.2, large variation was evident among individuals. Had it been feasible to follow discrete crust patches at Bamfield, where crust size of individuals was comparable to San Juan Islands crusts, but substratum consisted of consolidated rock, it is possible that patches would have been observed to be more static.

Although individual crust patches were much smaller at Sooke and Burrard Inlet than in the San Juan Islands and Bamfield, several boulders observed at Sooke were covered in crust fragments (Fig. 3.4E). This observation suggests patches on the same boulder may have once made up a single large continuous patch. There are of course other explanations such as habitat type and age of crust patches which may account for smaller patch size at Sooke and Burrard Inlet. "Resurrections" were suggested by this and the study of Paine *et al.* (1979), and are thought to occur as a result of algal tissue remaining among the rock matrix after crust patch disintegration (Paine *et al.* 1979, Kitting 1980).

The relatively low survivorship (61-72%) of crusts followed for up to 18 months has also not been reported previously. Paine *et al.* (1979) found 70% survivorship over 6-7 years, with many patches requiring a minimum of 4 years before undergoing fragmentation, and mortality being confined to the smaller individuals. In my study individual crusts began to break up within 6-8 months, and the smallest individuals (2.5-3.5 cm^2) did not seem any more susceptible to mortality than larger patches. In light of the phenomenon of crust resurrection, mortality of individual crusts can only be ascertained with confidence over a much longer time period. Furthermore, juvenile barnacle overgrowth at Burrard Inlet may account for the apparent mortality of many of the patches. The question of whether 'Petrocelis' really lives for decades (Paine *et al.* 1979) on boulder strewn shores therefore remains unanswered.

Recruitment is then also difficult to assess (recruit or resurrected crust?), especially when crust patches exist nearby. Also, the basal system of gametophytic *Mastocarpus* is indistinguishable from the crustose tetrasporophyte in the field (Dethier 1987). Misidentification of new crusts could occur either early in the spring when gametophytes are becoming newly established or when herbivore pressure is high and removes the upright portion of *Mastocarpus*. Nonetheless, considering only three new crust patches were observed while monitoring 60 crusts at two sites over 12-18 months, it seems recruitment is low as concluded by Dethier (1994) and Paine *et al.* (1979). Furthermore, in the northern part of the latitudinal distribution range in the northern hemisphere, direct development of the bladed phase of *Mastocarpus* from carpospores is believed to predominate over a heteromorphic alternation of generations (Polanshek and West 1977, Guiry and West 1983, Zupan and West 1988). Accordingly, crustose tetrasporophytes would not be expected to become established with any great frequency. With such low recruitment it seems puzzling that high rates of mortality would be the norm. Perhaps many of the crust patches which disappeared at Sooke and Burrard Inlet are still to be "resurrected" with time.

Mechanism for seasonal pattern of 'Petrocelis' abundance. It seems intuitive that spring and summer represent favourable periods for growth of crusts at Sooke and Burrard Inlet. Other macroalgae, such as *Ulva*, *Fucus*, the bladed phase of *Mastocarpus*, *Porphyra* and filamentous reds present on boulders, also experienced growth at this time. Paine *et al.* (1979), unlike this study, documented an increase in percent cover (for those crusts exhibiting a seasonal pattern) from roughly October to May with lowest percent cover occurring in the summer. This

summer decrease is purported to be due to the annual sloughing of central tissues. Dethier (1987) found some reproductive crusts to be greenish and mucilaginous, and believed this to be a result of the sloughing of loose filaments with tetraspores. Since fertile 'Petrocelis' has only been found in late October to January (pers. observation, Dethier 1987), this phenomenon does not coincide with the systematic sloughing of tissue observed by Paine *et al.* (1979) in the summer. In my study green mucilaginous tissue that appeared to be disintegrating was noted in central portions of some crust patches only in the fall of 1996 (these crusts were reproductive) and in August 1998. Consequently, decreases in crust surface area and fragmentation at Sooke and Burrard Inlet can not be attributed to *annual* episodes of sloughing of reproductive tissue or senescing internal tissues.

Tectura scutum, the predominant limpet at Sooke and Burrard Inlet, was observed in abundance on crusts in fall and winter, the period coinciding with loss of crust tissue. This led to speculation on the role of herbivory in reducing 'Petrocelis' crust abundance. Laboratory feeding experiments (Dethier 1994) and underwater listening techniques and observations (Kitting 1980) revealed that Washington and California *Tectura scutum* do readily feed on 'Petrocelis', and are capable of damaging and removing tissue. However, both Dethier (1994) and Kitting (1980) failed to suggest herbivory may play a role in decreasing 'Petrocelis' abundance. Kitting (1980) concluded that high densities of limpets on central California vertical rock faces did not decrease algal abundance detectably, but cited at least two cases where rock was exposed within a crust patch while limpets were observed feeding. Photographs showed that such damage persisted for a year or more while tissue was generated to slowly fill in the space. Dethier 's (1994) experimental limpet manipulations in the San Juan Islands revealed significantly more loss of crust percent cover in treatments with normal (and higher than normal) densities of limpets than in limpet exclusions.

What does the available evidence from this study suggest? *Tectura scutum* gut analysis was unsuccessful in revealing 'Petrocelis' filaments, but based on the above studies we can assume this limpet readily consumes and is capable of damaging 'Petrocelis'. An abundance of *T. scutum* on crusts in Sooke and Burrard Inlet photographs in the fall and winter (the period which coincides with loss of crust tissue) and low densities observed in the summer, suggest herbivore pressure may be seasonal. And yet, can variable herbivore pressure alone explain crust decrease (detected within just one month for some crust individuals), fragmentation within

6 - 8 months and mortality, when contradictory evidence is offered by Kitting (1980) and Dethier (1994)? A combination of factors may better support a mechanism for this phenomenon.

Herbivory may visibly decrease 'Petrocelis' abundance in winter in association with senescence of tissue of older crusts and abiotic factors. Environmental conditions are less favourable for growth in winter, and may actually tend to weaken central portions of crust tissues (*e.g.* the effect of higher than normal rainfall) making them more susceptible to grazing. Furthermore, environmental events such as higher than normal water temperature caused by El Niño may result in variable crust health from year to year. Senescence of tissue of older crusts may result in visible sloughing, or also contribute to weakening central portions of 'Petrocelis'. Other factors which may affect crust health and contribute to decreasing crust abundance include bacteria cover and barnacle settlement. Thick cover of bacteria was shown by Dethier's (1994) laboratory study to result in sloughing of 'Petrocelis' tissues. In contrast to Paine *et al.*'s (1979) study where the presence of 'Petrocelis' was concluded to inhibit the settlement of barnacles, at Burrard Inlet, where barnacle densities were high, the photographic time series unequivocally revealed the settlement of juvenile barnacles on 'Petrocelis'.

Failure to recover in spring and summer from tissue loss in fall and winter (*e.g.* extremely high herbivore pressure, adverse environmental conditions and/or extensive senescence of tissues) could result in a decay process whereby the more permanent peripheral tissue becomes discontinuous and eventually the isolated fragments disappear.

Further experimentation, *e.g.* limpet exclusion and *Tectura scutum* feeding behaviour studies, and observation over a longer time period are necessary to identify the key factors playing a role in this complex phenomenon at Sooke and Burrard Inlet.

Implications for 'Codiolum' survival and duration in host. On average, regeneration of lost crust tissue and growth of 'Petrocelis' occur from spring to early fall, coinciding with high densities of small endophytic 'Codiolum' cells (Figs 3.1, 3.2). Consequently 'Petrocelis' is abundantly available for colonisation by 'Codiolum'. It is unknown if dense barnacle settlement on 'Petrocelis' (as shown at Burrard Inlet) may interfere with 'Codiolum' colonisation. The decrease in crust surface area observed in fall and winter coincides with the reproductive phenology of 'Codiolum' (fertile 'Codiolum' cells were present from October – April, Chapter 2), implying that many endophytic cells may lose their host before they have had a chance to release zoospores. The question of whether 'Codiolum' can survive an epilithic or pelagic existence is

without answer. It is interesting to note that there exists synchrony of phenology between host and endophyte: 'Petrocelis' crust patches with divided tetraspores were observed from October/November – February/March, coinciding with fertile 'Codiolum'. What this also means is that some reproductive crust tissue is lost. This may, however, be of little consequence for 'Petrocelis' persistence if, as already mentioned, the foliose phase of *Mastocarpus* commonly develops directly from carpospores rather than the tetraspores of 'Petrocelis' in the northern reaches of *Mastocarpus* distribution (growth may play a larger role than sexual reproduction in the maintenance of 'Petrocelis'). Depending on herbivore pressure, environmental conditions affecting the health of crust tissues and the extent of any decay of tissue through senescence, 'Codiolum' survivorship may be quite variable from year to year. However, those 'Codiolum' present in crust portions which are consumed by limpets may be able to survive partial digestion in the herbivore gut. Defecation of still viable seaweeds is known from sea urchins (Santelices *et al.* 1983) and molluscs (Jernakoff 1985). The tendency for 'Petrocelis' to undergo conspicuous fluctuations in abundance at Burrard Inlet and Sooke, implies that it may not be as ideal a host as suggested by Paine *et al.* (1979).

The duration of 'Codiolum' of ≤ 1 year was suggested by size class data and the 'Codiolum' life history schematic (Fig. 2.13, Chapter 2). This is consistent with large seasonal decreases in 'Petrocelis' abundance: if host tissue loss occurs every fall / winter, the exit of 'Codiolum' zoospores and consequent termination of *Acrosiphonia*'s endophytic existence until new colonisation of 'Codiolum' every spring, is not surprising. Kornmann (1964) concluded 'Codiolum' persists for more than one year within 'Petrocelis' in Helgoland, Germany (Chapter 2). To my knowledge 'Petrocelis' seasonal patterns are unknown for Helgoland. The fact that 'Petrocelis' crust patches on boulders in the intertidal of southwestern British Columbia are not as static as they were believed to be, suggests endophytes have evolved a strategy whereby they minimise their time spent in the host, becoming reproductive when host tissue loss is most likely to occur. As such their life history can be considered to be synchronised with that of their host.

Mazzaella splendens *dynamics*. The seasonal changes in *Mazzaella splendens* density shown at Burrard Inlet, Sooke and Bamfield are similar to those observed by Dyck and DeWreede (1995) at Burrard Inlet and F. Shaughnessy (pers. comm.) at Bamfield in 1990-1991. *Mazzaella splendens* density peaks in the summer months when environmental conditions are favourable for growth, and decreases drastically in the winter. Lower light and temperature are

correlated with decreased abundance, and heavy winter storms dislodge blades. In relatively exposed environments such as Sooke and Bamfield *M. splendens* densities of < .1/m^2 are not uncommon in winter. Heavy fouling of blades in late summer by bryozoans, diatoms and other epiphytes may also affect survival of *M. splendens* blades through fall and winter. Although littorinid snails have not been shown to graze on *M. splendens*, (they may instead be grazing on diatoms covering the blades), distinct marks that resemble "chewing" have been observed. Furthermore, Kim and DeWreede (1996) showed that littorinids readily consume *Mazzaella cornucopiae* (Postels & Ruprecht) Hommersand, a related species found higher in the intertidal, and concluded that these snails have a significant effect on the abundance of *M. cornucopiae* at Bamfield. Herbivory, then, when combined with other factors may also play a role in reducing *M. splendens* densities.

Implications for 'Chlorochytrium' survival. The bladed red algal host, *Mazzaella splendens*, is present in high densities during 'Chlorochytrium' colonisation (Figs. 3.7, 3.8, 3.9). Factors which may reduce 'Chlorochytrium' survival during this time are episodes of bleaching of blades (desiccation and nutrient depletion) and epiphytisation which reduces available blade surface area. *Mazzaella splendens* densities decrease in the winter at the time when both *M. splendens* (90-100 % *M. splendens* blades collected from all three sites from October-February were reproductive) and 'Chlorochytrium' are reproductive. Again, as with fertile 'Petrocelis', loss of reproductive *M. splendens* blades is implied. This may not be very detrimental for maintenance of *M. splendens* populations, since some blades may survive long enough to release spores, and more importantly, the perennial basal crusts not lost give rise to large numbers of new blades (Hansen 1977). Furthermore, fertile *M. splendens* from the subtidal (not subjected to dislodgement by winter storms) also help in maintaining intertidal *M. splendens* populations.

When *Mazzaella splendens* blades are dislodged by winter storms many carry 'Chlorochytrium' which have not yet released zoospores. This phenomenon seems difficult to comprehend: how would such an endophyte / host relationship have evolved? There are several possibilities. Firstly, the dislodgement of *M. splendens* blades may serve as a trigger for 'Chlorochytrium' fertility. Alternatively, drift blades may be able to survive for quite some time with endophytes intact, or the endophytes may remain viable despite decay of drift blades. In years where winter storms are more severe than usual and dislodge a larger proportion of blades in the intertidal, subtidal *M. splendens* may still persist and provide host availability for

'Chlorochytrium' through the winter. And finally, 'Chlorochytrium' which have colonised the basal portion of *M. splendens* blades would be more likely to survive the winter.

Based on observations of *Mazzaella splendens* blades colonised by 'Chlorochytrium' that had been collected at Sooke in November and left in a seawater tank, it does not seem that *M. splendens* blade dislodgement triggers 'Chlorochytrium' to become reproductive. Nor do *M. splendens* blades remain healthy long enough for 'Chlorochytrium' to develop and release zoospores. However, the fact that 'Chlorochytrium' cells remained healthy-looking after two months within decaying fragments of *M. splendens* blades, and were capable of increasing in size and resembling pre-zoospore release cells, suggests 'Chlorochytrium' may be able to achieve reproductive status despite deterioration of its host. Additional evidence for this hypothesis is provided by the Bamfield experiment: *Acrosiphonia arcta* was found growing in the same tank where *M. splendens* blades colonised by 'Chlorochytrium' had been found dead one month prior in January 1998. Although further experimentation with replication is necessary to confirm this result, the real possibility exists that 'Chlorochytrium' can persist and become fertile, releasing zoospores, despite mortality of its host. If this is the case, it is also significant in terms of means of dispersal for *Acrosiphonia*. Russell (1967) showed that drifting fertile fragments of the Ectocarpaceae were able to stay alive for several months and produce sporangia. He and others (Hoffmann 1987, van den Hoek 1987) argued that drifting mature thalli are an important means of dispersal. However, the fact that *M. splendens* blades are not particularly buoyant and thus sink, must be kept in mind when considering endophyte survival and dispersal via drift thalli. Of course, as with 'Codiolum', the question of whether 'Chlorochytrium' can survive free-living, once its host has disappeared, is of extreme interest. The fact culture studies (Jónsson 1959a 1959b 1962 1966, Kornmann 1961 1964, Chihara 1969, Hudson 1974, Miyaji 1996) showed 'Chlorochytrium' and 'Codiolum' capable of settling on glass and growing independent of their host, does not rule out this possibility.

The presence of subtidal *Mazzaella splendens* year round (F. Shaughnessy, pers. comm.) could be important for 'Chlorochytrium' colonisation during years where winter storms are severe and leave little *M. splendens* attached in the intertidal. Although it was not possible to identify 'Chlorochytrium' cells in the basal crust of *M. splendens* (tissue being too thick), cells were often observed near the base of the blades. If generally more distal parts of the blade are lost, then endophytic cells located closer to the base stand a higher chance of surviving the winter within their host.

The life history schematic for 'Chlorochytrium' based on unicell size (Figure 2.7, Chapter 2) suggested an annual cycle for the endophytic cells. Like 'Codiolum', 'Chlorochytrium' in southwestern British Columbia, may have evolved to synchronise time spent in its host with the one year lifespan of blades of intertidal *Mazzaella splendens*. Even those *M. splendens* blades which persist through the winter, and thus enable 'Chlorochytrium' to become fertile within its host, are not believed to survive much longer than one year (pers. observation, pers. comm. L. Dyck). No data are available for the longevity of subtidal *M. splendens* other than the fact they are present in the winter. For those 'Chlorochytrium' which are vegetative at the time their host is dislodged from the substratum, evidence discussed in this chapter suggests they may still be capable of reproducing and releasing zoospores. Furthermore, should 'Chlorochytrium' colonise the basal crust of *M. splendens*, yet another means for 'Chlorochytrium' survival to reproduction exists.

This chapter has established host availability and illustrated the large number of factors and complexity of interactions that play a role in the survival and reproductive maturity of 'Codiolum' and 'Chlorochytrium', *Acrosiphonia*'s sporophytic phase. These host / endophyte relationships may be highly specific, *i.e.* obligate symbiotic associations involving a limited number of host species, or non-specific with endophytes employing a wide variety of algal hosts. Chapter 4 will investigate host specificity of *Acrosiphonia*'s sporophyte, and whether the natural dynamics of the endophyte can be generalised for the range of hosts.

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CHAPTER 4

HOST SPECIFICITY OF *ACROSIPHONIA*'S SPOROPHYTE, 'CHLOROCHYTRIUM' AND 'CODIOLUM'

INTRODUCTION

A number of red algal hosts have been reported for 'Chlorochytrium inclusum' (Table 4.1), whereas 'Codiolum petrocelidis' has only been found in the red algal crusts 'Petrocelis' spp. and *Haemescharia hennedyi*. It is unknown if all of the endophytic green unicells identified as 'Chlorochytrium' represent the sporophytic phase of *Acrosiphonia*. Nonetheless, the presence of putative 'Chlorochytrium' cells in such a wide range of red algal species, suggests *Acrosiphonia*'s sporophyte may exhibit relatively low host specificity. In this study the range of algal host species for 'Chlorochytrium' and 'Codiolum' in southwestern British Columbia was investigated.

Endophytes representing red, brown and green algae have been reported from numerous algal hosts (Garbary 1979, Boney 1980, Tam *et al.* 1986, Correa *et al.* 1987, Peters 1991, Ellertsdóttir & Peters 1997). Few studies, other than the extensive work on red algal parasites reviewed by Goff (1982), have, however, investigated host specificity of algae associated with other algae; fewer still have focused on host specificity of endophytes, and most of these were based on field observations. Recent cross-infection experiments, primarily with the green algal endophytes *Acrochaete operculata* Correa & Nielsen and *Acrochaete heteroclada* Correa & Nielsen (Correa & McLachlan 1991) and *Endophyton ramosum* Gardner (Sanchez *et al.* 1996), have more rigorously examined the range of hosts for algal endophytes. Colonisation of macroalgae by green algal endophytes has demonstrated low (Iima & Tatewaki 1987, Correa & McLachlan 1991) to high (Correa & McLachlan 1991) host specificity. Sanchez *et al.* (1996) showed that endophytes may colonise a greater variety of hosts in laboratory cross-infection experiments than in nature, since *Endophyton ramosum* was capable of colonising algae that have not been found colonised in the field.

The mechanisms involved in algal host specificity remain poorly understood. Host specificity may be ecologically determined, *i.e.* the endophyte requires a specific set of physiological conditions which are met in the micro-niche established on the host's surface (Dickson & Waaland 1985, Hansen 1986). On the other hand, since the endophyte must breach structurally and chemically diverse host components, by mechanical or enzymatic means or both,

Table 4.1 Red algal hosts reported for 'Chlorochytrium'. Note all herbarium specimens were examined by me for the presence of 'Chlorochytrium'.

Species	Family	Order	Location	Collector / Reference
Mazzaella splendens	Gigartinaceae	Gigartinales	Southwestern B.C. Puget Sound, Wash.	Chapters 2,3 Hudson, 1974
Schizymenia pacifica	Schizymeniaceae	Gigartinales	Puget Sound, Wash.	Hudson, 1974 Chihara, 1969
Mastocarpus papillatus	Phyllophoraceae	Gigartinales	Burrard Inlet, B.C. Puget Sound, Wash.	C. Borden pers.comm. Hudson, 1974
Polyides rotundus	Polyideaceae	Gigartinales	Helgoland, Germany	Kornmann, 1964
Weeksia sp.	Weeksiaceae	Cryptonemiales	Sitka, Alaska	Setchell & Gardner, 1920
Constantinea subulifera	Dumontiaceae	Cryptonemiales	Sooke, B.C.	UBC Herbarium #A33090, 1968 Freeman, 1899
Dilsea californica	Dumontiaceae	Cryptonemiales	Europe	Cohn, 1872
Farlowia sp.	Dumontiaceae	Cryptonemiales	Japan	Miyaji & Kurogi, 1976
Sarcophyllis arctica	Dumontiaceae	Cryptonemiales	Arctic Sea	Kjellman, 1883
Kallymenia sp.	Kallymeniaceae	Cryptonemiales	Vancouver Isl., B.C.	UBC Herbarium #A33546, 1968
Halymenia sp.	Cryptonemiaceae	Cryptonemiales	Ridley Isl., B.C.	UBC Herbarium #A64855, 1981
Palmaria mollis	Rhodymeniaceae	Rhodymeniales	Scandinavia Prince William Sound, Alaska	Printz, 1926 UBC Herbarium #A60616, 1979
Porphyra sp.	Bangiaceae	Bangiales	Vancouver Isl., B.C.	UBC Herbarium #A39186, 1969

interactions between the chemical and structural composition of the host may determine host specificity. For colonisation of red macroalgae by green endophytes, both structural (Bird *et al.*, 1981) and chemical (Correa & McLachlan 1991, Sanchez *et al.* 1996) characters have been implicated in host specificity.

Recent studies of red algal hosts have established a close relationship between susceptibility to colonisation and the type of polysaccharides present in the host's cell wall. *Endophyton ramosum*, the causative agent of green patch disease in *Mazzaella laminarioides* (Bory de Saint-Vincent) Fredericq, was found to rarely infect agarophytes in cross-infection trials (Sanchez *et al.* 1996). Similarly, *Acrochaete operculata*, a green endophyte, only colonised the carrageenophytes *Chondrus crispus* Stackhouse and *Iridaea cordata* (Turn.) Bory when subjected to infection experiments with both agarophytes and carrageenophytes (Correa & McLachlan 1991). Structurally similar hosts, but with different cell wall compositions were

differentially infected, whereas structurally different hosts, but with similar cell wall compositions, showed similar susceptibility to infection. These studies suggest endophytes may be capable of discriminating between agar and carrageenan-producing algal hosts.

In the Gigartinaceae the isomorphic generations, although structurally similar, have different carrageenan types in their cell walls (McCandless *et al.* 1975, 1983; DiNinno *et al.* 1979, Whyte *et al.* 1984, Correa-Diaz *et al.* 1990, Chopin *et al.* 1999). The sporophyte has λ-type carrageenans (and any combination of θ, α, ξ, π carrageenans) and the gametophyte κ-type carrageenans (including ι carrageenan). Although *Acrochaete operculata* penetrated the outer wall of sporophytic and gametophytic fronds of *Chondrus crispus* in Correa and McLachlan's study (1991), subsequent development did not occur in gametophytes. A similar association was established with the sporophytic phase of *Iridaea cordata*, indicating that the life history phase / carrageenan type of the host may determine susceptibility to colonisation by *A. operculata*. Other species in the Gigartinaceae and species with λ carrageenan were not tested. Although no cross-infection experiments were carried out with putative hosts for 'Chlorochytrium' and 'Codiolum' in my study, susceptibility of colonisation between agarophytes and carrageenophytes and between different phases of a single host species was determined by comparing endophyte densities.

To shed light on the host specificity of *Acrosiphonia*'s sporophyte in southwestern British Columbia this study (1) determines the degree of host specificity for 'Chlorochytrium' and 'Codiolum' by examination of the foliose Rhodophyte species and crustose species present at Burrard Inlet, Sooke and Bamfield; (2) considers the role of structural and chemical characters of the host versus host availability / chance settlement for determining host specificity and (3) speculates on the natural dynamics of 'Chlorochytrium' and 'Codiolum' over the range of their hosts.

MATERIALS AND METHODS

Putative host species for 'Chlorochytrium' and 'Codiolum'. Collection of foliose red algae and crustose algae for identification of hosts for *Acrosiphonia*'s sporophytic phase was carried out from July 1995 to November 1998. Due to time constraints while working in the low intertidal zone, difficulty in removing the thinner crusts from boulders and the fact many foliose

red algal species were present in low numbers, no specific protocol was adhered to for collection of putative hosts.

Bladed red algal species were collected haphazardly in the low to high intertidal zone (0.2 - 5.1m above zero tidal level) of the study sites at Burrard Inlet, Sooke and Bamfield whenever time permitted (approximately two or three times a year) and were representative of species present. Table 4.2 provides sampling dates at the three sites. The number of blades collected reflected the seasonal abundance of particular species. *Mazzaella linearis* (Setchell & Gardner) Fredericq and *Mazzaella cornucopiae* were collected outside the study site at Bamfield; *M. linearis* in the low intertidal zone in an area of higher wave exposure and *M. cornucopiae* in the high intertidal zone of a less wave-exposed site. At Sooke *Mazzaella sanguinea* (Setchell & Gardner) Hommersand was found as drift. Data from collections by students working under my guidance are included in this chapter: in May 1997 Jan Le Moux collected all foliose red algal species found at Burrard Inlet and Sooke, and in 1998 Dana Small's Honours project enabled a more thorough sampling of putative hosts for 'Chlorochytrium'. For the period February 1998 to November 1998 we focused sampling effort on the Sooke site (since the greatest densities of 'Chlorochytrium' in *Mazzaella splendens* were found there in 1997, Chapter 2). Sampling at Sooke was more intensive (eight occasions) than at Burrard Inlet or Bamfield (Table 4.2).

All red algal blades were identified to species where possible, wrapped in newspaper and frozen. Examination for the presence of 'Chlorochytrium' involved thawing the blades and using magnification. For the species *Chondracanthus exasperatus* (Harvey & Bailey) Hughey and *Mastocarpus papillatus* the thickness of the blades prevented detection of endophytic cells with a dissecting microscope. Rather than engage in labour intensive cross-sectioning of blades for 'Chlorochytrium' detection, blades were bleached in boiling water for a few seconds. Blades became semi-transparent (the red algal phycobilin pigments are more soluble in water than chlorophyll), enabling them to be examined microscopically.

Identity of the green unicells endophytic in the foliose red algal species collected was primarily based on morphological characters. Since high densities of cells were found only within *Schizymenia pacifica*, ITS sequencing was only employed for establishing the identity of endophytes in *Schizymenia*. The ITS1, ITS2 and 5.8s regions of endophytes from three isolates of *Schizymenia* were sequenced. The protocols for DNA extraction of *Mazzaella splendens* and 'Chlorochytrium' tissue (rather than the method requiring isolation of cells from host) and amplification and sequencing of 'Chlorochytrium' from *M. splendens* blades (Chapter 2), were

Table 4.2 Collection dates for putative host species for 'Chlorochytrium' at Burrard Inlet, Sooke and Bamfield and their relative abundance from (+) low to (++++) high numbers. A (-) indicates the species may have been present but was not collected.

Burrard Inlet

Species	July 95	Nov. 95	Jan. 96	Mar. 96	Jun 96	May 97	Apr. 98	July 98
Mazzaella splendens	++++	+++	++	+++	++++	++++	+++	++++
Mastocarpus papillatus	-	-	-	-	-	++	++	+++
Chondracanthus exasperatus	-	-	+	++	-	+	+	+
Polyneura latissima	-	++	+	-	+++	+++	+++	+++
Sparlingia pertusa	-	-	-	-	+	+	+	+
Constantinea subulifera	+	-	-	-	++	++	-	++
Porphyra sp.	-	-	-	+	-	+	-	-

Bamfield

Species	July 95	Sept.95	Apr. 96	Jun 96	Apr. 98	Aug. 98
Mazzaella splendens	++++	+++	+++	++++	+++	++++
Mazzaella lineare[1]	++++	+++	-	++++	-	-
Mazzaella heterocarpa	++	-	-	-	-	++
Mazzaella cornucopiae	++++	-	-	+++	-	+++
Mastocarpus papillatus	+++	+	++	+++	++	+++
Chondracanthus exasperatus	+++	-	+++	+++	+++	+++
Polyneura latissima	+++	-	-	+++	++	+++
Sparlingia pertusa	+++	+	-	++	++	++
Constantinea subulifera	+	-	-	+	-	+
Schizymenia pacifica	+	+	-	+	-	+

Sooke

Species	Mar. 96	July 96	May 97	Feb./ Mar. 98	Apr./ May 98	June 98	Jul/Aug 98	Nov. 98
Mazzaella splendens	+	++++	+++	+	++	++++	++++	+
Mazzaella heterocarpa	-	-	-	-	-	-	++	-
Mazzaella sanguinea[2]	-	-	-	-	-	-	-	+
Mastocarpus papillatus	-	+++	+++	+	++	+++	+++	-
Schizymenia pacifica	-	+	+	+	+	+	+	-
Polyneura latissima	-	-	++	-	++	++	++	-
Palmaria palmata	+++	++++	++++	+++	+++	++++	++++	+
Porphyra sp.	+	-	++	+	-	-	-	-
Smithora sp.	-	-	-	-	+++	-	-	-
Callophyllis firma	-	-	-	-	+	-	-	-
Rhodoglossum sp.	-	-	-	-	-	-	+	-
Cryptopleura sp.	-	-	-	-	+	-	-	-

[1] collected from very wave exposed site
[2] collected as drift

used. 'Chlorochytrium' density calculations were carried out as stated in Chapter 2 for cells within *M. splendens*. However, since a number of people were involved in estimating cell densities of different blades, it was necessary to ensure estimates among workers were comparable.

Thirteen patches of the crustose brown alga, *Ralfsia pacifica*, and 18 patches of the red crust, *Hildenbrandia occidentalis,* were collected randomly from the mid intertidal zone at Bamfield (1.4 - 1.9 m above zero tidal level) in October 1998. At Sooke 30 patches of *Ralfsia* were obtained throughout the intertidal zone (0.2 - 2.1 m above zero tidal level) in May 1998. These sampling dates were chosen to correspond to periods of high 1997 'Codiolum' densities found in 'Petrocelis' at Bamfield and Sooke (Chapter 2). Only 'Petrocelis' was collected from Burrard Inlet. Crusts were removed by scraping patches of approximately 5 x 5 mm with a razor blade; these crusts were then air dried and later hydrated for detection of 'Codiolum' cells. The same procedures described in Chapter 2 were carried out for 'Codiolum' detection and density calculation. Due to very low densities of endophytic cells, DNA analysis was not attempted; instead identification depended on cell morphology.

'Chlorochytrium' affinity for life history phase. Non-reproductive *Mazzaella splendens* blades, examined for 'Chlorochytrium', were analysed with resorcinol reagent (Dyck *et al.* 1985, Garbary & DeWreede 1988, Shaughnessy & DeWreede 1991) to determine their life history phase. A 5 mm diameter disk was removed from each *M. splendens* blade using a single-hole paper punch; disks were then air dried and tested with resorcinol. A red colour develops if κ-type carrageenans are present, indicating the blade is gametophytic, whereas the tetrasporophyte remains colourless, indicating the presence of λ-type carrageenans. A control of known reproductive phase was included in all resorcinol tests.

Data analysis. 'Chlorochytrium' densities within each host were compared with densities within *Mazzaella splendens* to determine if the host is as susceptible to colonisation as is *M. splendens*. Likewise, 'Codiolum' densities in *Hildenbrandia* and in *Ralfsia* were compared with densities in 'Petrocelis'. Independent samples *t* tests were performed using SPSS 9.0 for Windows (1999) for each host / *M. splendens* or 'Petrocelis' pair, as well as to determine whether 'Chlorochytrium' densities differed significantly among tetrasporophytic and gametophytic *Mazzaella splendens* blades. In several tests the assumption of equal variances was violated

(Levene Statistic), with transformations of the data failing to reduce heterogeneity to nonsignificant levels. All data, however, satisfied the Kolmogorov-Smirnov Test for normality, and *t* tests are sufficiently robust when sample sizes are large (≥ 30) and nearly equal (Zar 1996). In only a few cases, where foliose red algal species were scarce, sample sizes were small (*e.g.* n = 5, n = 8) and unequal to *M. splendens* sample size (approximately 30). Small sample size decreases the power of the test (Zar 1996), *i.e.* reduces the probability of detecting a difference between means (Type II error). Yet, non-parametric tests have an even greater probability of committing a Type II error, and thus were not considered for analysis.

RESULTS

'Chlorochytrium' hosts. Sixteen foliose red algal species, in addition to *Mazzaella splendens*, were examined for the presence of 'Chlorochytrium'. Table 4.3 lists these species and collection sites; relative abundance of each species is illustrated in Table 4.2. Green endophytic cells were found in four of the 16 species: *Mazzaella heterocarpa, Mazzaella sanguinea, Schizymenia pacifica* and *Sparlingia pertusa* Saunders [= *Rhodymenia pertusa* (Postels & Ruprecht) Agardh]. No endophytes were present in blades of *Mastocarpus papillatus, Constantinea subulifera, Palmaria mollis* or *Porphyra* sp.; all species which have been reported as hosts for 'Chlorochytrium' (Table 4.1).

Identity of endophytes within foliose red algae. All endophytes were identified to be 'Chlorochytrium inclusum' by morphological comparisons with 'Chlorochytrium' cells from *Mazzaella splendens* blades. Those cells found within *Schizymenia pacifica* from Sooke were present in high enough densities to conduct DNA analysis for confirmation of their identity. All three endophyte isolates from *Schizymenia* sequenced corresponded to 'Chlorochytrium' isolate sequences (sequences established in Chapter 1). Two of the endophyte isolates from *Schizymenia* were 100% identical to 'Chlorochytrium' isolates of the Alaskan *Acrosiphonia* sp. genotype. This genotype made up more than 50% of the 'Chlorochytrium' isolates sequenced from Sooke. The third endophyte isolate was identical to the 'Chlorochytrium' sequence corresponding to an *Acrosiphonia arcta* sequence. This genotype comprised approximately 30% of the 'Chlorochytrium' isolates from Sooke.

Table 4.3. 'Chlorochytrium' presence in putative host species. Note the much lower sample sizes of all species compared to *Mazzaella splendens*.

Species	Order	Site collected	Endophyte Present	n
Mazzaella splendens	Gigartinales	Burrard Inlet, Sooke, Bamfield	+	1840
Mazzaella linearis	Gigartinales	Bamfield	-	34
Mazzaella heterocarpa	Gigartinales	Sooke, Bamfield	+	13
Mazzaella cornucopiae	Gigartinales	Bamfield	-	67
Mazzaella sanguinea[1]	Gigartinales	Sooke	+	1
Schizymenia pacifica	Gigartinales	Sooke, Bamfield	+	40
Chondracanthus exasperatus	Gigartinales	Burrard Inlet, Bamfield	-	68
Mastocarpus papillatus	Gigartinales	Burrard Inlet, Sooke, Bamfield	-	282
Rhodoglossum sp.	Gigartinales	Sooke	-	3
Callophyllis firma	Cryptonemiales	Sooke	-	3
Constantinea subulifera	Cryptonemiales	Burrard Inlet, Bamfield	-	64
Palmaria palmata	Rhodymeniales	Sooke	-	123
Sparlingia pertusa	Rhodymeniales	Burrard Inlet, Bamfield	+	56
Smithora sp.	Bangiales	Sooke	-	600[2]
Porphyra sp.	Bangiales	Burrard Inlet, Sooke	-	28
Polyneura latissima	Ceramiales	Burrard Inlet, Sooke, Bamfield	-	104
Cryptopleura sp.	Ceramiales	Sooke	-	4

[1] collected as drift

[2] *Smithora* blades epiphytic on 4 blades of eelgrass

Mean densities of 'Chlorochytrium'. Table 4.4. provides mean densities of 'Chlorochytrium' for the different red algal species. *Sparlingia pertusa* was collected at both Bamfield and Burrard Inlet on several occasions (Table 4.2), but endophytes were only found in Bamfield blades collected in July 1995. The mean density of 0.02 cells cm^{-2} per blade (n = 16) is not significantly lower (p = 0.0156, t = 1.452) than Bamfield mean 'Chlorochytrium' density

Table 4.4 Mean densities of 'Chlorochytrium'. Note the lower sample sizes of species compared to *Mazzaella splendens*.

Species	Site collected	Date collected	Endophyte density (# cm^{-2})	n
Schizymenia pacifica	Sooke	May '97	19.9	5
Mazzaella splendens	Sooke	May '97	22.6	30
Schizymenia pacifica	Sooke	May '98	47.5	5
Mazzaella splendens	Sooke	May '98	29.4	30
Mazzaella heterocarpa	Sooke	July '98	4.8	8
Mazzaella splendens	Sooke	July '98	10.6	30
Sparlingia pertusa	Bamfield	July '95	0.02	16
Mazzaella splendens	Bamfield	July '97/'98	0.1 / 4.2	30
Mazzaella sanguinea	Sooke	Nov. '98	1.2	1
Mazzaella splendens	Sooke	Nov. '98	2.7	30

(n = 30) of 0.1 cells cm^{-2} within *Mazzaella splendens* for July 1997, but significantly lower ($p = 0.010$, $t = 2.742$) than 4.2 cells cm^{-2} (n = 32) for July 1998. A single large blade (40 cm in length) of *Mazzaella sanguinea* collected as drift at Sooke in November 1998 was colonised by 1.2 cells cm^{-2}; 2.7 cells cm^{-2} (n = 30) were found within *M. splendens* blades sampled at Sooke on the same day. *Mazzaella heterocarpa* collections from Sooke (n = 8) in July 1998 revealed colonisation by 'Chlorochytrium'. No significant difference ($p = 0.523$, $t = 0.646$) was found between mean densities of 'Chlorochytrium' cells within *M. heterocarpa* (4.8 cells cm^{-2}) and within *M. splendens* (10.6 cells cm^{-2}, n = 30) collected at the same time. Endophyte densities in *Schizymenia pacifica* collected at Sooke in May 1997 (19.9 cells cm^{-2}, n = 5) were not significantly different ($p = 0.897$, $t = 0.131$) from May 1997 'Chlorochytrium' densities in *M. splendens* (22.6 cells cm^{-2}, n = 27).

Since more intense sampling was carried out in 1998 (*i.e. Schizymenia* blades were collected in all seasons) and endophytes were found on more than one collection day, it was possible to compare seasonal abundance of endophytes within *M. splendens* and within *Schizymenia*: 'Chlorochytrium' abundance peaked in late April / early May and density declined

toward zero by autumn for both hosts. 'Chlorochytrium' densities in *Schizymenia* in May 1998 (47.5 cells cm^{-2}, n= 5) and in July 1998 (19.4 cells cm^{-2}, n = 12) did not differ significantly (p = 0.593, t = 0.561; p = 0.527, t = 0.642 respectively) from densities in *M. splendens* (29.4 cells cm^{-2}, n = 23; 10.6 cells cm^{-2}, n = 30 respectively). High variance in the data and small sample sizes could have prevented detection of any significance, but since the similarity of the seasonal trends is clearly visible in the graph (Fig. 4.1), there is reason to believe that seasonal abundance of 'Chlorochytrium' did not differ among *M. splendens* and *Schizymenia*. The small sample sizes for *Schizymenia* precluded endophyte density comparisons with *Mazzaella* for all sampling dates.

'Codiolum' hosts. Green unicells were found attached to and embedded within the filaments of the red algal crust, *Hildenbrandia occidentalis*, and the brown algal crust, *Ralfsia pacifica*. The unicells were identified as 'Codiolum' by morphological comparison with the green endophytes found in 'Petrocelis'. Due to very low densities (0-75 cells in 1 cm^2 patches), DNA extraction, amplification and sequencing for endophyte identification was not feasible. The mean densities of 'Codiolum' in *Hildenbrandia* (24 cells cm^{-2}, n = 18) and *Ralfsia* (12 cells cm^{-2}, n = 13) from Bamfield were significantly lower (p = 0.037, t = 2.247) than in 'Petrocelis' (199 cells cm^{-2}, n = 20) from Bamfield collected in fall 1997. Likewise mean 'Codiolum' density in *Ralfsia* (51 cells cm^{-2}, n = 30) was significantly lower (p = 0, t = 4.479) than 'Codiolum' density in 'Petrocelis' (11,760 cells cm^{-2}, n = 27) for Sooke samples collected in late spring 1997.

'Chlorochytrium' colonisation of gametophytic vs. tetrasporophytic phase. 'Chlorochytrium' densities in gametophytic *Mazzaella splendens* did not differ significantly (Table 4.5) from densities in tetrasporophytic *M. splendens* at Sooke and Burrard Inlet respectively. Tetrasporophytic *M. splendens* blades sampled at Bamfield, however, were colonised by significantly higher numbers (Table 4.5) of 'Chlorochytrium' than were gametophytic blades. When data from all three sites were combined 'Chlorochytrium' densities of tetrasporophytic and gametophytic *M. splendens* blades did not differ significantly (Table 4.5).

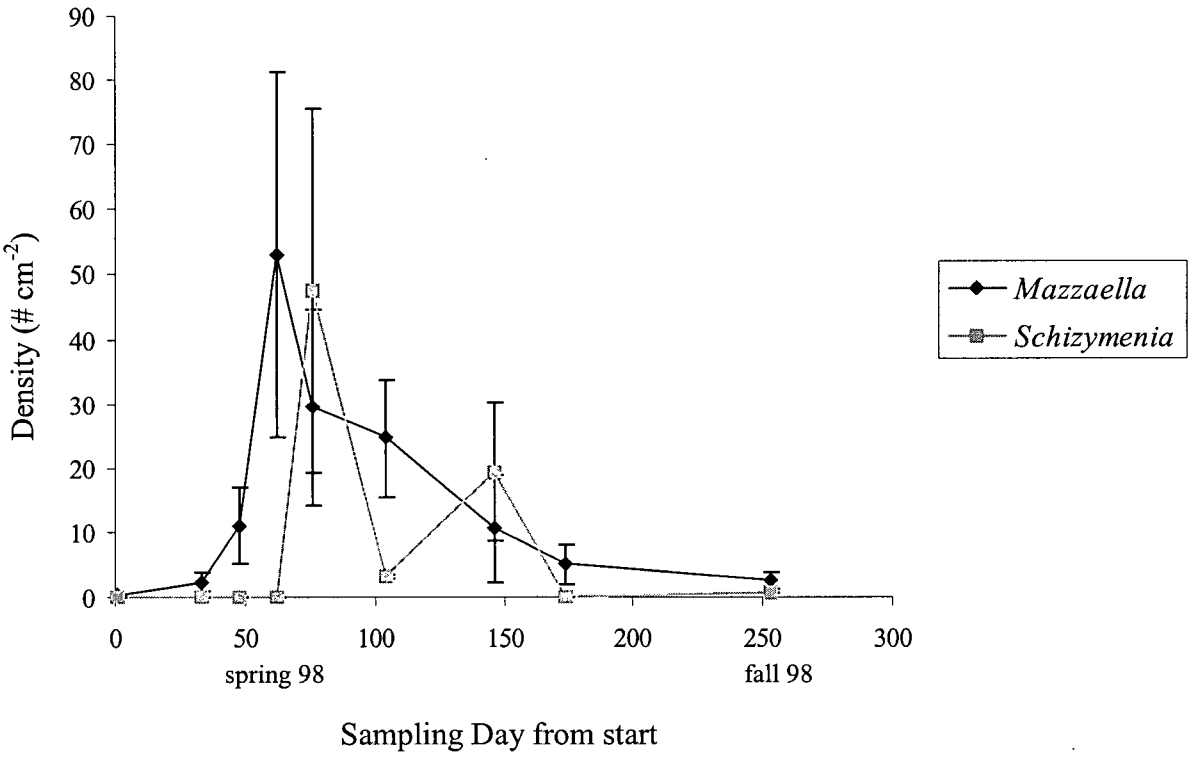

Figure 4.1 'Chlorochytrium' density in *Mazzaella splendens* and in *Schizymenia pacifica* at Sooke for 1998. Data are means ± S.E. from cells counted in 30 *M. splendens* blades and in 2-12 *S. pacifica* blades.

Table 4.5 'Chlorochytrium' densities in tetrasporophytic and gametophytic *Mazzaella splendens* blades. Results of independent samples *t*-tests performed for Burrard Inlet, Sooke and Bamfield and all three sites combined. The significant *t* value (*) indicates a statistically significant difference among endophyte densities in tetrasporophytic and gametophytic blades.

	n	Mean	S.D.	S.E.	*t*	p
Burrard Inlet						
tetrasporophyte	247	0.05529	0.3347	0.02130	1.820	0.069
gametophyte	288	0.1182	0.4619	0.02722		
Sooke						
tetrasporophyte	275	10.7810	36.7495	2.2161	0.300	0.765
gametophyte	319	9.7425	46.2328	2.5885		
Bamfield						
tetrasporophyte	235	4.9049	23.5178	1.5341	2.379*	0.018*
gametophyte	220	1.1781	4.7117	0.3177		
All 3 sites						
tetrasporophyte	757	5.4572	26.0892	0.9482	0.969	0.333
gametophyte	827	4.1126	29.1369	1.0132		

DISCUSSION

The results from this, including Small's (1998) and Le Moux's (1997) studies, combined with previous reports (Table. 4.1), suggest a wide range of hosts for *Acrosiphonia*'s sporophytic phase. This study, however, also suggests greater affinity for colonisation of 'Petrocelis', *Mazzaella splendens*, *Schizymenia pacifica* and *Mazzaella heterocarpa* than other foliose red and crustose algae present at Burrard Inlet, Sooke and Bamfield.

Three foliose red algae, *Mazzaella heterocarpa*, *Mazzaella sanguinea* and *Sparlingia pertusa*, and the two crustose algae, *Ralfsia pacifica* and *Hildenbrandia occidentalis*, are newly reported hosts for 'Chlorochytrium' and 'Codiolum'. This is the first study to report 'Codiolum' colonisation of a brown alga (= *Ralfsia*), which prompts the question of whether

'Chlorochytrium' inhabits other bladed, *e.g.* brown, algae. 'Chlorochytrium' has, however, never been reported in brown algae. *Laminaria saccharina*, the only foliose alga present at Kitsilano Beach, Vancouver, was intensely sampled (n = 45) in May, the time *Acrosiphonia* is fertile (Chapter 2), and no 'Chlorochytrium' cells were detected. Clearly 'Chlorochytrium' and 'Codiolum' are not host specific for a particular species, family or even taxonomic order; *Sparlingia pertusa*, unlike the other hosts in this study, is in the Rhodymeniales. In addition, if the identification of 'Chlorochytrium' in other studies is accepted (I have not examined type specimens nor did other authors base identifications on molecular work), the range of hosts for 'Chlorochytrium' extends to a number of species within the Cryptonemiales (Table 4.1). For host algae collected from outside the northeast Pacific, it is possible that 'Chlorochytrium' is the sporophyte of *Spongomorpha* (closely related genus to *Acrosiphonia* not found on our coast), and thus host specificities of *Spongomorpha*'s sporophyte would not necessarily be shared by *Acrosiphonia*'s sporophyte.

A number of host species for 'Chlorochytrium' reported in the literature, and collected in this study, were not found to be colonised by endophytic cells. The absence of 'Chlorochytrium' in *Mastocarpus papillatus* was surprising, since Hudson (1974) found *M. papillatus* blades with 'Chlorochytrium' in the Puget Sound area, Washington. A single blade of *M. papillatus* colonised by 'Chlorochytrium' was , however, collected by C.A. Borden in the vicinity of the study site at Burrard Inlet. The endophytes were identified by me to be 'Chlorochytrium'. 'Chlorochytrium' is also present in the sample of *Constantinea subulifera* from Sooke deposited in the UBC Herbarium in 1968. Unfortunately, *Constantinea* was not detected at Sooke during this study. Furthermore, 'Chlorochytrium' densities in other genera at both Bamfield and Burrard Inlet, where *Constantinea* was present (but in relatively low abundance), were much lower than at Sooke. The report of *Palmaria mollis* as a host was from Trondhjemsfijordes, Norway. In the only specimen (UBC herbarium specimen) from the northeast Pacific (Prince William Sound, Alaska) documented to be colonised by 'Chlorochytrium' I could not detect any endophytes, suggesting 'Chlorochytrium' may have been misidentified. It is interesting to note that a spherical green unicell, *Halochlorococcum porphyra* (Setchell & Gardner) West, Smith & McBride (= *Chlorochytrium porphyrae* Setchell & Gardner), is endophytic, embedded in the cell wall of some species of *Porphyra* from British Columbia to California (Setchell & Gardner 1920, Scagel 1966, Abbott & Hollenberg 1976). Reproductive and cytological characteristics

suggested to West *et al.* (1988) that the endophyte be placed in the genus *Halochlorococcum* (Chlorococcales), delineating it from 'Chlorochytrium'.

The absence of 'Chlorochytrium' in many of the foliose red algal species collected from Burrard Inlet, Sooke and Bamfield does not rule out the possibility that they are hosts. There are a number of reasons 'Chlorochytrium' many not have been found in the representatives I examined of these species; for example, (1) not all species were represented at Sooke where 'Chlorochytrium' densities were much higher than at Burrard Inlet and Bamfield, (2) some species were present in relatively low abundance during the time *Acrosiphonia* is fertile, translating to very low sample sizes and (3) some species may have a very patchy distribution and thus colonisation by 'Chlorochytrium' is less likely than for a species such as *Mazzaella splendens*, which is present throughout an area. Essentially, if algal species which are not readily available as hosts in a given area are found absent of endophytes, it is difficult to conclude they are incapable of being colonised by *Acrosiphonia*'s sporophyte. Laboratory cross-infection experiments would be valuable in determining a species' capability as a host. Based on the limited data of this study *Mazzaella splendens*, *Schizymenia pacifica*, *Mazzaella heterocarpa* and *Mazzaella sanguinea* were equally well colonised by 'Chlorochytrium'. 'Chlorochytrium' densities were not significantly different within *Schizymenia* and *M. heterocarpa* blades paired with *M. splendens* for a particular site and date; *M. sanguinea* is the exception since only a single blade was available for comparing 'Chlorochytrium' densities. Although 'Chlorochytrium' density in Bamfield *Sparlingia pertusa* was not significantly lower when compared to Bamfield *M. splendens*, dates of collections did not coincide, *i.e.* densities of 'Chlorochytrium' in *M. splendens* were not available for comparison in the year endophytes were found in *S. pertusa*. Furthermore, *S. pertusa* was not found to be colonised by endophytes in either of the years *M. splendens* was shown to be colonised. It is therefore very difficult to conclude anything about *S. pertusa*'s status as a host. 'Petrocelis' was shown to be colonised by a significantly higher number of 'Codiolum' cells than *Hildenbrandia* and *Ralfsia*, indicating it is preferentially colonised.

If some algal species are really not penetrated by 'Chlorochytrium' and 'Codiolum', or at least are less preferentially colonised, what determines which species are better hosts than others? One explanation for differential colonisation of algal blades and crusts by 'Chlorochytrium' and 'Codiolum' is variable structural composition among host species.

Upon examination of filaments among the three algal crusts, 'Petrocelis' filaments appear easier to penetrate than the more closely appressed filaments of *Ralfsia* and *Hildenbrandia*. Similarly, degree of host specificity among foliose red algae may be determined by how tightly cells are compacted. 'Chlorochytrium' cells were generally located in the cortex layer of *Mazzaella* species and *Schizymenia pacifica*, all of whose cortex is comprised of small relatively loosely packed cells which become progressively smaller towards the surface (Abbott & Hollenberg 1976). The medulla consists of loosely interwoven filaments. This does not, however, address how 'Chlorochytrium' actually penetrates the surface of blades. Other species from the northeast Pacific similarly constructed include *Mastocarpus papillatus, Constantinea subulifera, Chondracanthus exasperatus, Halymenia* sp., *Kallymenia* sp., *Weeksia* sp., all but *Chondracanthus* found colonised by 'Chlorochytrium'. The absence of reports of 'Chlorochytrium' within *Chondracanthus* is somewhat surprising. However, the blades are very thick, such that unless the bleaching technique used in this study is applied, the chance of detecting 'Chlorochytrium' cells is low. Furthermore, no *Chondracanthus* was available at Sooke , where (in this study) 'Chlorochytrium' densities were highest.

The cortex of *Palmaria mollis* and *Sparlingia pertusa* is only a few cells thick and the medulla is composed of large, compacted cuboidal cells, suggesting endophytic cells may have difficulty penetrating below the thin cortex (Fig. 4.2). None of the 123 *P. palmata* blades collected from Sooke were found to be inhabited by 'Chlorochytrium', and of the less than 10% of *S. pertusa* blades collected which contained 'Chlorochytrium', the latter was present in very low densities (0.01-0.1 cells cm^{-2} per blade). The representatives of the Bangiales, *Porphyra* sp. and *Smithora* sp., are structurally very different from the other foliose red algae. Parenchymatous in construction, a filamentous medulla is lacking, and cells of uniform size are densely packed. 'Chlorochytrium' cells have never been found in these genera, except that some *Porphyra* species are reported to be colonised by *Halochlorococcum porphyrae.*

The surfaces of many macroalgae are believed to be covered by a layer of material called the cuticle (Craigie 1990). This layer is chemically and structurally distinct from the cell wall, and is formed from protein-rich cellular secretions that presumably move through the cell wall to the thallus surface. Few studies (Hanic & Craigie 1969, Gerwick & Lang 1977, Pedersen *et al.* 1981) have, however, successfully characterised the cuticle of an algal species structurally and chemically. It is feasible that host cuticle thickness and / or chemical composition may play a role in ease of penetration by the endophyte.

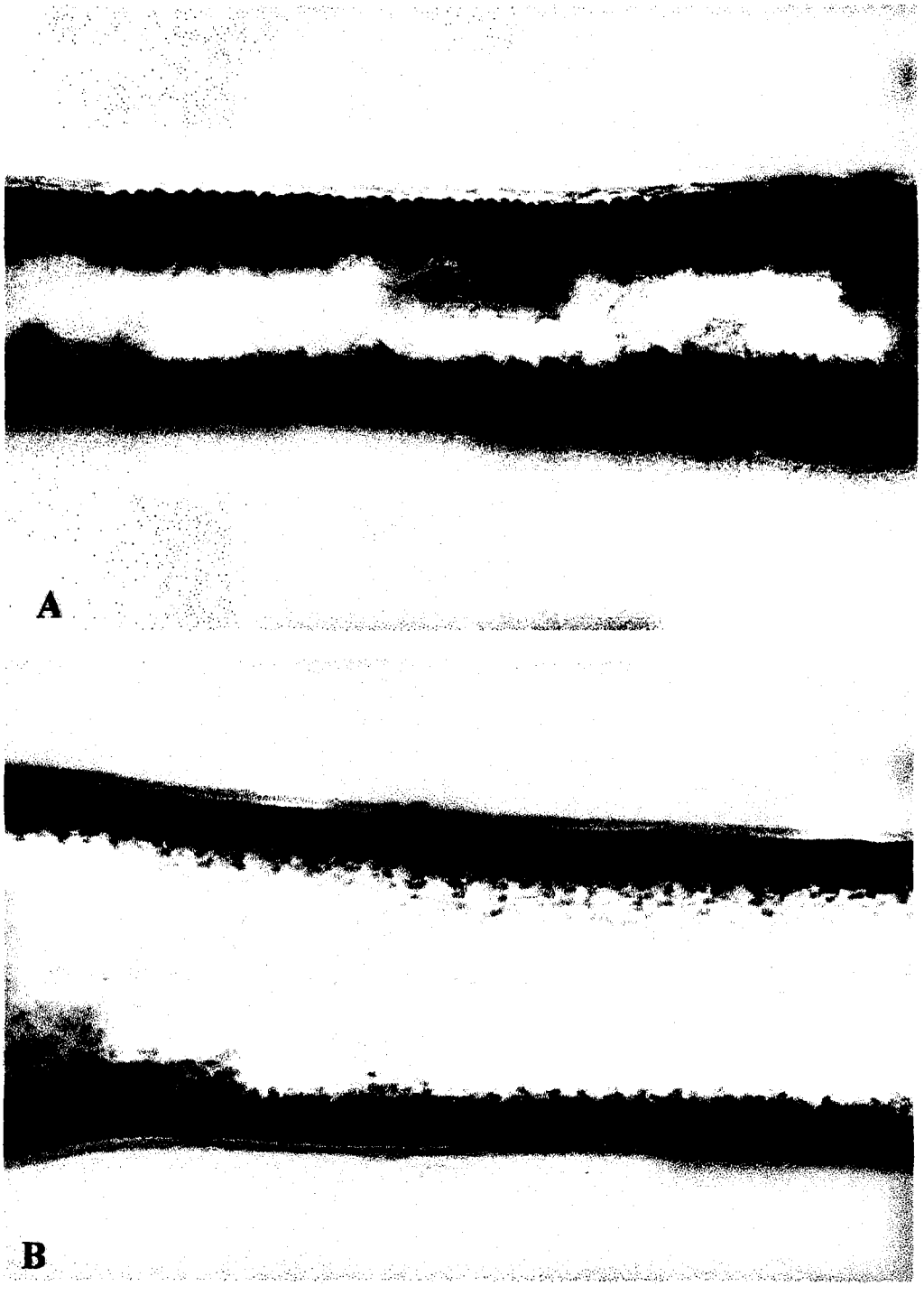

Figure 4.2 A comparison of blade cross-sections: *Sparlingia pertusa* (**A**) is characterised by a medulla comprised of large, compacted cuboidal cells whereas *Mazzaella splendens* (**B**) exhibits a medulla consisting of loosely interwoven filaments.

A second mechanism for host specificity considers variable chemical composition among host species. Experimental evidence (Correa & McLachlan 1991, Sanchez *et al.* 1996) strongly suggests that the green endophytes *Acrochaete operculata* (endophytic in *Chondrus crispus* and *Iridaea cordata*) and *Endophyton ramosum* (the causative agent of green patch disease in *Mazzaella laminarioides*) discriminate between hosts with different cell wall composition. Host specificity for both endophytes is suspected to be mediated by the presence of carrageenan in host cell walls. Cell wall composition of the algal species examined as hosts in my study is summarised in Table 4.6. The species are differentiated into carrageenophytes, agarophytes and carragar (agar / carrageenan hybrid) producing algae. Where no data were available for a particular species (studies tend to centre on species that contain large quantities of the commercially important polysaccharides), the cell wall composition was based on that of the taxonomic family the species is found in. From the table it is clear that the foliose species colonised by 'Chlorochytrium' in my study do not all share the same cell wall composition. However, those host species whose 'Chlorochytrium' densities were highest and not significantly different from *Mazzaella splendens*, were primarily carrageenophytes; *Schizymenia* being a producer of carragar. 'Codiolum's higher affinity for 'Petrocelis' than for *Hildenbrandia* can not be interpreted in terms of carrageenan presence, since the cell walls of neither *Hildenbrandia* nor any other members of the Hildenbrandiaceae have been examined. Although the presence of carrageenan may not dictate which species can or can not function as hosts for 'Chlorochytrium' (I have already suggested that 'Chlorochytrium' has a wide range of hosts from different taxonomic orders), the data suggest that carrageenophytes / carragar producing hosts are more readily penetrated by the endophyte. Furthermore, it may be that interactions between cell wall chemical composition and structural characteristics dictate differential endophyte success in colonising different host species.

Andrews *et al.* (1979) suggestion that algal life history phases might be differentially susceptible to colonisation by other organisms, and Correa and McLachlan's (1991) confirmation by experimental evidence (*Acrochaete operculata* infected blades of host species of the same life history phase and containing λ-type carrageenans), was not supported by my study. Among host species with heteromorphic life history phases (suspected to be chemically different), neither gametophyte nor sporophyte seemed to be consistently more colonised. *Mastocarpus papillatus*, the foliose gametophyte (κ-type carrageenan-containing), may be less colonised than 'Petrocelis', the crustose tetrasporophyte (λ-type carrageenan-containing);

Table 4.6 Cell wall composition of algal species examined for the presence of endophytes. Note all are members of the Rhodophyta, except *Ralfsia pacifica*, a brown alga.

Species	carrageenan / Agar	Reference	Endophyte present
Mazzaella spp.	κ/ι carrageenans G) λ/θ/α carrageenans (S)	McCandless *et al.* 1975, 1983; Whyte *et al.* 1984, Correa-Diaz *et al.* 1990, Chopin *et al.* 1999	+
Schizymenia pacifica	carragars	Whyte *et al.* 1984, Chopin *et al.* 1999	+
Mastocarpus papillatus	κ/ι carrageenans	Whyte *et al.* 1984, Correa-Diaz *et al.* 1990	+ (but not in my study)
'Petrocelis franciscana'	λ/ξ/π carrageenans	DiNinno *et al.* 1979, McCandless *et al.* 1983, Whyte *et al.* 1984, Correa-Diaz *et al.* 1990	+
Chondracanthus exasperatus	κ/ι carrageenans G) ξ carrageenans (S)	McCandless *et al.* 1983, Correa-Diaz *et al.* 1990	
Rhodoglossum sp.	κ carrageenans (G), λ carrageenans (S)	McCandless *et al.* 1983	
Callophyllis sp.	λ/θ/α carrageenans, carragasr	Chopin *et al.* 1999	
Constantinea subulifera	carragars	Chopin *et al.* 1999	+ (but not in my study)
Palmaria mollis	agar	Usov & Klochkova 1992	
Sparlingia pertusa	agar	Usov & Klochkova 1992	+
Porphyra sp.	agar	Usov & Klochkova 1992	
Polyneura latissima	agar	Craigie 1990	
Cryptopleura sp.	agar	Usov & Klochkova 1992	
Smithora sp.	agar	Rees & Conway 1962	
Hildenbrandia occidentalis	unknown	Hildenbrandiaceae unstudied	+
Ralfsia pacifica	absent		+

G = gametophyte
S = sporophyte

Schizymenia,on the other hand, recently established as a gametophyte alternating with the crust *Haematocelis* (DeCew *et al.* 1992) showed very high colonisation by 'Chlorochytrium'. The crustose phase, *Haematocelis*, has, unfortunately, not been examined for endophytes. This is also the case for *Farlowia*'s [bladed gametophyte found to be colonised by 'Chlorochytrium' in

Japan (Miyaji & Kurogi, 1976)] crustose tetrasporophytic phase. Of course, where phases are both structurally and chemically different, it is difficult to isolate whether host specificity is determined by interactions between the chemical and structural composition of the host or by only one property.

For host algal species of the Gigartinaceae where the isomorphic generations, although structurally similar, have different cell wall compositions (McCandles *et al.*, 1983), 'Chlorochytrium' did not appear to show a higher affinity for either the λ-type carrageenan-containing tetrasporophyte or κ-type carrageenan-containing gametophyte. When endophyte densities in tetrasporophytic and gametophytic blades of *Mazzaella splendens* obtained from all three study sites were compared, no significant difference was detected. However, examining densities site by site, 'Chlorochytrium' cells were found to be present in significantly higher numbers in tetrasporophytes than in gametophytes at Bamfield; no difference was found for Burrard Inlet or Sooke. This discrepancy may be site-specific, *e.g.* tetrasporophytic blades at Bamfield may be structurally different from those at Burrard Inlet or Sooke. Correa and McLachlan (1988) suggested tetrasporophytic blades in the Gigartinaceae have a thinner cuticle than gametophytic blades. If this were true at Bamfield, it could explain differential colonisation between the life history phases.

Host availability may be a factor in which algal species are colonised by 'Chlorochytrium' and 'Codiolum'. The endophytes may have evolved to colonise hosts present in abundance (whether by structural or chemical recognition), or chance settlement may dictate colonisation, *i.e.* if algal hosts are less abundant than others during the period *Acrosiphonia* is fertile, the endophyte will have less chance for colonisation. The hypothesis of chance settlement determining an endophyte's success in inhabiting its host, is weakened, however, because *Schizymenia pacifica* at Sooke is both densely colonised by 'Chlorochytrium' and very low in abundance (Table 4.2). Furthermore, *Ralfsia* is as abundant as 'Petrocelis' at Sooke, yet 'Codiolum' densities were significantly lower in *Ralfsia*. The hypothesis that a requirement of a host is its availability long enough for the endophyte to reach maturity, is also not supported here. *Mazzaella heterocarpa*, found to be densely colonised by 'Chlorochytrium' in July at Sooke, disappears from the intertidal zone by late summer / early fall (pers. observ.), long before 'Chlorochytrium' has been observed to be fertile (Chapter 2).

Since *Schizymenia pacifica* was the only host species collected over a one year period, and which was consistently found to be colonised by endophytes, it is difficult to speculate on

the natural dynamics of 'Chlorochytrium' and 'Codiolum' for their range of hosts. Nonetheless, the similar patterns of seasonal abundance for 'Chlorochytrium' endophytic in *Schizymenia* and in *Mazzaella splendens* (Fig. 4.1) do suggest similar life histories for 'Chlorochytrium' and 'Codiolum' within hosts which are present year round. Nonetheless, more intensive and frequent sampling of putative hosts would be necessary to attempt generalising about the life history of *Acrosiphonia*'s sporophyte for its range of hosts.

Based on evidence presented in this chapter, *Acrosiphonia*'s unicellular sporophyte employs a wide range of hosts. The evidence also suggests that 'Codiolum' has a greater affinity for 'Petrocelis' than for *Hildenbrandia* and *Ralfsia*, and 'Chlorochytrium' colonises some foliose red algal species more readily than others. Clearly, laboratory cross-infection experiments accompanying field observations of endophyte colonisation would greatly improve our understanding of host specificity of 'Chlorochytrium' and 'Codiolum'. Both structural characteristics and cell wall composition of the host may play a role in differential colonisation. Availability of the host species does not appear to be an important factor in host specificity, as seen by high densities of 'Chlorochytrium' in both *Schizymenia* (present in low abundance year round) and *Mazzaella heterocarpa* (a seasonal species absent late summer / fall until winter, R. Scrosati pers. comm.). Endophyte survival in *M. heterocarpa*, which disappears prior to 'Chlorochytrium' fertility, may be possible through 'Chlorochytrium' survival in drift blades and in the basal crust of *M. heterocarpa* (as was suggested for non-fertile endophytes in *M. splendens* blades dislodged by winter storms). In general, these results further support a bet-hedging strategy for *Acrosiphonia*'s sporophytic phase. 'Chlorochytrium' and 'Codiolum' may have evolved to preferentially colonise and synchronise their life histories with hosts which are abundant and available long enough for the endophyte to reach maturity (*M. splendens* and 'Petrocelis', Chapter 3) , but also seem capable of colonising a range of less 'suitable' hosts to compensate for the absence of primary hosts. In addition, some sporophytes of *Acrosiphonia* may survive epilithically or planktonically. All of these mechanisms for endophyte survival are significant since conditions which affect host availability (already mentioned in Chapter 3) are variable, *e.g.* herbivore pressure may fluctuate or winter storms vary in intensity.

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GENERAL CONCLUSIONS

This thesis has demonstrated the value of combining molecular studies with field work to expand our understanding of complex algal life histories and endophyte / host associations. This approach was successful in the identification of the alternate life history phase of *Acrosiphonia* and in revealing the natural dynamics of the life cycle in southern British Columbia. The relationship of *Acrosiphonia*'s gametophyte and sporophyte had never been examined in nature; even fundamental studies on distribution, seasonal abundance and reproductive phenology of both phases were lacking. A unicellular sporophyte, found to be endophytic in a wide range of crustose and foliose red algae for a duration of ≤ one year, was shown to alternate with a free-living filamentous gametophyte that is abundant in the rocky intertidal zone in spring and summer. Host availability and specificity were identified as important factors in the survivorship of the sporophytic endophytes. In addition, examination of the evolutionary relevance of *Acrosiphonia*'s complex life history led to speculation that heteromorphy may have evolved as a bet-hedging strategy.

DNA sequencing proved to be a powerful tool in the identification of the spherical 'Chlorochytrium' cells endophytic in *Mazzaella splendens* and the stalked 'Codiolum' cells found in 'Petrocelis'. DNA sequences of endophyte / *Acrosiphonia* pairs provided conclusive evidence that the unicells were the sporophytes of *Acrosiphonia* and not of other genera in the Codiolales reported to exhibit 'Codiolum' phases in their life histories. Lack of information in the ITS sequence data did not permit resolution of infrageneric relationships within *Acrosiphonia*. The morphological species, *A. arcta* and *A. coalita* did, however, show consistent ITS sequence variation. A number of interesting questions regarding *Acrosiphonia* and endophyte genotypes were generated in the study. These are detailed in the discussion of Chapter 1. Regardless of the number of *Acrosiphonia* and endophyte genotypes found, the sequence data showed that the morphologically different endophytes, 'Chlorochytrium' and 'Codiolum', can be produced by the same *Acrosiphonia* species, *A. arcta*. This supports Kornmann's hypothesis that 'Chlorochytrium' and 'Codiolum' represent alternate phenotypes of the same sporophyte. The sporophyte of *A. coalita* was, however, only found to be associated with 'Codiolum', suggesting it may be host specific in southern British Columbia, only colonising crusts.

Host specificity examined in Chapter 4 focused on the range of foliose (red) and crustose hosts for *Acrosiphonia*'s sporophyte at Burrard Inlet, Sooke and Bamfield. Host specificity of

sporophytes of individual *Acrosiphonia* "species" was not further examined. Two general conclusions were drawn: firstly that the endophytic sporophytes colonise a wide range of hosts, and secondly that 'Codiolum' demonstrates a greater affinity for 'Petrocelis' than for other coexisting crustose algae, and 'Chlorochytrium' colonises some foliose red algal species more readily than others. Foliose brown and green algae have never been reported colonised by 'Chlorochytrium'. Endophyte density data from Chapter 4 suggested that variable structural composition among host species, *i.e.* how closely appressed crust filaments are and the degree of compactness of the cells of the cortex and medulla of foliose red algae, may be important for ease of penetration by the endophyte. Host cell wall composition is also suspected to play a role in preferential colonisation. Carrageenophytes and carragar producing hosts were colonised by higher densities of 'Chlorochytrium' than were agarophytes. The hypothesis that host life history phases might be differentially susceptible to colonisation by endophytes was not supported in my study; among host species with an alternation of generations neither gametophytes nor sporophytes seemed to be consistently more colonised.

The relationship between the unicellular endophytes and filamentous *Acrosiphonia* plants was established in nature. *Acrosiphonia arcta* and *A. coalita*, identified both morphologically and by ITS sequences, comprised the majority of *Acrosiphonia* plants in southwestern British Columbia, abundant in the low to mid intertidal zone throughout the spring and summer. *Acrosiphonia*'s gametophyte became reproductive almost immediately after establishment, and endophyte colonisation of its host occurred subsequently, one to three months after *Acrosiphonia* appeared in the intertidal zone. Colonisation may occur as two major events, corresponding to two *Acrosiphonia* pulses, or continuously over the spring and summer. Although counter-intuitive, 'Chlorochytrium' colonisation was shown to occur earlier than 'Codiolum' colonisation. This was unexpected since *A. coalita*, associated only with 'Codiolum', was observed to become fertile before *A. arcta*. The endophytic sporophytes remain within their hosts, even long after *Acrosiphonia* has disappeared from the intertidal zone, maturing during the winter and releasing zoospores in winter and spring. Clearly the two morphologically distinct phases of *Acrosiphonia* differ with respect to tolerance of environmental conditions.

The success in colonisation and survival of *Acrosiphonia*'s endophytic sporophyte is largely determined by host availability. *Mazzaella splendens*, found consistently to be densely colonised by 'Chlorochytrium' in spring and summer, is the dominant foliose red alga at all study sites. Likewise, 'Petrocelis franciscana', abundantly colonised by 'Codiolum', is a conspicuous

crust in the intertidal zone along the southern coast of British Columbia. However, seasonal fluctuations in abundance were shown to occur for both hosts: a decrease in *Mazzaella* density was noted in the winter, primarily due to winter storms dislodging blades; loss of tissue of 'Petrocelis' crusts also occurred in (fall) winter and is attributed to a combination of herbivory, adverse environmental conditions such as rainfall, water temperature and light levels and tissue decay from senescence. Release of endophyte zoospores appears to coincide with the decrease in host abundance, such that the endophytes spend ≤ one year in their host. In the case of 'Chlorochytrium', *M. splendens* blade dislodgement prior to endophyte maturity may be compensated by the survival of 'Chlorochytrium' in drift blades, endophytes persisting in perennial basal crusts of *Mazzaella* not dislodged and an epilithic or planktonic existence for 'Chlorochytrium'. 'Codiolum' survivorship may be affected by the ability of cells present in 'Petrocelis' tissue consumed by limpets to survive partial digestion and the survival of cells epilithically or planktonically.

Acrosiphonia's success in southern British Columbia has been indicated by gametophytic filamentous plants alternating with endophytic sporophytes at three environmentally different sites. Although variable seasonality and abundance of both phases is exhibited at different sites (see Chapter 2 discussion for details), this study suggests adaptability of *Acrosiphonia* to a range of habitats. I suggest that *Acrosiphonia*'s success may be attributed to its complex heteromorphic life history, which evolved as a bet-hedging strategy: (1) two morphologically different phases have adapted to a seasonally variable environment (no evidence is available suggesting an adaptation to grazing pressure); (2) the endophytic sporophyte phase (at least of *Acrosiphonia arcta*) successfully colonises both foliose red algae and crustose algae; (3) 'Chlorochytrium' and 'Codiolum' appear to have evolved to synchronise their duration as endophytes with host availability and (4) 'Chlorochytrium' and 'Codiolum', although showing higher affinity for some hosts, seem to exhibit relatively low host specificity and may exist epilithically or planktonically.

Suggested Future Research

As is generally the case in scientific investigations, many questions remain unanswered in this study of the life history of *Acrosiphonia*. The major areas where additional research could potentially clarify aspects of the relationship of *Acrosiphonia*'s sporophyte and gametophyte in southwestern British Columbia are highlighted below.

Firstly, there is great need for a molecular study to resolve the taxonomic confusion of the genus. The ITS sequence data from this study lacked sufficient variation for resolution among *Acrosiphonia* species. A multiallelic population level approach or selection of a more variable gene, *e.g.* the even faster evolving IGS (intergenic spacer) region of the nuclear ribosomal DNA cistron, could enable the number of *Acrosiphonia* species and their corresponding endophytic sporophytes in British Columbia to be determined. If different *Acrosiphonia* species exhibit variable seasonal abundance patterns, reproductive phenology or host specificity (colonising only crusts of foliose algae), the life histories of individual species could be established.

Another aspect of interest which commands further attention is the significance of the role the endophytic sporophytes play in *Acrosiphonia*'s life history. Since 'Chlorochytrium' and 'Codiolum' become reproductive at a time when host availability has been shown to decrease, are other factors, yet undiscovered, involved? Perhaps the unicellular sporophytes can survive epilithically (or planktonically) as do those of *Urospora*, and as is suggested by free-living 'Chlorochytrium' and 'Codiolum' in culture and the presence of filamentous *Acrosiphonia* plants in a habitat devoid of hosts. Perhaps the relative contribution of sexual reproduction is low compared to asexual or vegetative propagation for maintenance of *Acrosiphonia* populations. As yet, it remains unknown if *Acrosiphonia* rhizoid overwintering is a means of propagation, and if filamentous gametophytic plants can give rise to haploid zoospores in nature that survive the winter. Some of these questions could be experimentally tested through the use of sterilised settling plates placed in the intertidal zone or in large outdoor tanks, as described in Chapter 3 for testing the ability of 'Chlorochytrium' to become fertile, despite mortality of its host. The feasibility of survival of 'Chlorochytrium' in drift host blades also needs further investigation.

An area of study where nothing is known is the mechanism for entry of 'Chlorochytrium' and 'Codiolum' into their hosts and the exit of zoospores. Does host penetration occur by mechanical means, *e.g.* where surfaces are wounded or the cuticle of bladed algae is disrupted by growth, or by enzymatic hydrolysis or by both? Scanning electron microscopy, as well as experimental scarification of host blade surfaces are two techniques which may shed light on how *Acrosiphonia*'s endophytic sporophytes get in and out of their hosts. With regard to the range of hosts employed by 'Chlorochytrium' and 'Codiolum' in southern British Columbia, more intensive sampling of putative hosts accompanied by laboratory cross-infection experiments,

would contribute to increased understanding of host specificity of *Acrosiphonia*'s endophytic sporophytes.

Lastly, herbivory as a factor in *Acrosiphonia*'s life history along British Columbia's coast remains to be investigated. As mentioned in this thesis, herbivore pressure is speculated to be an evolutionary force in the heteromorphy of algae. Nothing is known of differential susceptibility of *Acrosiphonia*, *Mazzaella splendens* and 'Petrocelis' to grazing. Furthermore, the role of herbivory in reducing 'Petrocelis' crust abundance (as suggested by the photographic time series and abundance of limpets in this study) requires additional study. Feeding experiments and manipulative grazer exclusion experiments may be useful.

In conclusion, this research identified the alternate life history phase of *Acrosiphonia*, and established the natural dynamics of the life cycle in southern British Columbia, through a combination of molecular and field studies. The value of utilising molecular tools to solve ecological questions has been made evident. The thesis contributes not only to our understanding of complex algal life histories and endophyte / host associations, but also addresses the adaptive significance of heteromorphic life cycles among algae. Increased knowledge of algal life histories in nature is vital for the cultivation or harvesting of economically important algae, the control / eradication of detrimentally invasive seaweeds and our comprehension of marine intertidal communities, biodiversity and ultimately conservation.

* **NOTE:** Alignments for isolate sequences in phylogenetic analysis (Chapter 1) have been entered in TreeBASE.

APPENDIX A: *Acrosiphonia* percent cover means. Numbers in parentheses are sampling days from start.

Burrard Inlet

Sampling Date	n	Mean	S.D.	S.E.
March 5/97 (100)	30	0.673	1.853	0.338
March 28/97 (123)	30	2.383	3.486	0.637
April 9/97 (135)	30	9.483	16.782	3.06
April 26/97 (152)	30	12.741	13.777	2.515
May 8/97 (164)	30	7.458	7.841	1.432
May 23/97 (179)	30	1.792	2.890	0.528
April 28/98 (519)	30	26.533	21.603	3.944
May 12/98 (533)	30	10.667	13.498	2.464
June 9/98 (563)	30	4.275	9.359	1.709

Sooke

Sampling Date	n	Mean	S.D.	S.E.
March 7/97 (118)	60	7.150	13.725	1.772
March 29/97 (138)	60	8.921	16.482	2.128
April 10/97 (160)	90	10.108	15.126	1.594
April 28/97 (178)	90	5.247	11.986	1.263
May 25/97 (205)	90	4.806	11.333	1.195
June 24/97 (235)	90	3.683	9.327	0.983
July 19/97 (260)	90	2.094	6.148	0.648
August 19/97 (291)	90	6.322	18.019	1.899
May 1/98 (545)	90	2.294	4.055	0.427
May 14/98 (559)	90	1.653	3.749	0.395
June 11/98 (577)	90	3.0	10.162	1.071

Bamfield

Sampling Date	n	Mean	S.D.	S.E.
May 24/97 (209)	60	4.112	10.828	1.398
June 22/97 (238)	60	0.867	2.327	0.3001
July 20/97 (266)	30	0.117	0.639	0.117
May 26/98 (576)	30	8.333	18.613	3.340
June 27/98 (608)	30	8.390	12.669	2.313
July 21/98 (632)	30	2.9333	6.061	1.107

APPENDIX B: 'Chlorochytrium' Density Means. Numbers in parentheses are sampling days from start.

Burrard Inlet

Sampling Date	n	Mean	S.D.	S.E.
April 9/97 (136)	30	0	0	0
April 26/97 (152)	30	0	0	0
May 23/97 (180)	33	0.0046	0.0164	0.0029
July 18/97 *236)	30	0	0	0
Sept. 14/97 (294)	29	0.0018	0.0069	0.0013
April 28/98 (519)	30	0	0	0
May 12/98 (534)	28	0.0370	0.0809	0.0153
June 9/98 (564)	29	0.6640	1.1850	0.2200
July 9/98 (594)	29	0.6550	0.9310	0.1730
Aug. 8/98 (624)	31	0.2160	0.3100	0.0557
Nov. 3/98 (711)	31	0.0491	0.0690	0.0124

Sooke

Sampling Date	n	Mean	S.D.	S.E.
March 29/97 (138)	27	0	0	0
April 10/97 (160)	29	12.160	11.6730	2.1680
April 28/97 (178)	27	22.571	43.8800	8.4450
May 25/97 (205)	27	23.578	35.0690	6.7490
June 24/97 (235)	28	6.304	8.3810	1.5840
July 19/97 (260)	25	5.788	11.6650	2.3330
August 19/97 (291)	29	23.3810	71.2710	13.2350
Sept. 16/97 (319)	30	2.3410	3.7110	0.6780
Oct. 17/97 (350)	39	3.3530	5.4870	0.879
Nov. 30/97 (394	32	0.4160	1.8740	0.3310
Jan. 11/98 (435)	31	1.3230	3.7200	0.6680
Feb. 25/98 (481)	33	0.1070	0.2240	0.0390
April 1/98 (516)	30	1.5160	5.2470	0.9580
April 16/98 (531)	29	9.1310	26.5590	4.9320
May 1/98 (546)	27	53.0290	146.3050	28.157
May 14/98 (559)	23	29.4420	73.7240	15.373
June 11/98 (577)	33	22.4070	48.6160	8.463
July 23/98 (620)	30	10.6110	45.6520	8.334
August 20/98 (648)	30	5.2220	17.0210	3.107
Nov. 7/98 (725)	28	2.6790	6.6390	1.254

Bamfield

Sampling Date	n	Mean	S. D.	S. E.
Oct. 27/96 (1)	26	7.2180	18.4110	3.6110
Dec. 9/96 (43)	32	9.3330	33.4260	5.9090
Feb. 5/97 (101)	32	0.1750	0.5190	0.0918
May 24/97 (209)	25	0	0	0
June 22/97 (238)	22	0.0020	0.0080	0.0017
July 20/97 (266)	32	0.1020	0.3120	0.05520
Oct. 17/97 (355)	32	0.6390	1.8390	0.3250
Dec. 2/97 (401)	32	0.1250	0.4310	0.0762
Feb. 28/98 (489)	28	0	0	0
March 26/98 (515)	32	0	0	0
April 25/98 (545)	24	0	0	0
May 26/98 (576)	32	0.9850	3.6190	0.6400
June 27/98 (608)	29	6.7650	19.9040	3.6960
July 21/98 (632)	30	4.2540	8.4590	1.5440
Sept. 6/98 (679)	29	4.2770	16.1210	2.9940
Oct. 6/98 (710)	29	14.2360	47.6950	8.8570

APPENDIX C: 'Codiolum' Density Means. Numbers in parentheses are sampling days from start.

Burrard Inlet

Sampling Date	n	Mean	S. D.	S. E.
Nov. 24/96 (1)	23	23523.910	21242.994	4429.470
Jan. 10/97 (48)	24	7965.833	5319.667	1085.877
March 5/97 (101)	30	926.333	2350.968	429.226
March 28/97 (124)	30	80.833	129.768	23.692
April 9/97 (136)	30	545.167	1303.724	238.026
April 26/97 (153)	30	576.667	522.961	95.479
May 23/97 (180)	27	11597.590	14691.292	2827.340
June 22/97 (210)	26	16510.000	16023.279	3142.423
July 18/97 (236)	30	11514.170	11357.357	2073.560
August 17/97 (266)	26	8048.462	13277.291	2603.891
Sept. 14/97 (294)	30	4968.333	4303.546	785.716
Oct. 18/97 (328)	26	3535.192	4882.857	957.607
Nov. 14/97 (355)	26	1843.846	5356.893	1050.573
Dec. 15/97 (386)	30	8.333	21.105	3.853
Jan. 28/98 (430)	26	1437.308	3531.957	692.674
Feb. 24/98 (457)	15	96.667	258.579	66.765
March 30/98 (491)	29	11.207	25.518	4.739
April 28/98 (520)	18	924.444	1663.357	392.057
May 12/98 (534)	25	8939.000	9937.591	1987.518
June 9/98 (564)	26	33094.23	30372.782	5956.593
July 9/98 (594)	28	93333.040	84676.282	16002.310
August 8/98 (624)	30	99111.000	82123.760	14993.680
Nov. 3/98 (711)	29	15602.610	38725.265	1562.825

Sooke

Sampling Date	n	Mean	S. D.	S. E.
Nov. 11/96 (1)	29	4699.138	4866.868	903.755
Jan. 8/97 (59)	25	1011.600	1417.323	283.466
March 7/97 (118)	29	276.724	507.793	94.295
March 29/97 (138)	29	37.931	56.149	10.427
April 10/97 (160)	27	783.333	1080.074	207.860
April 28/97 (178)	27	4329.815	3385.810	651.599
May 25/97 (205)	26	12038.080	13773.900	2701.284
June 24/97 (235)	29	17027.070	10837.888	2012.545
July 19/97 (260)	29	13376.550	7418.535	1377.587
August 19/97 (291)	29	14903.620	13403.218	2488.915
Sept. 16/97 (319)	28	12915.000	9376.810	1772.050
Oct. 17/97 (350)	28	13091.790	8870.571	1676.380
Nov. 30/97 (394	29	10166.210	8925.600	1657.442
Jan. 11/98 (435)	22	6196.364	8771.494	1870.089
Feb. 25/98 (481)	29	903.103	809.151	150.256
April 1/98 (516)	29	462.069	389.346	72.300
April 16/98 (531)	29	1323.621	1896.518	352.175
May 1/98 (546)	29	661.379	621.078	115.331
May 14/98 (559)	27	8568.704	7859.414	1512.545
June 11/98 (577)	28	5601.786	6548.875	1237.621
July 23/98 (620)	29	23031.720	12569.741	2334.142
August 20/98 (648)	29	20197.070	17634.758	3274.692
Nov. 7/98 (725)	29	7341.724	10601.194	1968.592

Bamfield

Sampling Date	n	Mean	S. D.	S. E.
Oct. 27/96 (1)	30	491.667	746.640	136.317
Dec. 9/96 (43)	23	559.783	836.651	174.454
Feb. 5/97 (101)	10	140.000	295.851	93.556
May 24/97 (209)	22	32.954	101.590	21.659
June 22/97 (238)	30	86.667	178.926	32.667
July 20/97 (266)	30	134.167	236.942	43.259
Oct. 17/97 (355)	20	198.750	371.384	83.044
Dec. 2/97 (401)	30	255.833	403.684	73.702
Feb. 28/98 (489)	9	8.333	17.678	5.893
March 26/98 (515)	7	0	0	0
April 25/98 (545)	25	4.000	9.354	1.871
May 26/98 (576)	26	16.346	78.403	15.376
June 27/98 (608)	28	5.357	12.467	2.356
July 21/98 (632)	30	33.333	99.856	18.231
Sept. 6/98 (679)	30	109.167	213.396	38.961
Oct. 6/98 (710)	26	2947.500	2654.369	520.564

APPENDIX D: Surface area (cm^2) of individual 'Petrocelis' crusts at Burrard Inlet for one year.

Aug '97	Oct '97	Dec '97	Feb '98	March '98	May '98	June '98	August '98
2.396	2.9956	1.83016	3.3407	1.6899	11.424	6.991	10.196
2.65	4.3704	5.3954	0.1742	6.89	5.0889	10.408	7.0534
3.129	15.635	13.024	11.006	2.5585	7.423	4.375	8.859
2.312	6.743	3.5905	7.0098	3.0686	6.782	4.397	13.732
1.953	5.222	11.1214	1.4475	2.096	14.8008	4.545	10.172
1.97	7.57	0.32677	1.607	1.8166	5.4889	15.995	1.606
3.444	9.3018	4.366	2.1678	2.765	11.01	4.672	7.708
1.7067	42.367	3.3225	6.963	3.492	2.504	18.1125	5.486
12.83	4.435	3.3406	3.778	6.499	10.908	7.47	10.6
2.9267	8.372	5.896	3.804	7.1093	11.082	16.053	5.875
9.4325	5.412	0.7069	3.688	0	0	6.95	8.69
2.232859	0.79056	3.936	2.241	0	0	6.502	0
20.488	17.0685	1.7374	2.708		0	0.8103	0
11.948	3.9236	10.152	5.752		0	3.0938	0
3.09766	3.416	3.071	0.8754		0	0	0
7.96	23.632	2.2705	0.6103		0	0	0
4.617	7.577	2.4733	5.8465		0	0	0
2.9686	4.906	10.16	0		0	0	0
16.199	2.1215	15.57			0	0	
2.741	8.468	1.207				0	
18.635	13.347	2.068				0	
6.446	4.111	4.85					
5.247	1.078	0.43					
3.669	1.3027	11.486					
	15.908	0					
	1.551	0					
n = 24	n = 26	n = 26	n = 18	n = 12	n = 19	n = 21	n = 18

APPENDIX E: Surface area (cm^2) of individual 'Petrocelis' crusts at Sooke from summer 1997 to fall 1998.

Crust #	May-Jul 97	Sep-97	Oct-97	Jan-98	Dec-98	Feb-98	Apr-98	May-98	Jun-98	Jul-98	Aug-98	Nov-98
1				25.59	17.09				25.41			12.42
2	11.23		10.3	9.13	2.09	0		0	0	0	0	0
3	21.81	23.92	27.07		19.29	12.26	17.01	17.01	17.39	16.95		18.69
4	19.39	24.89	26.42			18.54	22.77	22.77	23.02	23.85	25.5	33.07
5	10.36	16.58	16.58	16.54	16.36	16.07	15.01	15.01	18.12	21.77	25.02	22.7
6	14.51	12.95	12.67		11.76	11.67	12.2			14.06		17
7	10.79	12.07	9.58		9.62	9.66	10.36	10.36	12.37	12.56	12.89	12.99
8	18.69	24.26	28.51	27.67	0.53	0	0	0	0	0	0	0
9	46.4		44.99	32.07	4.04							
10	7.76		10.53	10.29	7.76	5.61		6.77				
11	6.72		6.41				5	4.4				
12	9.85	12.82	13.2	13.76	6.89	4.3		4.75				
13	16.95	18.06	17.53		17.02	16.46	19.05	19.05		20.95		
14	8.34	9.68	9.73	10.19	2.22	0.54		0	0	0	0	0
15	29.34	30.07	37.67	35.74	36.13	35.7	33.99	33.99	21.02	20.65	14.28	0.6
16	19.17		17.98	16.51	16.89	17.22	19.13	19.13	19.24	19.19	23.47	22.69
17	4.55			17.05		13.48	15.1	15.1	15.36			12.13
18	2.58		5.87	6	6.65	5.87	5.91	5.91		6		
19	9.97	11.15	10.09		6.18	6.22	5.11	5.11	9	9.17	8.93	13.22
20	24.77		36.01	16	5.53	5.1	0	0	0	0	0	0
21	3.2	3.67	3.54	3.77	3.84	3.72			4.23			
22	20.44		24.72		25.11	32.66	34.39	34.39				
23	4.67	4.81	5.34	4.39			0	0	0	0		0
24	2306		37.7	33.6	4.55		0	0	0	0	0	0
25	2.91		4.45		4.73		5.44	5.44	5.94	7.01	7.34	7.89
26	8.2		9.51	6.05	6.33	5.96	4.52	4.52				5.8
27	14.76	21.79	21.99		21.87	22.01	21.85	21.85	24.06	24.63	24.47	26.13
28	11.1	13.08	12.85		12.96	12.54	14.64	14.64		16.4	15.89	15.96
29	10.87		14.2		15.68	14.68	15.87	15.87	16.17	15.87	14.19	16.93
30	27.36		33.94	24.26	2.57	2.89	2.75	2.75	2			0.78
n	30	15	28	18	25	24	23	26	20	20	15	22