The structure and function of hairs of chaetophorales

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Frontispiece - *Draparnaldia glomerata* incubated in Na-β-glycerophosphate and stained using a lead capture technique. The black deposits indicate alkaline phosphatase activity.

The Structure and Function of Hairs of Chaetophorales

by

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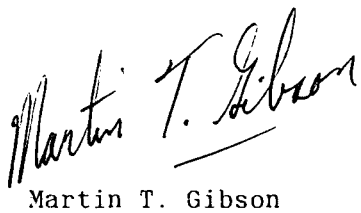
This thesis is entirely the result of my own work. It has not been accepted for any other degree and is not being submitted for any other degree.



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ABSTRACT

A study was carried out on the relationship between hair formation and water chemistry in three genera of Chaetophorales (especially Stigeoclonium).

The relationship between environmental chemistry, algal morphology and phosphatase activity for 32 samples of natural populations in N-E. England was investigated. Two indices of hair formation were significantly correlated with aqueous P (-ve), algal N : P ratio (+ve) and phosphatase activity (+ve). Other correlations included: algal P v. aqueous P (+ve); algal P v. phosphatase activity (-ve); algal N : P ratio v. aqueous P (-ve). All algal samples with a P content of 0.96% (by weight) and above lacked hairs; all with a P content of 0.53% and below had hairs. All algal samples with N : P ratios of 5.58 and below lacked hairs; all with ratios of 6.46 and above had hairs.

The influence of pH on phosphatase activity of six natural populations showed three markedly different responses, but the pH optima of three laboratory strains of Stigeoclonium were similar; pH had little influence on the phosphatase activity of two laboratory strains of Draparnaldia. Phosphatase activity was localized on hairs of Chaetophora and Draparnaldia, but the results were not clear-cut for Stigeoclonium.

Elemental deficiencies gave a similar pattern of the extent of hair formation in 13 strains: - P > - N > - Fe = - S > - Ca > - Mg > control. Elemental additions to five strains resulted in zoospore release; the greatest amount was released when N or P was added to N- or P-limited cultures. Five strains were grown to P-limitation in batch culture; the maximum P composition ranged from 2.4 - 6.4 % dry weight; hair formation commenced when algal P had fallen to about 1 % dry weight, long before any culture had ceased to grow or started to appear unhealthy. The strains were shown to be capable of utilizing a number of organic P sources.

The possible role of hairs and similarities between hair-forming blue-green algae and Chaetophorales are discussed.

ABBREVIATIONS

°C	degrees Celcius
g	gramme
mg	milligramme
µg	microgramme
l	litre
ml	millilitre
m	metre
cm	centimetre
µm	micrometre
nm	nanometre
d	day
h	hour
min	minute
s	second
M	molar
mM	millimolar
µM	micromolar
µmol	micromole
m-equiv	milli-equivalent
n	number of samples
P	phosphorus
FRP	filtrable reactive phosphorus
TFP	total filtrable phosphorus
P_i	inorganic phosphate
PAR	photosynthetically active radiation
s.d.	standard deviation
$\bar{x}$	mean
µS	microsiemens
chl α	chlorophyll α
DMGA	dimethyl glutaric acid
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid

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CONTENTS

List of Tables

List of figures

1. INTRODUCTION

1.1 General introduction

Chemical analyses of water are usually undertaken to assess either the suitability of water for human needs or the effect of human activities on the environment. As the measurements are often used to assess biological needs it seems logical to use biological systems in the assessment procedure. Much work has been carried out using species presence or absence to give a broad indication of water quality and destructive chemical analyses of biological material are now widely accepted in environmental studies, since they give an integrated or average reading over time.

Plant morphology is often related to environmental factors and Sinclair (1977) pointed out that the development of hairs in a certain alga might have potential as an indicator of nutrient status. This feature could be of particular value if hairs occurred in response to specific nutrient deficiencies.

1.2 What is an algal hair?

Hairs in prokaryotes were defined by Bornet & Flahault (1886) as a series of narrow, elongated cells containing very little protoplasm and incapable of further growth. Sinclair & Whitton (1977) redefined hairs as a region of the trichome where the cells are narrow, elongated, highly vacuolated and usually apparently colourless; this definition allows easy recognition of hairs by microscopy alone.

According to DeBoer & Whoriskey (1983) a hair in eukaryotic algae usually refers to a sterile, filamentous outgrowth from the surface of the

thallus; this definition is good for many marine species but is not general enough to include filamentous algae. Whitton and Harding (1978) defined hairs in Chaetophorales as a terminal portion of a filament composed of one or more narrow and elongated cells lacking any visible chloroplast; again this allows easy recognition by light microscopy alone.

Huber (1892) defined a hair (pilum) as a hyaline, elongated vegetative cell or series of cells in each of which a nucleus is present. He differentiated this from a seta, which he described as a hairlike outgrowth of an algal cell which does not contain a nucleus; these are present in members of the Coleochaetaceae and Chaetosphaeridiaceae. Setae can usually be distinguished from hairs by observation alone, as most authors agree that they consist of only one cell or part of a cell. Some authors use the words hair and seta interchangeably (e.g. Pickett-Heaps, 1975), which can cause confusion.

1.3 Occurrence of hairs

Hairs can be produced by members of the Chlorophyta, Cyanophyta, Phaeophyta and Rhodophyta. Hair formation has usually been considered to be influenced environmentally in those species genetically capable of such development (DeBoer & Whoriskey, 1983; Fritsch, 1935; Pickett-Heaps, 1975). This is not universally accepted, as morphological differences between strains of *Anabaena* were shown by Stulp & Stam (1984) to be a reflection of their genetic structure, which was interpreted by the authors as indicating that such differences were genetically controlled. This interpretation did not seem to agree with the results of many studies (see below) and is undermined to a large extent by the findings of Golden *et al.* (1985) who demonstrated the loss of a section of chromosome immediately prior to heterocyst formation in a strain of *Anabaena*. If this type of chromosomal

change is associated with other morphological changes then the genetic differences observed by Stulp & Stam may be a reflection of changes in genetic structure caused by environmental factors.

1.31 Hairs in Chlorophyta

1.311 Hairs in the Chaetophoraceae

Hair development in the green algae is most marked in the Chaetophoraceae. Probably the most common observation is that hair presence increases with low combined nitrogen and this has naturally lead to suggestions that nitrogen deficiency causes hair formation in this family. As early as 1930 Uspenskaya noted that the number and length of hairs of *Draparnaldia glomerata* increased as the amount of combined nitrogen in the environment decreased. This trend was reported independently, for the same species, by Soumalainen (1933). In a later paper Uspenskaya (1936) reported hairs of *Stigeoclonium tenue* also increased when combined nitrogen was low. Abbas & Godward (1963) found that the number and length of hairs of *S. amoenum* and *D. plumosa* increased as nitrogen was depleted in the culture medium. Yarish (1976) reported that N deficiency caused an increase in hairiness of *Entocladia viridis* and *E. flastrae*. Whitton & Harding (1978), in culture studies of eleven strains of *S. tenue* and one of *Chaetophora incrassata*, and Franke (1982) in culture studies of five strains each of the species *S. tenue*, *S. aestivale* and *S. farctum*, found nitrogen deficiency caused hair formation in all of the isolates.

The other common limiting nutrient, phosphorus (Section 1.5), has also been implicated in hair formation in this group. Whitton & Harding (1978) reported that P deficiency gave the longest hairs in all the strains they studied; Franke (1982) reported formation under similar conditions for the

strains he studied but did not comment on hair length. Yarish (1976) found phosphorus deficiency gave rise to hairs in *Entocladia viridis*, *E. flastrae* and *E. ramulosa*; the last of these did not form hairs under nitrogen deficient conditions.

More general evidence supporting hair formation under nutrient deficient conditions comes from Godward's (1942) observation that hairs were present in old cultures of *Stigeoclonium amoenum*. Nielsen (1972) found that hairs were developed when *Phaeophila dendroides* and *P. tenuis* were transferred from an artificial medium to 3% sea water and Moestrup (1969) noted that the hairs of *Bulbocoleon piliferum* disappeared when it was transferred from sea water to enriched medium. Tupa (1974) grew *Aphanochaete magna* on slides in different natural environments and found growth was poorest and hairs most abundant in lake water cultures; she suggested this was due to low nitrogen concentration and noted that hairs increased the surface area so could aid nutrient uptake. Tupa (1974) also stated that Klebs (1896), Cholnoky (1929) and Vischer (1933) found fewer hairs on *Stigeoclonium* in running waters. The hairs of *Draparnaldia champlainensis* protrude from the mucilage which lead Cook (1970) to postulate an uptake function for them.

In two papers Johnstone (1978a, 1978b) investigated the effects of physical and chemical factors on the morphology of *Draparnaldia* in culture. He found that physical factors such as light intensity and duration had a profound affect on morphology, including hair formation, but hairs were not produced when phosphorus was depleted or absent from the growth medium.

1.312 Hairs in other green algae

The hairs of members of the Coleochaetaceae are outgrowths of pigmented vegetative cells so should probably be referred to as setae (Section 1.2). McBride (1974) noted high golgi activity in the seta-bearing cells of

Coleochaete scutata which lead him to suggest they had a secretory function.

Unicellular hairs are a characteristic of *Bulbochaete* (Fritsch, 1935) and the ultrastructure of this genus has been studied in some detail. Fraser & Gunning (1974) found high golgi activity in the hairs of *B. hiloensis* which lead them to suggest a secretory role for these structures. The cells at the base of the hairs were shown to be connected to the hairs by many plasmodesmata (Pickett-Heaps, 1975).

Within the Siphonales the genera *Acetabularia* and *Codium* have hairs or hair-like structures. The whorls of *Acetabularia* consists of sterile, unicellular hairs produced before the cap. these hairs have traces of pigment which are progressively reduced with distance from the main axis (Gibor, 1973). He also demonstrated the ability of these hairs to concentrate neutral red stain and calculated the surface area of one whorl of *A. mediterranea* to be almost equal to that of the main axis, which would aid uptake. The whorls of *A. mediterranea* are longer when the nitrogen concentration is low and Adamich *et al.* (1975) suggested this aided absorption as the surface area to volume ratio was increased. Head & Carpenter (1975) found *Codium fragile* had hair cover and were light green in low nitrogen environments but were non-hairy and dark green when nitrogen was abundant. When they put hairy material in sea water the hairs remained, but when combined nitrogen was added the hairs were lost.

1.32 Hairs in Rhodophyta

According to Fritsch (1945), early workers suggested one function of hairs of Rhodophyceae could be light shielding as hairs were more commonly observed at high light intensities (Berthold, 1882; Oltmanns, 1923). According to DeBoer & Whoriskey (1983), West (1971) considered light

intensity to be a major factor in the development of hairs in *Acrochaetium proskaueri* but DeBoer & Whoriskey found that light intensities in the range 40 to 210 µmol photons m^{-2} s^{-1} had relatively little effect on the abundance, length or distribution of *Ceramium rubrum* hairs. Berthold (1882) said that nutrients could be involved in hair formation as nutrient utilization was more rapid at high light intensities, other authors also implicated hairs in nutrient uptake (Kylin, 1917; Oltmanns, 1923; Rosvinge, 1903). Kylin (1917) found germlings of *Dumontia filiformis* produced hairs when KNO_3 was absent from the growth medium and also observed that hairs were produced by *Stilophora rhizoides* and *Asperococcus bullosus* if they were kept in the same medium for ten days whereas no hairs were produced, but growth increased, if the medium was changed daily. Hair initiation in *Ceramium rubrum* was stimulated by external NH_4^+ concentrations less than 0.5 µM and inhibited by concentrations greater than 20 µM (DeBoer & Whoriskey, 1983). In the same study, hairy thalli were found to have an ammonium uptake rate about two times greater than non-hairy algae. The increase in surface area due to hairs in conjunction with increased diffusive uptake sites on the hairs was proposed as an explanation of these results; the authors felt hairs were probably adaptive to take advantage of intermittant bursts of nutrients.

Dromgoole and Booth (1985) could not induce or suppress hair formation in isolated fragments of *Gelidium caulacantheum* by altering the nutrient status and changes in incident light made little difference to hair production so they discounted light screening as a possible function of these hairs. They suggested the hairs could protect against damage by U.V. light, heat and the activity of herbivores but further investigation was necessary.

Ultrastructural studies have shown complex pit connections between the mother and hair cells of *Nemalion helmenthoides*; this, and the presence of Golgi vesicles in the hairs, lead Duckett *et al.* (1974) to propose these hairs as a possible secretory route. Similar high Golgi activity and a pore at the base of hairs of *Ceramium rubrum* lead Chamberlain (1974) to the same conclusion.

1.33 Hairs in Phaeophyta

Fritsch (1945) described some genera with hairs in each of the nine orders of Phaeophyta. He quoted Berthold (1882) as suggesting these could function as a light shield but agreed with other authors (Oltmanns, 1923; Wille, 1897; Sauvageau, 1896) that they were more likely to be involved in nutrient or gas absorption. *Scytosiphon lomentaria* grown in blue light produced hairs irrespective of photosynthesis and growth (Dring & Lüning, 1974): the authors concluded that this was a specific morphogenetic response to the light quality.

1.34 Hairs in Cyanophyta

Extensive reviews of the literature on hairs in Cyanophyta are presented by Sinclair (1977) and by Whitton (in press). Hairs in this group are most characteristic of the Rivulariaceae (Bourrelly, 1970; Sinclair, 1977) but can occur in some members of the Loefgreniaceae, Nostochopsidaceae and Mastigocladaceae (Geitler, 1932; Sinclair, 1977). Until quite recently, hairs were often assumed to be dead or dying cells (Fuhs, 1973) despite the rather striking cellular differentiation associated with hair formation. As many members of this group can fix nitrogen there are few cases of nitrogen deficiency leading to hair formation, though Sinclair (1977) found no hair production in a *Calothrix* grown in an atmosphere devoid of

nitrogen. Kirby (1975) noticed that hairs were more common when the external phosphorus concentration was low and Sinclair (1977) reported that, in cultures of 13 strains grown to elemental deficiency, hairs were most common and longest under phosphorus deficiency. Livingstone *et al.* (1983) studied a strain of *Calothrix parietina* (Durham culture D550) isolated from an upland stream and concluded that hairs were the site of localized phosphatase activity which probably allowed utilization of organically bound phosphorus. The isolate was grown in culture to P deficiency; hair formation began at an internal phosphorus content of about 1% of dry weight. Surface phosphatase activity was detectable at about the same time as hair formation started and the strain was capable of utilizing a number of organic phosphorus forms.

1.4 Chaetophorales

There is no general consensus about the classification of Chaetophorales. The order Chaetophorales is recognized by many authors (Printz, 1964; Fritsch, 1935; Bourrelly, 1972; Bold & Wynne, 1978) and contains the family Chaetophoraceae. Stewart *et al.* (1973) redefined some of the orders within the Chlorophyta on the grounds of cytological findings and kept the order Chaetophorales; they included the families Chaetophoraceae, Aphanochaetaceae and Schizomeridaceae in this order. Other authors have included the Chaetophoraceae within the order Ulotrichales (Smith, 1950; Fott, 1971) and Pickett-Heaps (1975) stated it was doubtful whether the Chaetophorales were distinctive enough to be set apart from the simpler Ulotrichales merely because they have more than one plane of cell division; he pointed out that genera such as *Ulothrix*, *Stigeoclonium*, *Chaetophora*, *Draparnaldia*, and *Fritschiella* show an obvious gradation of morphological properties. Printz (1964) had yet another approach, putting

the families Chaetophoraceae and Ulotrichaceae in the order Chaetophorales. Whichever classification is used, the division is based on the grounds that the vegetative cells differentiate into a basal and a prostrate system. Fritsch (1935) went so far as to say that the Chaetophorales were the only algal forms to look to for a clue to the origin of higher plants, since they exhibit heterotrichous development.

Although the classification of Stewart *et al.* (1973) is probably the most sound, it is not complete, so Printz's (1964) flora is preferred for all identification in the present study. This study deals mostly with three genera of the Chaetophoraceae which are described below.

1.41 The genus *Stigeoclonium*

Stigeoclonium is generally recognized as being the simplest member of the Chaetophoraceae, closely resembling *Ulothrix*. This genus is widely distributed, being typical of both organically and heavy metal polluted waters (McLean, 1974; Harding & Whitton, 1976; de Vries *et al.*, 1983, 1985) and upland streams (Harding & Whitton, 1976). It can grow attached to stones and woodwork or epiphytically on aquatic angiosperms (Bold & Wynne, 1978).

Each plant consists of a basal and erect system; both systems have considerable phenotypic plasticity (Cox & Bold, 1966; McLean & Benson-Evans, 1977; Whitton & Harding, 1978; Franke & ten Cate, 1980). The erect system has uniseriate axes and, usually, alternate or opposite branches. The width of cells of the erect system decreases gradually along the filament, and ends in bluntly pointed or hair cells. Each chloroplast is a parietal plate covering most of the cell wall, especially in the cells of the branches. There are reports of strains having a thin mucilage (Prescott, 1962).

Asexual reproduction is by biflagellate or quadriflagellate zoospores or the formation of resistant akinetes; these may be produced in great numbers under certain conditions, such as transfer to artificial medium (Cox & Bold, 1966; de Vries *et al.*, 1983). Reports of the sexual reproduction of this genus are rare (Franke & ten Cate, 1980) and the conflicting accounts were summarized by Cox & Bold (1966).

1.42 The genus *Chaetophora*

Less widely distributed than *Stigeoclonium* (Bold & Wynne, 1978) this genus is found in running or quiet fresh waters and is often most abundant in winter (Round, 1981). The colonies are often recognizable macroscopically because they are enclosed by a visually obvious gelatinous mucilage of firm consistency which may contain carbonate crystals (Round, 1971). The larger cells of the axial filaments contain band shaped chloroplasts and may appear pluriseriate. The cells of the branches have massive chloroplasts and have blunt tips or end in long multicellular hairs which protrude from the mucilage.

Quadriflagellate zoospores are produced, and the biflagellate gametes are isogamous (Bold & Wynne, 1978). The life cycle has been elucidated in culture.

1.43 The genus *Draparnaldia*

Prescott (1962), in his key to algae of the Great Lakes, said species of the genus *Draparnaldia* seem to prefer cold water, so are often collected in Spring. Each colony has an obvious main axis of large cylindrical cells 1.5 to 2 times their diameter in length. Branches originate at one point near the transverse wall of the axial cells and rebranch to form whorls. A copious mucilage surrounds each colony, but

unlike in *Chaetophora*, this is not firm in consistency. The axial cells have band shaped chloroplasts while the cells of the branches have massive chloroplasts and end in blunt tips or long multicellular hairs which protrude from the mucilage. A high degree of phenotypic plasticity has been demonstrated in cultured material (Johnstone, 1978a, 1978b).

Quadriflagellate zoospores arise from the cells of the branches in asexual reproduction and, as in *Stigeoclonium*, these may be produced in large numbers (Johnstone, 1978a). According to Bold & Wynne (1978) the sexual reproduction of this genus has not been studied for more than 60 years; in sexual reproduction quadriflagellate gametes are reported to form thick-walled zygotes which germinate to form two or four young filaments.

1.5 Nitrogen and phosphorus as limiting nutrients

Nutrients are said to be limiting when the growth or a metabolic process of an organism is dependent on the rate of supply of the nutrient. Many studies of nutrient limited growth have been carried out (see Kuhl, 1974; Healey, 1982) and it has been demonstrated that growth may be limited by more than one nutrient (see, for example, de Vries *et al.*, 1985). There is still controversy over how applicable the concept of nutrient limitation is to natural populations as physical and other factors are also important. No clear relationship between growth rate and external nitrogen or phosphorus concentrations has been found for eukaryotes in nature (Caperon & Meyer, 1972; Rhee, 1973; Healey, 1982); Brown & Button (1979) suggested this could be because limiting concentrations are below current detection limits. On a global scale, phosphorus is more likely to be limiting than nitrogen in freshwaters (Wetzel, 1975).

1.51 Nitrogen in the environment

Nitrogen is an abundant element but much is bound in rocks and unavailable to the biosphere. All but about 0.04% of the nitrogen that is available to the bioshpere is in the form of N_2 gas, so nitrogen fixation by prokaryotes is an important source of organic nitrogen in natural waters (Söderlund & Rosswall, 1982). Nitrogen is most stable as NO_3^- in oxidized waters but significant quantities of NH_4^+ can occur in areas where biological matter is degraded. Both NO_3^- and NH_4^+ can be assimilated by most algae and many can also assimilate NO_2^-, urea and amino acids (de Vries & Kamphof, 1984; Wheeler *et al.*, 1974; Jackson & Williams, 1985) and Grobbelaar (1983) found that 95% of the nitrate nitrogen on suspended particles was available to algae in the Amazon River.

1.52 Phosphorus in the environment

Phosphorus can enter the aquatic environment from a number of sources including the weathering of apatite, the degradation of biological systems and anthropogenic input (Stumm & Morgan, 1981). Many forms of phosphorus can be carried in the dissolved, colloidal or suspended load. Recent studies (Viner, 1984) show that fine clay particles often carry *c.* 60% of the analyzable phosphorus of the sediments. In solution P has been shown to be present in many compounds (Lean, 1973; Stevens, 1980) and the organic forms are often significant portions of the total dissolved phosphorus (TDP); this reflects the importance of P in biological systems. The inorganic dissolved phosphorus fraction is not well understood, hence analytical techniques will often define what is considered to be dissolved. The standard methods of analyses use a molybdate reagent which is very acidic and is thought to hydrolyze some polyphosphates (Stevens & Stewart, 1982); radiotracer studies and bioassays have indicated that the

orthophosphate may be over-estimated by up to 60%. The terms filtrable reactive phosphorus (FRP: Strickland & Parsons, 1968) and molybdate-reactive phosphorus (MRP: Broberg, 1985; Klotz, 1985) have been used to define the fraction sometimes referred to as orthophosphate. Enzyme hydrolyzable phosphorus (EHP: Klotz, 1985) has been used to describe the fraction released by enzymic digestion less the FRP fraction and total filtrable phosphorus (TFP: Strickland & Parsons, 1968) is used to define the fraction produced by acid-persulphate digestions; the use of such terms helps to remind us of our ignorance of the system under investigation.

1.521 Biologically available phosphorus

The forms of P most commonly utilized by biological systems are HPO_4^{2-} and $H_2PO_4^-$, the ionic forms of phosphorus which predominate according to the pH. Many organisms have two uptake systems for phosphate, one being 'diffusive' and the other 'rapid' (Ducet *et al.*, 1977). The rapid uptake system occurs when the internal phosphorus concentration is low, and presumably growth limiting. Both these uptake systems require energy but Whitton (1967) described colonies of *Nostoc* which appeared to passively take up P when the external concentration was very high.

Many different substrates can be utilized as P sources by algae which are capable of alkaline phosphatase activity (Ihlenfeldt & Gibson, 1975; Livingstone *et al.*, 1983; Al-Mousawi, 1984). In natural waters both high and low molecular weight organic phosphorus fractions have been identified (Minear, 1972; Lean, 1973) and at least a part of each fraction has been shown to be available to some algae (Lin, 1977; Taft *et al.*, 1977). Kuenzler (1965) showed that the phosphate from glucose-6-phosphate could be assimilated by many marine algae. Livingstone *et al.* (1983) found that *Calothrix parietina* (D550) could utilize six different substrates as

sources of phosphorus. Al-Mousawi (1984) found 10 substances could be utilized by nine strains of blue-green algae isolated from an Iraqi rice field and *Anacystis nidulans* (Durham culture 33, CCAP 1405/1); a strain of *Anabaena cylindrica* (Durham culture 2, CCAP 1403/2) could utilize 9 of the 10. A colloidal phosphorus fraction found in lakes (Lean, 1973) which is excreted by algae has been found to be unavailable to the algae (Lean & Nalewajko, 1976; Olsson & Jansson, 1984). Most of the compounds used by algae are soluble but 84% of the particulate P was available to algae in the River Amazon (Grobbelaar, 1983).

Broberg (1985) stated that the availability of different phosphorus compounds to algae is dependent on the algal enzyme pool, the phosphorus status of the alga, the orthophosphate uptake rate, the nature of the P-compounds and environmental conditions.

1.53 Environmental N : P ratios

Assays of lake water by Chiaudani & Vighi (1974), using *Selenastrum*, showed that if the N : P ratio was below 7 then N was limiting but if it was above 10 then P was limiting: Storch & Dietrich (1979) found the same critical ratio for P limitation in studies on phytoplankton. Few studies on this subject have been carried out on running water (Elwood *et al.*, 1985; Klotz, 1985) and flow has been shown to be an important factor in the rate of nutrient uptake. A number of studies have shown that increased flow rates increase the rate of nutrient uptake (Whitford, 1960; Whitford & Schumacher, 1961; Lock & John, 1979) but at high nutrient concentrations this is not always the case, presumably because the demand is satisfied by the concentration (Lock & John, 1979).

1.54 Algal N : P ratios

There have been many attempts to use the algal nitrogen and phosphorus concentration to determine which element is growth limiting. Unfortunately, the numbers produced by these studies are often difficult to compare because of the units of measurement used. Continuous cultures of phytoplankton often use the cell quotient (Q_o) which is the amount of nutrient per cell (e.g. Droop, 1975; Auer & Canale, 1982). Another common measure in phytoplankton studies is the ratio of C : N : P (see Goldman *et al.*, 1979), either by atoms or by weight. The final common measurement commonly used is N or P as a proportion of dry weight, this may be presented as weight for weight (e.g. Birch *et al.*, 1981) or as a percentage (e.g. Livingstone *et al.*, 1983). Parsons *et al.* (1984) provide factors for converting phytoplankton carbon values into dry weight; such factors should help make comparisons between different measures easier.

For oceanic phytoplankton populations the Redfield ratio (see Goldman *et al.*, 1979) is often used to determine which nutrient is limiting: an N : P ratio of less than 15.5 (by atoms) indicates N is probably limiting while a ratio greater than 15.5 indicates P is probably limiting. In studies on lakes, Gibson (1971) found that N was limiting if the algal N : P ratio was below 4.5 (by weight) and P was limiting if the ratio was above 7. Using *Stigeoclonium helveticum* as an assay organism de Vries & Hotting (1985) found the critical algal N : P ratio was 6.2 (by weight): ratios below this value indicated N limitation and above it indicated P limitation. Under conditions of P limitation the N : P ratio can be up to 35 : 1 (Nalewajko & Lean, 1980).

1.55 Algal P composition

Kuhl (1974) pointed out that the P composition of algae fluctuated widely depending on whether they were growing under P limitation. Lund (1956) found the P content of *Asterionella formosa* dropped from 1.66 µg mg dry weight^{-1} at the start of the spring bloom to 0.9 µg mg dry weight^{-1} at its peak but in cultures the content ranged from 14 - 4.6 µg mg dry wt^{-1} depending on the external P concentration. P concentration ranges of 79.8 - 5.9 and 3.6 - 1.82 µg mg dry wt^{-1} have been quoted for *Scenedesmus quadricauda* and *Navicula pelliculosa*, respectively (Nalewajko & Lean, 1980). Healey (1982) tabulated the P contents of a number of blue-green algae; they ranged from 18.3 - 0.3 µg mg dry wt^{-1}. He noted the minimum content was similar to that found in some eukaryotic algae. Gerloff & Skoog (1954) defined the critical concentration of a nutrient as the minimum tissue content that is necessary for maximum growth in a particular species: Birch *et al.* (1981) found this was 3.3 µg mg dry wt^{-1} for P in an estuarine *Cladophora*. The minimum viable algal P concentration of this alga was 0.5 µg mg dry wt^{-1} (Gordon *et al.*, 1981). A strain of *Stigeoclonium tenue* grown in culture had maximal and minimal P contents of about 70 and 8.5 µg P/µg C (Rosemarin, 1982).

Phosphorus within the cell is often described as being in different compartments, and interchange may take place between some of these compartments. The turnover and cycling of the terminal phosphate group of ATP is very rapid, but the phosphate of DNA is effectively separated from the other cell constituents. Fitzgerald & Nelson (1966) used a hot water extraction to try to determine the surplus phosphorus with an alga. They used the ratio of surplus to total algal phosphorus to determine whether phosphorus was limiting. Models of uptake and growth which account for the

internal pools have been quite successful (Tilman, 1977; Auer & Canale, 1982).

1.6 Phosphatases

Phosphatases catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt & Laskowski, 1961; Feder, 1973) and under some circumstances certain phosphatases act as transferases by catalyzing the transfer of phosphate from one substrate to another (Stadtman, 1961). They are often divided into acid and alkaline phosphatases according to their pH optima.

Two groups of phosphatases occur:

1) Constitutive phosphatases are intracellular and are involved in many processes such as photosynthesis and respiration (Kuhl, 1973);
2) Inducible phosphatases which are only synthesized under certain conditions.

The inducible phosphatases may be classified as either:

1) surface phosphatases;
2) alkaline or acid phosphatases;
3) phosphomonoesterases.

Induced phosphatases are usually associated with the cell surface, often being firmly bound (Kuenzler & Perras, 1965), and Livingstone *et al.* (1983) demonstrated that the activity was localized on hairs of *Calothrix parietina* (D550).

Surface phosphatases release orthophosphate, which can then be assimilated by the algae (Kuenzler, 1965). Although they are often classed

as phosphomonoesterases, phosphatases are noted for their lack of specificity so such classification is not always adequate.

The inducible surface phosphatase activity is usually associated with phosphorus limitation (Healey, 1982) and is widely used as an indicator of nutrient status (Healey, 1982; Nalewajko & Lean, 1980; Khoja *et al.*, 1984). Many studies have shown surface alkaline phosphatase activity to be very low at high external phosphate concentrations but increase manyfold when the external phosphate concentration drops (Fitzgerald & Nelson, 1966; Healey, 1973; Ihlenfeld & Gibson, 1975). Many authors however have found that while phosphatase activity is not a good indicator of the external phosphate concentration (Fitzgerald & Nelson, 1966; Perry, 1972; Vincent, 1981; Klotz, 1985) it may reflect the algal phosphorus content. Grusky & Aaronson (1969) found high phosphatase activity in senescent cells irrespective of the external P concentration and Klotz (1985) noted that, since algae in nature vary in age, the phosphatase activity would not necessarily reflect the nutrient condition, especially between spates in running waters.

The addition of phosphate to algae with high alkaline phosphatase activity has been shown to reduce or completely inhibit this activity (Price, 1962; Healey, 1973); this, however, may be by dilution with new growth rather than by end product repression (Fitzgerald, 1969).

The pH optima of alkaline phosphatases from most organisms fall within the range 8 to 11 (Kuenzler & Perras, 1965; Heath & Cooke, 1975; Doonan & Jensen; Rivkin & Swift, 1980; Al-Mousawi, 1984) but Livingstone & Whitton (1983) showed the optimum of a *Calothrix parietina* may have been higher. The ionic requirements of phosphatase enzymes are quite variable. The activity of phosphatases in many algae can be enhanced by the addition of Ca^{2+} (Healey, 1973; Healey & Hendzel, 1975; Doonan & Jensen, 1979). The

effect of Mg^{2+} on phosphatase activity is less clear. It has been reported to slightly enhance activity in the blue-green alga *Plectonema boryanum* (Doonan & Jensen, 1979), but had no effect on the chrysophyte *Ochromonas danica* (Patni & Aaronson, 1974); Al-Mousawi (1984) reported an inhibitory effect when Mg^{2+} was used in the assay medium of two strains of blue-green algae. Zinc may slightly inhibit activity (Doonan & Jensen, 1980; Ihlenfeldt & Gibson, 1975) but has been reported as an activator in a multicellular marine alga (Walther & Fries, 1976). There are also reports of cobalt, iodine, manganese and potassium being stimulatory (Ihlenfeldt & Gibson, 1975; Doonan & Jensen, 1979) and cobalt and manganese being inhibitory (Walther & Fries, 1976).

Lean (1973) noted that many naturally occurring organophosphorus compounds had bonds very similar to the high energy phosphate bonds of ATP. This means that the cleaving of phosphorus from such compounds would release energy though there appears to be no evidence that this can be used by the organism for other purposes. As many phosphatases can catalyze reactions even when the organisms are killed by chemical fixation (e.g. Livingstone *et al.*, 1983) the reactions are obviously exergonic and the enzymes must be quite robust.

1.7 Algae as biological indicators

Palmer (1959) demonstrated that algal species could be used as indicators of clean or polluted water and produced lists of indicator assemblages. It has been pointed out that algal assemblages, such as the diatom frequency distributions of Patrick (1971), probably give more information than species lists (Trainor, 1984).

Whitton (1984) distinguished monitors from indicators: a population is used as an indicator if presence or absence data is collected and used as a

monitor if subjected to numerical analyses. He pointed out that chemical analyses of algal material could give an integrated picture of pollution and could also give a better indication of the chemical fraction likely to affect the ecosystem than chemical water analyses.

1.71 *Stigeoclonium* as an indicator

The presence of *Stigeoclonium* has often been taken as indicative of organic pollution (Butcher, 1955; Palmer, 1959; McLean, 1974; de Vries & Kamphof, 1984), but it does occur in many unpolluted and heavy metal polluted waters (McLean, 1974; Harding & Whitton, 1976). McLean & Benson-Evans (1974) found *Stigeoclonium tenue* was more abundant in polluted waters but it was not restricted to organically polluted environments and was therefore not a good indicator of organic pollution.

1.8 Algae as assay organisms

Algae have been used as assay organisms in a number of studies, especially in the determination of B group vitamins (Swift, 1984) and a standard assay procedure has been developed using *Selenastrum* (Bartsch, 1971). Whatever species is used the assay techniques are similar in theory: sterile water samples are inoculated with the algae which is then incubated for a number of days. The growth, or lack of it, is a measure of available nutrients or toxins (Trainor, 1984).

1.81 *Stigeoclonium* as an assay organism

Because of difficulties in quantifying the growth of attached algae they are rarely used as assay organisms, but some assays using *Stigeoclonium* have been carried out. *Stigeoclonium* has often been reported to be heavy metal tolerant (Whitton, 1970; McLean, 1974; Harding & Whitton, 1976).

Harding & Whitton (1976) assayed 34 strains from 33 sites and confirmed different levels of zinc resistance occurred which appeared to be dependent on genetic adaptation. In a later paper (Harding & Whitton, 1977) they found that increased pH, Ca, Mg or P slightly decreased the toxicity of zinc to a non-tolerant population but greatly decreased toxicity in a tolerant population.

Trotter & Hendricks (1976a, 1976b) devised and described an assay system based on *Stigeoclonium subsecundum* grown on glass slides (which they termed "stigeometers") held in a flow through tank. In a later paper growth under intermittant chlorination was reported to be dependent on the initial biomass at the beginning of the assay (Trotter *et al.*, 1978).

De Vries *et al.* (1983) used a strain of *Stigeoclonium helveticum* to determine whether N or P was limiting in polder ditches. They determined a critical ratio of inorganic N : P of 6.2: any water with a ratio higher was P limited and any with a ratio lower was N limited. As they worked on nutrient rich waters the use of the term limited could be misleading as some other environmental factor may limit growth in nature. In a later study de Vries & Hotting (1985) found that the growth potential of the assay organism on water collected downstream from a sewage outflow increased up to a certain distance away from the outflow and then decreased again. Using the same strain, de Vries & Ouboter (1985) showed that sterilization of water samples for assay removed some chemical species from the water. Filtration of water samples removed 78 - 98% of the Fe^{3+} and up to 20% of the total phosphorus; autoclaving precipitated salts and up to 20% of the the total phosphorus was again removed. When both treatments were applied the removal was increased by up to 60%. In the same paper the authors point out that Franke & den Oude (1983) used *S. aestivale* from an environment very high in NH_4^+-N, which was probably a highly adapted

ecotype, so their warning against the use as of *Stigeoclonium* an assay organism would probably not apply generally.

1.9 Aims of the project

The hairs of members of the Chaetophoraceae are easily identified if present and their formation appears to be dependent on environmental factors (Section 1.31). The majority of studies indicate nutrient depletion has a role in hair formation, with deficiencies of the two most common limiting nutrients, N and P, giving the most abundant hairs. This study aimed to try and answer the following questions:

1) Is the presence of hairs in nature correlated with a specific chemical deficiency?

2) Does the relationship between alkaline phosphatase activity and the hairs of blue-green algae hold for Chaetophoraceae?

3) Is the formation of hairs related to the internal nutrient concentration of the alga?

4) Can the presence or absence of hairs be used as a reliable indicator of the nutrient status of an environment?

5) Do hairs have any unique functions?

Both natural populations and cultured material were used in the study. The aims of specific experiments are presented in the introduction to each chapter.

2 MATERIALS AND METHODS

2.1 Computing

Four computer systems were used during the study. Routine calculations were performed on a Sinclair QL (Sinclair Research Ltd) using the Abacus spreadsheet program (Psion Ltd). Statistical analyses were carried out using MIDAS (Fox & Guire, 1976) running on an IBM 4341-11 or an Amdahl 470/V8 mainframe operating under the Michigan Terminal System; the Durham database of river environmental data was held on an IBM 360/370 running under the same operating system. The Durham Culture Collection records were initially held on the mainframe but were transferred to a Research Machines Nimbus XN16 operating under MS-DOS; the database software used was Superfile (Southdata, London).

2.12 Durham computer databases and coding systems

2.121 Algal cultures

All unialgal cultures within the Durham culture collection are allocated a unique three digit number. The number has no taxonomic significance but is a reference for use in a computer database. The database holds information concerning details of sample collection and culture conditions.

2.122 River environmental database

Physical, chemical and biological data from previous field studies are stored in a standardized format database. Each stream and river studied has a unique four figure number and name allocated as described in Holmes and Whitton (1981a). All species are given a unique six digit number as described in Whitton *et al.* (1978). The first two digits define the

phylum, the second two the genus and the third two the species. If the species can not be determined the filament is subdivided into width categories. The database was compiled over a number of years and information which aided in the assessment of field study sites was obtained from it.

2.2 Chemicals and water

Most of the chemicals used in this study were Analar grade supplied by BDH, Poole, England; all other chemicals are listed in Table 2.1 with their grade and suppliers. Originally deionized water was produced by passing double distilled water through an Elgastat (model DCF-330) deionizer. Later in the study higher grade deionized water was produced by a Millipore Ultrapure Water system, fed with single distilled water.

2.3 Common procedures

2.31 Mass determination

Mass was measured on a Mettler H51 five place balance if accuracy was essential, routine measurements were made on a top-pan balance (Mettler P1200 or Sartorius, type 1474). All references to weight refer to mass.

2.32 pH

For laboratory work pH was measured using an EIL combination electrode and EIL pH meter (model 7050). All probes were calibrated with BDH standard buffer solutions immediately before a reading was taken; the pH of the buffers was arranged so that one was higher and one lower than the pH of the solution under investigation. The temperature of the buffers and solution being measured were the same.

Table 2.1 Specification and suppliers of chemicals other than BDH, Analar grade.

chemical	specification	supplier
glycine	chromatography grade	BDH, U.K.
β-glycerophosphate (sodium salt)	general purpose reagent	
phosphatidylcholine (lecithin) from egg		
$Na_2SiO_3.5H_2O$	technical grade	
agar		Difco Labs., U.K.
peptone		
nutrient agar		Oxoid, U.K.
tryptone		
yeast extract		
DNA, sodium salt from herring sperm, type VII	Sigma grade	Sigma Chemical Co., U.K.
glucose-1-phosphate		
HEPES		
inositol hexaphosphate (sodium salt)		
ρ-nitrophenyl phosphate		
$NaH_2P_2O_7$ (pyrophosphate)	practical grade	
$Na_3P_3O_9$ (metaphosphate)		
$Na_5P_3O_{10}$ (polyphosphate)		
osmium tetroxide		TAAB Labs, Reading
Spurr's Resin Kit		

2.33 Light

A Macam Lightmeter (model Q101) was used for all light measurements in the laboratory; light was not measured in the field. Photosynthetically active radiation was selected with a filter and readings were recorded as photon flux density (µmol photon m^{-2} s^{-1} PAR). As the light sensor was quite large it could not be placed inside flasks for measuring the true incident light, so all light measurements refer to the light incident on the surface of the relevant culture vessels.

2.34 Absorption

All colorimetric analyses were carried out using a Shimadzu Digital Double-beam Spectrophotometer (model UV-150-2); wavelengths were checked using the hydrogen emission line at 656.3 nm. Glass cuvettes of 1 - 10 cm path length were used for all readings in the visible and infra-red range of the spectrum; for readings in the ultra-violet range of the spectrum 1 cm quartz (Spectrosil) cells were used.

2.4 Standard culture techniques

2.41 General

To avoid contamination all isolation and sub-culturing work was carried out in a Microflow Pathfinder laminar flow cabinet conforming to B.S. 5295 class 1. Aseptic techniques were used throughout and working surfaces were swabbed with alcohol before and after use. If a microscope was needed during the procedure a Nikon Stereoscopic Zoom microscope (model SMZ-2) was used inside the laminar flow cabinet.

2.42 Preparation of glassware and utensils

Detergent washing was avoided if possible and any vessels which were detergent washed were thoroughly rinsed in tap water and twice in distilled water before acid washing. Containers and utensils were soaked in dilute acid and rinsed at least six times in distilled water before use. The acid used for washing stock, isolation and many experimental culture vessels was 4% HNO_3 aqueous. This was replaced by 10% H_2SO_4 aqueous for all experiments and analyses investigating phosphorus and/or nitrogen and the normal rinsing was supplemented by two additional rinses in double distilled deionized water or Milli-Q reagent grade water (see Section 2.2).

Glassware and utensils used for chlorophyll analyses were washed in detergent and rinsed thoroughly in distilled water or 90% methanol; these were never acid washed.

Silicon rubber stoppers (Sanko Plastics Co., Japan) used for culture flasks were soaked in 2% Decon 90 (Decon Laboratories Ltd, Hove), phosphate-free detergent, rinsed in hot water and then rinsed six times in distilled water before use.

2.43 Sterilization

Culture media and utensils were sterilized by autoclaving at 121°C (10^5 Pascal) for 20 min. Solutions with components destabilized by heat were filter-sterilized by filtration through pre-sterilized Millipore cellulose acetate filters; 0.2 µm filters were preferred but occasionally it was necessary to use 0.45 µm filters if resistance to filtration was high. Wire loops were pre-sterilized in a bunsen flame, isolation needles were dipped in alcohol and flamed very briefly.

2.44 Media

2.441 Stock culture medium (Chu 10-F)

The medium used for all stock culturing and most experimental work was a modification of formula No. 10 given by Chu (1942) and will be referred to as Chu 10-F. The concentration of combined nitrogen was increased to 16 mg l^{-1}, the extra nitrogen was added as $NaNO_3$ because additional KNO_3 caused precipitation during autoclaving.

The increased nitrogen level is in the range shown by de Vries & Kamphof (1984) to be optimal for some strains of *Stigeoclonium*. Preliminary investigations of field study areas indicated that the phosphorus concentration was below 1 mg l^{-1} at most sites so Chu's lower phosphorus concentration of 1.8 mg l^{-1} was used for stock cultures and either 1.0 or 0.5 mg l^{-1} for inoculation and most experimental media. Carbonate was also decreased and a chelator (EDTA) was added to increase the availability of Fe^{+}_{3} and decrease the precipitation of iron-phosphate salts. Micro-nutrients were added using the AC recipe of Kratz & Myers (1955) with the manganese concentration reduced.

The original medium relied upon the buffering capacity of orthophosphate to maintain the pH, however as phosphate was often reduced or depleted in experiments it was necessary to include another buffer. HEPES was chosen as the buffer because of its reported lack of interference in biological systems (Smith & Foy, 1974) and its low ionic complexation capacity (Good *et al.*, 1966). The pH (7.5) used was found to be within one s.d. of the mean pH for natural populations of Chaetophorales sampled in this study (from data of Table 4.2).

The medium was prepared according to a set recipe using concentrated stock solutions (Sinclair, 1977) as listed in Table 2.2. The pH of c. 500 ml of distilled water containing 0.6 g HEPES was adjusted to 7.5 with NaOH

before nutrient stocks were added; this gave a final HEPES concentration of 0.25 mM. The use of NaOH caused a marked increase in the amount of sodium in the medium, but no solution to this problem could be found. Stock solutions were stored in the dark at 4°C. The elemental composition of the standard media is presented in Table 2.3. It was found that autoclaving (10^5 Pascal, 20 min) after all the stock solutions were added caused negligible precipitation. Media were always left to cool at room temperature in the laboratory for at least six hours after autoclaving; this allowed re-equilibration with atmospheric gases. For large quantities of media (i.e. 1 l or over) at least sixteen hours were allowed for the cooling and re-equilibration.

2.4411 B-vitamins

The above medium was sometimes supplemented with B-vitamins: B_1 at 200 µg l^{-1} and B_{12} at 20 µg l^{-1}. These were added to autoclaved media from filter-sterilized stocks.

2.442 Chu 10-D

For preliminary investigations into deficiencies the Chu medium of Harding & Whitton (1976) was used; the recipe and elemental composition of this medium are listed in Tables 2.2 and 2.3, respectively.

2.443 $BBMPTB_{12}$

To study some aspects of taxonomy the $BBMPTB_{12}$ medium of Cox & Bold (1966) was used. Incubation was carried out in 250-ml flasks capped with cotton wool.

Table 2.2 Concentration of mineral salts used in Chu 10-D (Harding & Whitton, 1976) and Chu 10-F.

salt	Chu 10-D mg l^{-1}	Chu 10-F mg l^{-1}
$NaNO_3$	-	55.64
$Ca(NO_3)_2.4H_2O$	40.0	40.0
KH_2PO_4	8.0	4.49
$MgSO_4.7H_2O$	25.0	25.0
$Na_2SiO_3.5H_2O$	10.88	10.88
$NaHCO_3$	15.85	15.85
$FeCl_3.6H_2O$	2.42	2.42
$NaEDTA.2H_2O$	3.18	3.18
$MnCl_2.4H_2O$	0.045	0.045
$NaMoO_4.2H_2O$	0.007	0.007
$ZnSO_4.7H_2O$	0.056	0.056
$CuSO_4.H_2O$	0.02	0.02
$CoSO_4.5H_2O$	0.01	0.01
H_3BO_4	0.72	0.72
NaOH	c. 60	c. 60
HEPES	6.0	6.0

Table 2.3 Elemental compositions of media and complementary salts used in deficiency studies.

element	Chu 10-D mg l^{-1}	Chu 10-F mg l^{-1}	substitute salt
Na	c. 40	c. 48	
Ca	9.78	9.78	$NaNO_3$
N	6.83	16.0	$CaCl_2.6H_2O$
Mg	2.47	2.47	Na_2SO_4
K	2.24	2.24	
S	3.25	3.25	$MgCl_2.6H_2O$
Cl	0.96	0.96	
P	1.78	1.0	KCl
B	1.44	1.44	
Si	1.44	1.44	
Fe	0.5	0.5	none
Zn	0.013	0.013	
Cu	0.005	0.005	
Mo	0.0028	0.0028	
Co	0.002	0.002	
EDTA	2.78	2.78	

2.444 Experimental media

The standard medium was almost invariably the control for experiments investigating nutrient deficiencies or nutrient sources. The ionic background of each treatment was kept as near constant as possible by using substitute salt solutions to supply the complementary ions present in the omitted salt stocks; the concentration of the complementary ion was equal to that in the control. The substitute salts were those used by Whitton & Harding (1978) and are listed in Table 2.3. If nutrient concentrations were reduced the new concentration of an element, in mg l^{-1}, is given in brackets (e.g. Chu 10-F (0.1P) would be Chu 10-F with 0.1 mg l^{-1} P).

Substitute nutrient sources are listed in Table 2.4, many of these could not be autoclaved so all were added to the autoclaved medium after filter sterilization but Al-Mousawi (1984) demonstrated the stability of the substances under normal culture conditions. For these experiments the control would be treated in a similar manner, with the nutrient under consideration added after autoclaving. Any ionic substitutes were added before autoclaving the test media.

2.45 Culture vessels

2.451 Batch cultures

Batch culture vessels were always made of Pyrex glass. Incubation was carried out in either 10 ml of media in 60 ml capacity boiling tubes capped with Axa closures (Axa Ltd.) or 25 or 50 ml media in 100 ml conical flasks capped with silicon rubber stoppers (type S-28, Sanko Plastics Co.). If additions to the media were necessary 0.5 ml was added to boiling tubes and 1 ml was added to conical flasks; dilution of the medium was overcome by preparing the additives in medium lacking the ion being studied. Stock

Table 2.4 Sole sources of phosphorus other than KH_2PO_4.

β-glycerophosphate (sodium salt)

DNA (herring sperm)

glucose-1-phosphate

inositol hexaphosphate (sodium salt)

lecithin (phosphatidylcholine)

ρ-nitrophenyl phosphate

metaphosphate ($Na_3P_3O_9$)

polyphosphate ($Na_5P_3O_{10}$)

pyrophosphate ($Na_2P_2O_7$)

cultures were grown in 100 ml straight-necked, conical flasks and capped with silicon rubber stoppers (type C-30, Sanko Plastics Co.).

Solid media were produced by the addition of 1% w/v agar before autoclaving. The media were allowed to cool to about 50°C before pouring into sterile petri dishes (Sterilin) or 13 mm diameter glass boiling tubes. The latter were capped with silicon rubber stoppers (type C-20, Sanko Plastics Co.) and allowed to cool at an incline to produce 'slopes'.

2.452 Continuous cultures

The flasks described by Walsby (1967) were used for continuous cultures, these were made of boro-silicate glass. The solute concentration was reduced to one-fifth that of normal Chu 10-F. The dilution rate was such that the volume of the liquid in the container flowed through once per day, giving a phosphorus input of 0.1 mg d^{-1}. The algae were grown on glass microscope slides (see Section 2.47) resting at an angle of about 45° on

the indentation in the bottom of the flask. Individual slides were removed periodically to keep the biomass approximately constant during growth.

2.46 Incubation Conditions

2.461 Stock cultures

Stock cultures were maintained under continuous illumination (c. 30 µmol photon m^{-2} s^{-1} PAR supplied by Phillips warm white fluorescent tubes) at 20°C; subcultures were made every 6-8 weeks. Agar slopes of stock cultures were maintained under continuous illumination (15 µmol photon m^{-2} s^{-1} PAR) at 5°C; sub-culturing was carried out annually.

2.462 Batch cultures

Experimental cultures were incubated in Gallenkamp shaker tanks. Light was supplied from below by Phillips fluorescent tubes. Water temperature was maintained thermostatically at 20°C ± 0.5°C. Vessels were moved about 60 mm horizontally about 80 times a minute. Flasks were suspended by the neck with the base about 1 cm into the water, this gave an average light intensity of about 85 µmol photon m^{-2} s^{-1} PAR at the surface of the water. The light intensity varied with position in the growth tank so culture vessels were 'randomized' every other day to reduce the effects of this. Boiling tubes were incubated in the same growth tanks but were held at an angle of about 60° in a wire rack which reduced the average light intensity to about 65 µmol photon m^{-2} s^{-1} PAR.

2.463 Continuous cultures

Continuous cultures were incubated under continuous illumination at 20°C and stirred by an overhead rotary stirrer with stainless steel blades. As the light was supplied by a circular Phillips warm white fluorescent

tube the intensity within the vessel would vary, but at the surface it was 110 µmol photon m^{-2} s^{-1} PAR. The average speed of the water was about 0.4 m s^{-1}, but this was also subject to variation.

2.464 Taxonomic studies

Some studies of taxonomy used the methods of Cox & Bold (1966). The flasks for these studies were kept at 22°C and 100 µmol photon m^{-2} s^{-1} PAR in a growth cabinet. Illumination was provided from lights mounted above the flasks.

Table 2.5 Summary of algal incubation conditions

growth chamber	temperature (°C)	average light intensity (µmol photon m^{-2} s^{-1} PAR)
growth room	20	30
shaker tanks (flasks)	20	85
shaker tanks (tubes)	20	65
incubator	5	15
growth cabinet	22	100

2.47 Inoculation

Three inoculation strategies were used. For routine subculturing clumps of algae were transferred using a sterile wire loop; for experimental work a uniform inoculum was necessary.

For batch cultures the uniform inoculum was prepared by adding exponentially growing algal material to a known volume of sterile distilled water or media in an autoclaved blender unit; the material was always grown

under physical conditions similar to those of the experiment. The blender (MSE Overhead Blender) was run at full speed for 60 s and the resulting suspension filtered through a glass wool plug. The filtrate consisted of filaments with an average length of about 6 - 10 cells. A 1 ml sample was pipetted onto a Rafter Counting slide and at least 100 cells were counted; this gives a 95% confidence limit of <20% for each count if the cells are randomly distributed (Lund *et al.*, 1958). The total volume was adjusted to give an inoculum concentration that would produce a final concentration of 2.0×10^4 cells l^{-1}. The inoculum volume was always less than 2.5% of the experimental incubation volume.

For semi-continuous cultures microscope slides were roughened with emery paper before being acid washed. Each acid washed slide was then placed in 100 ml of medium in a 250 ml flask so that it inclined at about 45°. The media covered about half of the slide. Each flask was inoculated by the method given above and incubated for ten days under conditions of light and temperature similar to the average of those of the continuous culture vessel. The slides were transferred aseptically into the culture vessel and allowed to incubate for 48 h before the flow of medium was started.

2.48 Harvesting

The method used for harvesting depended upon the purpose of the experiment but three methods were generally used.

1) For routine morphological studies algal material was collected on a wire loop.

2) If material was to be used for electron microscopy or biochemical assays algae were carefully removed from the culture vessel using a pasteur pipette.

3) For analyses requiring all of the contents of the flask (e.g. dry weight, algal phosphorus determinations) algae attached to the flask were dislodged with a rubber tipped glass rod and the entire contents of the flask was passed through a pre-washed, pre-weighed GF/C filter under suction. If the medium was to be analyzed the acid washed collection flask was rinsed twice with a small amount of filtrate before a sample was taken. The culture vessel was rinsed with distilled water at least three times; the washings were passed through the collecting filter. The filter was dried at 105°C for 24 h and transferred to a desiccator for cooling; the desiccator was stored out of direct light.

2.49 Isolation and purification

Many methods for isolating and purifying algae were tried but the most effective was a modification of Hoshaw & Rosowski (1973) and Weidman et al. (1964). This became the sole method used for all the algae isolated by the author.

Algal material from the field was placed in acid washed snap cap vials containing a small quantity of the source water (typically <2 ml). The vials were transported to the laboratory on ice. The material was washed in distilled water and small sections of filament were removed with a micro-pipette. Each section was washed in six droplets of water held on an inverted petri dish lid, and placed in boiling tubes containing Chu 10-F. The resulting cultures were incubated in stock conditions (Section 2.461) until growth was obvious. The material was then transferred to fresh Chu 10-F and sonicated for 60 s at maximum power in a Soniprep 150. A drop of the sonicated suspension was removed with a sterile hypodermic (Gillette Surgical Ltd, U.K.) and sprayed onto agar plates using a air stream sterilized by passage through a 0.2 µm membrane filter. Plates were

incubated, upside down, under stock culture conditions for between 3 - 14 d before examination under a dissecting microscope. Apparently clean filaments were removed from the agar using a very fine sterile needle and placed into fresh Chu 10-F. Each culture was tested for bacterial contamination (method 2, Section 2.410) and if no pure cultures were found the sonication sequence was repeated. Most cultures were rendered unialgal after the first sequence and axenic after three or four.

2.410 Tests for purity

Stock cultures of axenic strains were tested for contamination after initial purification and before each subculturing. Each experimental inoculum was also tested. Only the second method of those that follow was used for every test.

1) Algal material was examined microscopically; if no obvious contamination could be seen further tests were carried out.

2) Streak plates of media and fragmented algal filaments were prepared following the methods of Hoshaw & Rosowski (1973), omitting the malt extract and casamino acid treatments. The plates were incubated in the dark at 32°C for 1 week. If no bacterial growth was seen inocula were considered pure but stock cultures were occasionally tested by method 3.

3) Normal liquid media was enriched with 0.05% sucrose, inoculated with algal material using a wire loop and incubated under stock conditions for 2 weeks. If the liquid did not become cloudy or have obvious bacterial flocks the cultures were re-examined as described in method 1.

2.5 Microscopy

2.51 Light microscopy

In the laboratory material was examined under a Nikon Fluorophot, type 109, fitted with a Nikon micrometer eyepiece. Light micrographs were taken using a Nikon M-350 automatic exposure camera. Either Ilford XP1 or Kodak Technical Pan 2415 film was used for black and white pictures, Kodak Ektachrome Tungsten Professional film was used for colour slides. To study the basal systems of *Stigeoclonium* strains an inverted microscope (G. Baker, London) was used; this did not have a camera.

To avoid osmotic effects live samples were mounted in a drop of the liquid in which they had been growing, for fixed material distilled water was found to be adequate. Sizes were measured using a micrometer eyepiece, for most purposes one division was 2.5 µm but if objects less than 5 µm were being measured a higher power objective was used so each division was 1 µm.

All slides were screened for obvious morphological features before any measurements were taken. Cell counts were made as random as possible by indiscriminate movement of the slide.

2.511 Morphology

2.5111 Definition of a hair

Hairs were identified using the definition of Whitton & Harding (1978) as "a terminal portion of the filament composed of one or more narrow and elongated cells lacking any visible chloroplast".

2.5112 Morphological scoring

The % hairiness of a colony was defined as the percentage of cells which were hairs, this was determined from a count of 100 cells selected at

Table 2.6 Trait frequency scale for morphological scoring of hairs and branching. Ten terminal cells were counted for column A; if hairs were obvious but none were counted a plus sign (+) was used. Averages were of ten counts.

A	B	C	D	
number of filaments ending in a hairs	Average length of hair (no. of cells)	average no. of cells between each branch	length of branch (no. of cells)	score
0	0	-	-	0
< 1	-	-	-	+
1	< 2	> 20	> 20	1
2 - 3	2 - 3	15 - 20	15 - 20	2
4 - 5	4 - 5	10 - 15	10 - 15	3
5 - 7	5 - 7	5 - 10	5 - 10	4
7 - 10	> 7	< 5	< 5	5

total hairiness = A + B

total branching = C + D

random. The percentage of terminal cells which were hairs was counted in a similar way and will be referred to as % hair terminal cells throughout this work. The statistical basis of such counts is given in Section 2.47.

The quick method used a variation of the semi-logarithmic scoring strategy of Tansley & Crisp (1926) and is presented in Table 2.6. Ten terminal cells were counted to determine the score for 'terminal hairiness', the number of cells in each of ten hairs was then counted to determine the 'hair length'; these scores added together give the 'total hairiness'. An index of branching was also used with the number of cells between any ten branches counted and the length of any ten branches (as number of cells) counted; these two numbers were added together to give the 'total branching'.

2.52 Electron microscopy

Fixed material (section 2.53) was washed twice in distilled water for fifteen minutes prior to staining in 1% osmium tetroxide for 1 h at room temperature. Stained material was washed in distilled water before dehydration was carried out according to the schedule given in Table 2.7. Dehydrated material was soaked for 2-4 h or overnight in 1 : 1 alcohol : Spurr resin (Spurr, 1969) and then resin alone for 4 h or overnight. Material was then set at 70°C in fresh Spurr resin.

Ultrathin sections were cut using glass knives on an LKB Ultrotome III ultra-microtome and collected on acid etched (4% HCl for 1min) copper/paladium grids. Section were then stained for 20 min in uranyl acetate, rinsed with distilled water and counterstained stained in lead citrate for the same length of time (Reynolds, 1963). (On one occasion 1%

toluidine blue and 1% borax were substituted for these stains.) The grids were then washed in distilled water, dried and examined under a Phillips EM 400 transmission electron microscope operated according to the manufacturers instructions. Kodak Electron Microscopy film was used for all electron-micrographs.

2.53 Fixation procedure

The following solution was used for routine fixation:

2.5% v/v glutaraldehyde

1.0% v/v formaldehyde

0.05 M Na-cacodylate (aqueous).

The pH was adjusted to 7.2 using NaOH. Material was incubated in the fixative solution for one hour at room temperature; this was followed by a distilled water rinse and an overnight rinse in Cacodylate buffer (pH 7.2, 4°C). Three five minute washings in 0.05 M HEPES-NaOH buffer (pH 7.5) completed the fixation.

Table 2.7 - Dehydration schedule for electron microscopy.

% alcohol	soaking time	no. of repeats
12.5	5	2
25	5	2
37.5	5	2
50	5	2
67.5	5	2
75	5	2
100	10	3

2.54 Staining techniques

2.541 Lead capture

Live or fixed material was incubated for one hour at room temperature in the following medium:

$Pb(NO_3)_2$	2 mM
$Ca(NO_3)_2.4H_2O$	2 mM
Na-β-glycerophosphate	2 mM
HEPES-NaOH (pH 8.0)	0.05 M.

Material was either prepared for electron microscopy using the standard fixation and embedding procedures or light microscopy by incubating in 1% $(NH_4)_2S$ for one minute and then washing 3 times in distilled water; the latter technique was essentially that of Gomori (1939).

2.542 Other light microscope stains

Nuclear material was stained using either DAPI or acridine orange according to the methods of O'Brien & McCully (1981).

Neutral red has been used to test the uptake of the sterile whorls of *Acetabularia* (Gibor, 1973), but its suitability for this is questionable (O'Brien & McCully, 1981). However, the technique is uncomplicated and indicates the extent of the vacuole so the uptake of neutral red was investigated by mounting filaments in a 10^{-5} M solution.

2.543 Ultra-cytological phosphate localization

Cytoplasmic distribution of phosphate was studied using a modification of the procedure of Voříšek and Zachleder (1984). Algal material was plunged into ice cold fixative solution (see above) and supplied alternately with 100 mM $MgCl_2$ and 100 mM $CaCl_2$. The remainder of the

fixation procedure was as above; fixed material was prepared for electron microscopy using the usual methods.

2.6 Studies of natural populations

2.61 Sample collection and preparation

2.611 On site

A number of physical and chemical factors were measured at the time of sampling. Current speed was measured using an OTT current meter. Conductivity was measured using either an Electronic Switchgear (model MC1 mark V) conductivity meter or a WTW (model FC91) portable conductivity meter with temperature display; all conductivity readings were corrected to 25°C. Total alkalinity (Mackereth *et al.*, 1978) and pH were measured using either an Orion Research (model 407A) pH/specific ion meter or a WTW (model PH91) pH meter with temperature display. When available, one of the WTW meters was used to measure temperature, in preference to a mercury thermometer.

2.612 Algal samples

Algal material was collected using forceps. Morphological scoring was carried out, on site, with the aid of a Cooke-McArthur field microscope fitted with a micrometer eyepiece. A sample of material was placed in an acid washed snap cap vial and kept on ice for transport back to the laboratory. As soon as possible after arrival at the laboratory the material was suspended in distilled water. Pieces of algae were identified under a dissecting microscope and transferred to clean distilled water using forceps; this process was repeated twice. Washed live material was used for alkaline phosphatase assays or microscopic studies.

For the determination of algal N and/or P content washed algal material was collected on pre-washed, pre-weighed GF/C filters, rinsed once with distilled water and dried at 105°C for 24 h. Dried material was cooled in a desiccator weighed and then digested and analyzed by the method of Section 2.752.

2.613 Water samples

Water was collected in H_2SO_4 washed, iodized polypropylene bottles; each bottle was rinsed three times with sample and filled expelling all air. Samples were kept on ice for transport to the laboratory where a known volume of each sample was passed through a GF/C filter. About 20 ml was allowed to pass through the filter before a sub-sample was collected for nutrient analysis. Water samples were analyzed according to Section 2.751.

2.62 Statistical treatments

Data from field surveys were divided into three groups- biological, physiological and physical and chemical. The biological data were a criterion set and the physical and chemical data a predictor set (Green, 1979), the physiological data were used as a predictor set with the biological data and a criterion set with the physical and chemical data.

Many environmental variables need to be transformed to meet the requirements of specific statistical techniques; in many analyses a normal frequency distribution is assumed. A sample was considered normally distributed if ± 1 S.D. accounted for 68% of all observations and ± 2 S.D. 96% (Statistical Research Laboratory, 1976). If logarithmic transformation was used and the sample contained zero values then 1 was added to every value before transformation. The transformations used are listed with the data.

The product moment correlation coefficient (r) was calculated for continuous data, for other data types Spearman's rank correlation coefficient (r_s) was employed. The latter method uses untransformed data and no assumptions are made concerning the distribution of these data. If hairs were obvious but not common enough to score morphologically they were assigned an arbitrary value of 0.5 for rank correlations, such samples are indicated by a plus sign (+) in tables.

Multiple and stepwise linear regression analyses were carried out for each criterion variable. The latter technique is highly recommended by Draper & Smith (1981) as it adjusts both independent and dependent variables not yet in the regression equation with those already present, taking into account intercorrelations within the set of independent variables. Because intercorrelations are accounted for it is often necessary to reduce a group of correlated independent variables to one variable representative of the group. If analysis of the correlation matrix indicated reductions were appropriate then the most representative member of the correlated group was taken as the variable which gave the highest correlation coefficient in regression analyses. It is necessary to bear in mind the inconsistencies involved with using multiple regression analyses (a Model I technique) with Model II data (Sokal & Rohlf, 1981).

2.7 Chemical analyses

2.71 Nitrite

Nitrite was determined by the modified Griess-Ilosvay method of Mackereth *et al.* (1978).

2.72 Nitrate

Nitrate (FRN) was reduced to nitrite by means of the spongy cadmium method of Elliot & Porter (1971) (as given in Mackereth *et al.*, 1978) and analyzed as for nitrite.

2.73 Filtrable reactive phosphorus (FRP)

Filtrable reactive phosphorus (FRP) was determined according to the method of Murphy & Riley (1962) as modified by Eisenreich *et al.* (1975).

2.74 Enzyme hydrolysis for total filtrable phosphorus (TFP)

Enzymatic hydrolysis of organic phosphates was carried out according to the method of Strickland & Parsons (1968) but β-glycerophosphate was substituted for ribose-5-phosphate as a test compound for complete hydrolysis. (This digestion should account for monophosphate esters and some fraction of any linear inorganic polyphosphates, little reaction with polynucleotides or inorganic pyrophosphates has been reported: Strickland & Parsons, 1968.) The total phosphorus of each digestion included both FRP and the phosphorus released by the digestion and was used as the measure of TFP for all natural waters.

2.75 Total N & P

2.751 Liquids

Digestions for the determination of total phosphorus were carried out according to the method of Eisenreich *et al.* (1975) or a modification of the method of Ebina *et al.* (1983); the latter allowed for determination of both total P and total N from one sample. In the latter method 25 ml of water sample was passed through a GF/C filter and then mixed with 25 ml of Milli-Q reagent grade water containing 20 g l^{-1} $K_2S_2O_8$ and 3.0 g l^{-1} NaOH

in 125 ml Pyrex flasks. The flasks were capped with aluminium foil and immediately autoclaved at 10^5 Pascal for 30 min. After cooling the digestion mixture orthophosphate was determined as above but with the digestion acid reduced to account for the acidity of the reaction solution; the acid : molybdate ratio was kept within the optimum range calculated by Eisenreich *et al.* (1975). Nitrate in the digestion mixture was determined either by absorbance at 220nm (American Public Health Association, 1980) or by the method given above. In the former method absorbance at 275nm was used to test for interference from undigested organic matter, any samples with absorbance values above 0.005 were rejected. The latter method required neutralization with NaOH.

2.752 Solids

Solid material was added to 50 ml of Milli-Q reagent grade water containing 20 g l^{-1} $K_2S_2O_8$ and 3.0 g l^{-1} NaOH in 100 ml Pyrex flasks; if the material was on GF/C filters both the material and filter were placed in the flask. The flasks were capped with aluminium foil and autoclaved as above. After autoclaving, the reaction mixture was allowed to cool before filtration through a GF/C filter. The remainder of the analysis is as above.

2.753 Test of digestion

To test the efficiency of the digestion of Ebina *et al.* in Sections 2.571 and 2.572 solutions of known N and P concentration were prepared, three replicates of two preparations of each solution were digested and analyzed. The concentrations were kept within the limits suggested by Ebina *et al.* (1983). No suitable reference material was available to test the digestion of solid material so the N and P content of five replicates

of four different algal strains were analyzed. Each alga was harvested during exponential growth and the nitrogen and phosphorus content determined from the difference between the initial and final concentrations of the respective nutrient; the percentage recovery of the digestion was then determined (Table 2.8).

2.76 Hot water extractable phosphorus

The hot water extractable phosphorus was determined using the method of Fitzgerald & Nelson (1966) but using the FRP analysis above.

Table 2.8 - Percentage recovery of nitrogen and phosphorus using a modification of the digestion method of Ebina *et al.* (1983).

compound	mean %age recovery	s.d.	n
Phosphorus:			
β-glycerophosphate	88.9	3.37	6
glucose-6-phosphate	93.4	7.42	6
phytate	100.5	6.36	6
K_2HPO_4	97.1	3.35	6
pyrophosphate	97.1	4.71	6
cultured algae	96.9	4.71	20
Nitrogen:			
glycine	98.6	4.02	6
HEPES	98.4	5.52	6
NH_4Cl	103.0	7.65	6
KNO3	101.7	4.82	6
urea	97.0	6.80	6
cultured algae	96.2	4.82	20

2.8 Chlorophyll *a* analysis

The basic method used was based on that of Lorenzen (1965) but the extraction was carried out using 90% methanol. Algae were collected on GF/C filters (Marker *et al.*, 1980) which were placed in McCartney bottles containing 10ml of solvent. The containers were placed in a water bath at 70°C and the liquid was allowed to boil for 10min and then cooled to room temperature overnight in the dark as recommended by Sartory & Grobbelaar (1984). Extracts were passed through GF/C filters to reduce turbidity and absorbance was measured at 665nm and 750nm. Extracts of field material were acidified with HCl to give a final concentration of 10 M; absorbance was recorded after 1 h.

The amount of chlorophyll within cultured material was assumed to account for a constant proportion of the total absorbance. In the field this assumption is not justifiable so the amount of absorbance due to phaeopigments must be considered. Unfortunately, both over- and under-estimates of measurements of chlorophyll have been reported (Bidigare *et al.*, 1985) and all the figures presented must be considered in the light of these findings. 4% of the absorption was assumed to be due to chlorophyll *b*.

2.81 Calculation of chlorophyll *a*

For culture work the mass of chlorophyll *a* was determined using the following equation:

$$C = E_a \times k \times V/l \quad (1)$$

for studies with material from natural populations this was expanded to:

$$C = (E_b - E_a) \times (R/R-1) \times k \times V/l \quad (2)$$

where:

C = concentration of chlorophyll in µg

E_b = absorbance of extract at 665nm before acidification less the absorbance at 750nm

E_a = absorbance of extract at 665nm after acidification less the absorbance at 750nm

R = maximum acid ratio for extracts containing no phaeopigments

k = 1000 x the reciprocal of the specific absorption coefficient of chlorophyll a at 665nm

v = volume of solvent used to extract the sample (in ml)

l = path length of the spectrophotometer cuvette (in cm).

The specific absorption coefficient and maximum acid ratio used were those of Marker *et al.* (1980).

2.9 Assay for phosphatase activity

The alkaline phosphatase activity (APA) of algal material was assayed using a modification of the colorimetric technique described in Sigma Technical Bulletin number 104 (Sigma Chemical Co., 1974). The assay was run on whole material except when the pH optima of isolated strains was determined, when a homogenate was produced by sonication for 60 s at full power in a Soniprep 150.

Material was washed in distilled water and incubated at 20°C in universal bottles (c. 25 ml) containing 1.0 ml buffer and 0.5 ml of source water. Routine analyses were carried out at pH 10.3 using 2-amino-2-methyl-1-propanol buffer (1.5 M). For some parts of the study a range of pH conditions were assayed for each sample with duplicate buffers at each pH to reduce the effects of any individual buffer on the reaction (Table 2.9).

After 10 min, 0.5 ml of substrate solution (ρ-nitrophenyl phosphate) was added to give a final concentration of 2.7 mM. The reaction was terminated by adding 10 ml of 0.05 M NaOH; this was usually after 1 h., but if the activity was low, after 2 h. The mixture was kept on ice for as much of the remainder of the procedure as was practicable (Healey, 1973). The entire mixture was filtered through GF/C filters and the absorbance of the filtrate read at 410 nm. For most assays the filter was analyzed for chlorophyll **a** and APA was determined as the amount of ρ-nitrophenol liberated per hour for each microgram of chlorophyll a. For some laboratory studies the initial amount of material was kept approximately the same between samples and the samples were scored on a 0-**V** scale; with 0 having no colour and **V** being the darkest yellow.

Table 2.9 - Buffers used for the study of alkaline phosphatase activity.

pH	buffer	molarity
7	3,3 dimethylglutaric acid - NaOH	0.05
7	HEPES - NaOH	0.025
8	glycine - NaOH	0.05
8	HEPES - NaOH	0.025
9	glycine - NaOH	0.05
9	Borax - HCl	0.05
10	glycine - NaOH	0.05
10	borax - NaOH	0.05
11	glycine - NaOH	0.05
11	borax - NaOH	0.05
12	glycine - NaOH	0.05
12	KCl - NaOH	0.1

3. AREAS OF FIELD STUDY

3.1 Introduction

This chapter presents background information on sites which were visited in a survey of algal hairs in the environment (see Chapter 4). The reaches were selected from a list accessed through the Durham Environmental Database; details of the reach numbers and dates of sampling are presented with physical and chemical data in Fig 4.1. Other workers have published data collected at some of the reaches studied here (e.g. Harding & Whitton, 1976, 1977; Holmes & Whitton 1981a, 1981b)

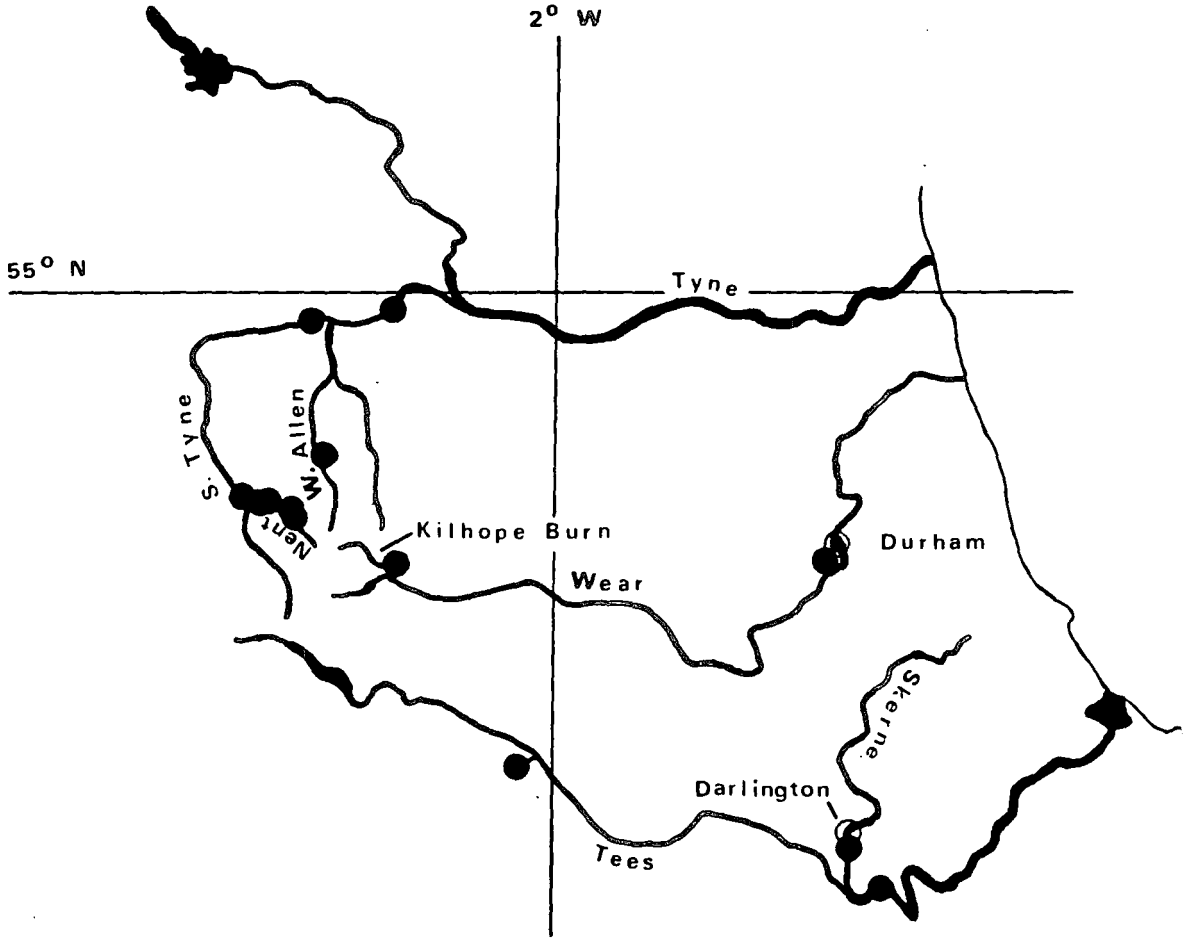
3.2 General background to areas of field study

The waters of the Tees, Tyne and Wear River catchments have been the subject of a wide range of studies (Horne, 1977) and are all easily accessible from the University of Durham. A map of the area is given in Fig. 3.1 showing sites which were visited in the survey of Chapter 4.

The three river systems studied rise in the Northern Pennine Ore Field and flow eastwards to the North Sea. The geology of the area is described by Dunham (1948). The upland areas consist of predominantly Carboniferous rock on a basement of folded Ordovician slates containing granitic intrusions. Magnesian Limestone and a metamorphosed limestone occur in the catchments, the former being more soluble gives a high input of Ca^{2+} and Mg^{2+} to waters which come into contact with it.

Anthropogenic influence comes mainly from agricultural and mining activities in the non-tidal portions of all the river systems studied, the main exception being the River Skerne (see Section 3.32). Mineral veins are reported to have been mined since pre-Roman times (Raistrick &

Fig. 3.1 River systems of sampling area. • denotes site visited in studies of Chapter 4.


2° W
55° N
Tyne
S. Tyne
W. Allen
Nent
Kilhope Burn
Wear
Durham
Skerne
Darlington
Tees

Jennings, 1965) but most of the mining activity took place in the nineteenth century. Many mine drainage waters empty into the three river systems, most being from disused mines (Harding & Whitton, 1976), polluting many of the upland streams with heavy metals; heavy metals are also introduced by seepage from heaps of mine tailings (Say & Whitton, 1981).

The upland areas are mainly covered by peat and heather moors, with pasture giving way to crops in the lower reaches (Whitton & Crisp, 1984). In general the upland areas have higher rainfall, more persistent cloud cover and lower temperatures than the lowland areas. The banks of many of the upland tributaries have little or no tree cover, but such cover increases towards the lower reaches.

3.3 Main Study Areas

3.31 Croft Kettle

This was the only still water site visited in the present study. It is characterized by very hard water and low concentrations of inorganic phosphorus (Livingstone pers. comm.). *Chaetophora incrassata* grows attached to wood and reeds around the edge of the pond and hair forming members of the Rivulariaceae also occur at this site (Hudson *et al.*, 1971).

3.32 Tees Catchment

3.321 Station Quarry outflow - tributary of River Tees

Station Quarry outflow is a narrow, slow flowing stream with highly calacareous water *Draparnaldia glomerata* is common in spring and autumn in this stream but *Batrachospermum* species are often dominant in summer; colonies of Rivulariaceae are present throughout the year.

3.322 River Skerne - tributary of River Tees

The River Skerne rises in the Durham Coalfield and is characterized by high concentrations of calcium and magnesium derived from Magnesian Limestone (Whitton & Crisp, 1984). The main site studied was below Darlington and could therefore be expected to have a high load of organic matter. Heavy metal pollution was found by Harding & Whitton (1976) to be low at a site somewhat higher up the catchment. Nutrient concentrations were consistently high in this river and, presumably, were never growth rate limiting.

3.323 The River Tees

The River Tees and its tributaries have recently been reviewed by Whitton & Crisp (1984). *Stigeoclonium tenue* has been reported at least three different sites (Holmes & Whitton, 1981a) and *S. falklandicum* at two (Holmes & Whitton, 1981b) sites in the river. Two streams in upland areas of the catchment were found to have low dissolved phosphorus concentrations of which a substantial proportion was 'organic phosphorus' (Livingstone & Whitton, 1983).

3.33 Tyne catchment

3.331 Rampgill Level - tributary of River Nent

Rampgill Level flows into the Nent from a tunnel; the stream is exposed for about one metre of its path and then flows underground until its mouth, which was the point of sampling. Harding & Whitton (1976) reported almost constant temperature year round with *Stigeoclonium tenue* the dominant alga throughout the year. They also reported high concentrations of heavy metals and low concentrations of phosphorus in the water.

3.332 River Nent - tributary of River South Tyne

The Nent catchment was the site of much lead and zinc mining in the last century and Harding & Whitton (1976) reported high concentrations of heavy metals, especially zinc and cadmium at two sites on the River Nent. These authors said *Stigeoclonium tenue* was the dominant organism in this river in both spring and summer.

3.333 River West Allen -tributary of River South Tyne

According to Wehr (1984) the River West Allen is a small upland stream influenced by a number of old lead mines along its length. Tailings from some of the disused mines can be seen along the banks of the river in some areas. Wehr said the river was frequently coloured brown with humic material and much of it is shaded during summer by several large trees.

3.334 River South Tyne - tributary of River Tyne

The presence of *Stigeoclonium tenue* in the South Tyne was reported by Harding & whitton (1976) and Holmes & Whitton (1981b). The water is often coloured brown with humic material and, although it must receive a large input of heavy metals from the River Nent, the site studied by Harding & Whitton did not have very high concentrations of zinc.

3.34 Wear catchment

3.341 Kilhope Burn - tributary of River Wear

Kilhope Burn has a high concentration of zinc (Harding & Whitton, 1976) for the site visited in this study. Harding & Whitton reported that *Stigeoclonium tenue* was often present but was once killed by some unknown factor; they postulated re-inoculation by algal populations from unpolluted

sites higher up the catchment. This is the only site visited in the present study which has an average pH below 7.

3.342 Hollingside Stream - tributary of River Wear

This stream has low concentrations of heavy metals and relatively hard water (Harding & Whitton, 1977); a sewage works outflow empties into the stream just above the reach visited in this study. *Stigeoclonium tenue* occurs in spring but is overrun by sewage fungus assemblage in late spring.

4. FIELD ANALYSES

4.1 Introduction

The aim of initial field work was to find which environmental factors, if any, were linked to hair formation. As phosphorus is often the most important limiting nutrient in freshwater systems (Section 1.5), and high ratios of organic- to inorganic-P are associated with blue-green algal hairs, particular attention was placed on collecting environmental phosphorus data.

4.2 Hairs, environment and algal physiology

4.21 Data Set

The data collection procedures were given in Section 2.6 and background information on the sites visited was presented in Chapter 3. The variables studied and transformations used in statistical analyses are listed in Table 4.1. Details of the sites studied and the environmental data are listed in Table 4.2, with descriptive statistics of the latter listed in Table 4.3. A total of 32 populations were sampled from different sites on 4 different dates. Sampling was only carried out at sites where Chaetophorales were present, 30 samples of *Stigeoclonium*, one of *Draparnaldia* and one of *Chaetophora* were collected. Nitrate in the water ranged over almost two and phosphate well over three orders of magnitude. A correlation matrix between some of the environmental variables (Table 4.4) indicates significant relationships between many of the variables with particularly close correlation between the two phosphorus fractions. Fig. 4.1 is a scatter diagram of TFP against FRP, the dashed line indicates where all the P is FRP. TFP exceeded FRP for all but 6 samples and was

Table 4.1 List of variables studied in survey of natural populations. The types of transformation used in statistical analyses are listed for continuous variables.

environmental variables	unit	transformation
temperature	°C	$\log_{10}$
current speed	m s^{-1}	$\log_{10}$
conductivity	µS cm^{-1}	$\log_{10}$
pH	-	none
NO_3-N	µg l^{-1}	$\log_{10}$
FRP	µg l^{-1}	$\log_{10}$
TFP	µg l^{-1}	$\log_{10}$
morphological variables		
% hairiness		
% terminal hairs		
physiological variables		
algal N	% dry weight	none
algal P	% dry weight	none
APA	µmol *p*-nitrophenol µg chl a^{-1} h^{-1}	square root

Table 4.2 Sites, dates and selected environmental data for algal samples collected for main study (see also Table 4.5).

sample number	site	grid ref.	stream - reach	date	current speed ($m\ s^{-1}$)	pH	NO_3-N (all $mg\ l^{-1}$)	TFP	FRP
1	Croft Kettle	NZ281113	-	3/6/85	-	7.6	100	645	12
2	R. Tees, Hurworth	NZ312100	0009-80	20/6/85	0.629	7.6	4160	1270	693
3	Kilhope Burn	NY809432	0013-12	3/6/85	0.434	5.5	138	10	10
4				5/6/85	0.249	5.3	400	11	9
5				20/6/85	0.256	5.5	275	16	16
6	R. Skerne, South Park	NZ284134	0025-85	3/6/85	0.460	8.0	39900	1680	872
7				5/6/85	0.503	7.7	37000	4610	1710
8				20/6/85	0.603	8.1	10800	4640	3100
9	R. Nent, above Levels	NY782435	0048-11	26/6/84	0.230	7.7	164	10	8
10				3/6/85	0.377	7.6	175	11	10
11				5/6/85	0.350	7.8	188	10	10
12				20/6/85	0.297	7.3	313	17	17
13	R. Nent, below Levels	NY782435	0048-12	26/6/84	0.250	8.0	390	7	7
14	R. Nent, Gossipgate	NY723467	0048-90	26/6/84	0.230	8.2	100	63	51
15	R. Nent, above South Tyne	NY716467	0048-99	26/6/84	0.270	8.0	91	8	7
16				3/6/85	0.354	8.3	175	30	20
17				5/6/85	0.288	8.3	198	27	16
28				20/6/85	0.255	8.2	296	17	12
29	R. South Tyne, below Nent	NY716469	0055-31	26/6/84	0.220	8.1	64	10	8
20				3/6/85	0.354	8.5	150	11	8
21				5/6/85	0.277	8.3	163	26	15
22				20/6/85	0.218	8.2	925	29	29
23	R. South Tyne, Crow Hall	NY795646	0055-80	26/6/84	0.550	8.3	291	38	21
24	R. South Tyne, Hayden Bridge	NY844644	0055-85	26/6/84	0.600	8.3	327	62	44
25	R. West Allen	NY802453	0085-10	26/6/84	0.350	8.2	182	5	3
26				3/6/85	<0.070	8.2	138	12	9
27	Rampgill Level	NY782435	0096-01	26/6/84	0.550	7.5	50	13	7
28				3/6/85	0.877	7.7	100	31	18
29				5/6/85	0.671	8.1	125	36	16
30				20/6/85	0.899	7.9	163	31	31
31	Hollingside Stream, below culvert	NZ272418	0376-65	3/6/85	0.601	6.8	36250	10600	989
32	Station Quarry Outflow	NY948255	0377-01	20/6/85	0.178	6.8	1312	63	54

Table 4.3 Descriptive statistics of environmental and morphological variables: n = 32.

variable	unit	min	max	$\bar{x}$
current speed	$m\ s^{-1}$	0	0.899	0.387
temperature	°C	8.7	19.0	13.7
conductivity	$\mu S\ cm^{-1}$	18.1	2550	536
pH		5.26	8.47	7.70
NO_3-N	$\mu g\ l^{-1}$	50	39900	4220
FRP	$\mu g\ l^{-1}$	3	9890	711
TFP	$\mu g\ l^{-1}$	5	10600	546

Table 4.4 Product moment correlation matrix of transformed environmental variables: n = 32.
(* = p0.05; ** = p <0.01; *** = p <0.001).

variable	temperature	current speed	conductivity	pH	NO_3-N	FRP	TFP
temperature	1.000	-0.943	0.350*	0.165	-0.094	0.354*	0.379*
current speed		1.000	0.408*	0.014	0.180	0.412*	0.448*
conductivity			1.000	0.165	0.542**	0.542**	0.588***
pH				1.000	-0.063	-0.127	-0.161
NO_3-N					1.000	0.868***	0.883***
FRP						1.000	0.991***
TFP							1.000

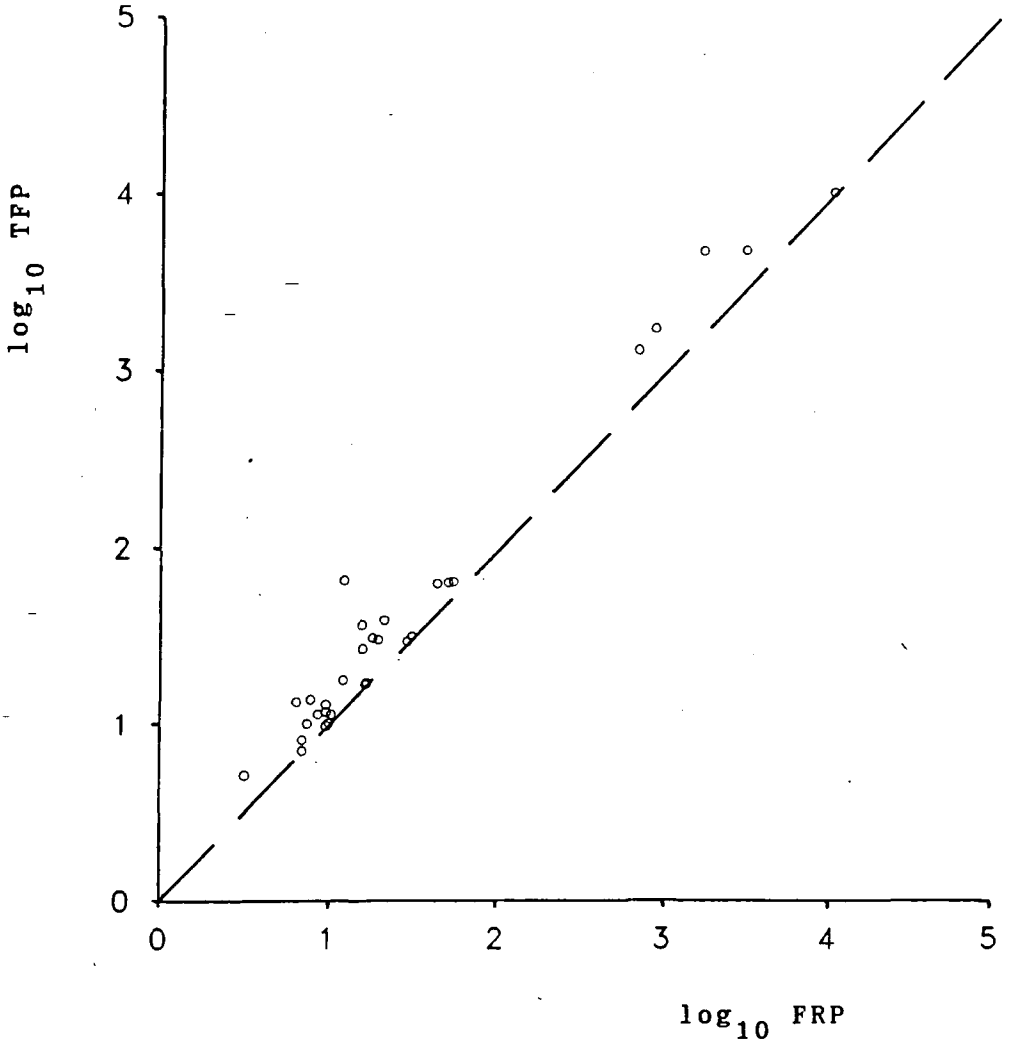


Fig. 4.1 Scattergram showing relationship between FRP and TFP from sites of field studies (n=32; see Table 4.2). Line indicates FRP=TFP, values above the line indicate available organic P.

over 20% greater for 21 samples. There was was also a highly significant correlation between nitrate and the two phosphorus fractions.

The biological data for each sample are listed in Table 4.5 with descriptive statistics given in Table 4.6. Algal N and P composition was determined for only 30 of the 32 samples: *Chaetophora* was not analyzed because of difficulties due to calcite and insufficient *Stigeoclonium* was present at 0142-50 for analysis. The large difference between the two indices of hairiness at 0096-01 occur because the algae at this site had very long branches with only one or two hair cells at their ends. A wide range of algal N and P compositions is show by the samples with the maximum exceeding the minimum by factors of 5.5 and 19.3, respectively.

Table 4.7 gives the ranges of physical, chemical and physiological variables for hairy and non-hairy algae. There was no overlap in algal P composition between the hairy and non-hairy populations: all the hairy populations had 0.605% P or less while all the non-hairy populations had 0.961% P or more. The range of algal N compositions was similar for the hairy and non-hairy populations, although the upper limit was slightly greater in the former. The algal N : P ratio ranged from 3.65 to 35.6 by weight, as with algal P the hairy and non-hairy populations were separate: all the hairy populations had ratios of 6.46 and above and all non-hairy populations had ratios of 5.58 and below.

4.22 Correlation of algal environmental variables

The relationship between the indices of hair formation and the physical and chemical factors are presented in Table 4.8. There were significant negative correlations for % hairiness with current speed, conductivity and both phosphorus fractions; for % hair terminal cells the pattern was

Table 4.5 Samples studied (see Table 4.2) with list of taxa and details of morphology, phosphatase activity (μmol *p*-nitrophenol μg chl a^{-1} h^{-1}), algal N, P and N : P.

sample number	taxon	% hairiness	% hair terminal cells	phosphatase	algal N (%dry wt)	algal P (% dry wt)	algal N : P
1	*Chaetophora*	35	24	0.038	-	-	-
2	*Stigeoclonium*	0	0	0	5.11	0.961	5.23
3	*Stigeoclonium*	13	55	0.0001	5.42	0.340	15.96
4	*Stigeoclonium*	28	55	0.0021	5.21	0.303	17.20
5	*Stigeoclonium*	28	48	0.006	8.41	0.271	31.04
6	*Stigeoclonium*	0	0	0	5.46	1.08	5.04
7	*Stigeoclonium*	0	0	0	6.83	1.87	3.65
8	*Stigeoclonium*	0	0	0	5.47	1.21	4.52
9	*Stigeoclonium*	48	94	0.047	2.46	0.125	19.72
10	*Stigeoclonium*	50	100	0.016	3.36	0.116	29.0
11	*Stigeoclonium*	50	100	0.0078	3.84	0.108	35.6
12	*Stigeoclonium*	45	90	0.028	5.10	0.188	27.15
13	*Stigeoclonium*	47	97	0.065	1.52	0.097	15.73
14	*Stigeoclonium*	7	3	0.005	3.32	0.189	17.57
15	*Stigeoclonium*	8	3	0.012	8.40	0.352	23.86
16	*Stigeoclonium*	23	40	0.0035	5.63	0.538	10.48
17	*Stigeoclonium*	20	30	0.001	5.14	0.486	10.57
18	*Stigeoclonium*	25	33	0.0043	5.23	0.738	7.08
19	*Stigeoclonium*	12	15	0.012	3.91	0.605	6.46
20	*Stigeoclonium*	15	51	0.0053	5.99	0.344	17.41
21	*Stigeoclonium*	20	30	0.0048	2.95	0.277	10.66
22	*Stigeoclonium*	24	33	0.0015	5.23	0.401	13.04
23	*Stigeoclonium*	0	0	0.001	5.74	1.02	5.58
24	*Stigeoclonium*	0	0	0	5.20	1.01	5.12
25	*Stigeoclonium*	34	96	0.037	6.00	0.290	20.69
26	*Stigeoclonium*	21	39	0.0053	3.47	0.250	13.89
27	*Stigeoclonium*	1	27	0.025	2.76	0.166	16.60
28	*Stigeoclonium*	+	98	0.004	5.03	0.456	11.04
29	*Stigeoclonium*	2	36	0.005	4.47	0.376	11.89
30	*Stigeoclonium*	+	10	0.0047	4.90	0.300	16.36
31	*Stigeoclonium*	0	0	0	-	-	-
32	*Draparnaldia*	19	25	0.027	4.23	0.256	16.54

Table 4.6 Descriptive statistics of environmental and morphological variables.

variable	unit	n	min	max	$\bar{x}$
% hairiness		32	0	50	18.1
% terminal hairs		32	0	100	39.3
algal N	% dry weight	30	1.52	8.41	4.85
algal P	% dry weight	30	0.097	1.87	0.491
N : P	(by weight)	30	3.65	35.6	14.81
APA	µmol *p*-nitrophenol µg chl a^{-1} l^{-1}	32	<0.0001	0.0650	0.0116

Table 4.7 Ranges of selected environmental variables for hairy (n=25) and non-hairy (n=7) field populations of Chaetophorales.

variable	hairy			non-hairy		
	min		max	min		max
NO_3-N (µg l^{-1})	50	-	925	291	-	39900
FRP (µg l^{-1})	3	-	54	21	-	9890
TFP (µg l^{-1})	5	-	63	38	-	10600
current	>0.005	-	0.99	0.46	-	0.693
algal P (% dry wt.)	0.097	-	0.605	0.961	-	1.87
algal N (% dry wt.)	1.52	-	8.40	5.11	-	6.83
N : P (by weight)	6.46	-	35.6	3.65	-	5.58

Table 4.8 Spearman's rank correlation of % hairiness and % terminal hairs with environmental variables: n = 32. (* = p <0.05; ** = p <0.01; *** = p <0.001)

	% hairiness	% terminal hairs
current speed	-0.6531***	-0.2578
temperature	-0.2849	-0.5362**
conductivity	-0.5866**	-0.5940**
pH	-0.0789	-0.1476
NO_3-N	-0.1127	-0.2573
FRP	-0.5774**	-0.6031***
TFP	-0.6435***	-0.7118***

Table 4.9 Correlations between algal N, algal P and the algal N : P ratio with environmental variables: n = 30. (* = p <0.05; ** = p <0.01; *** = p <0.001)

	algal N	algal P	N : P
temperature	0.291	0.560**	-0.367*
current speed	0.135	0.405*	-0.329
conductivity	-0.181	0.505**	-0.596***
pH	-0.240	0.185	-0.360
NO_3-N	0.288	0.723***	-0.414*
FRP	0.235	0.787***	-0.526***
TFP	0.226	0.825***	-0.586***

similar but the current was not significantly correlated while the temperature was.

Algal P and the N : P ratio were also correlated with a number of environmental variables (Table 4.9) but algal N was not. The significant correlations between algal P and the two phosphorus fractions and nitrate were expected, but the lack of correlation between algal N and nitrate in the water was surprising. The relationship between the two indices of hairiness and the algal nutrient composition are listed in Table 4.10; both indices were highly negatively correlated with algal P and highly positively correlated with the N : P ratio.

4.23 Phosphatase activity

Phosphatase activity was detectable in 26 of the 32 samples when assayed at pH 10.3. There was only one non-hairy sample (0367-65) which which had detectable activity, and this was at a low level (0.001 µmol *p*-nitrophenol µg chl a^{-1} h^{-1}). No examples of hairy algae without activity were found. One hairy population did have very low activity (0013-12, 3.6.85, 0.001 µmol *p*-nitrophenol µg chl a^{-1} h^{-1}) but other samples from this site had activity at least an order of magnitude higher even though the chemistry and morphology was similar on all occasions. Phosphatase activity had significant negative correlations with a number of environmental variables, including nitrate and the two phosphate fractions (Table 4.11). Phosphatase activity was significantly negatively correlated with both algal N and algal P composition while the N : P ratio and the two indices of hairiness were significantly positively correlated (Table 4.12). When one sample from each site was used in these analyses the pattern of correlations was similar, but the significance levels were reduced. If

Table 4.10 Correlation of algal N, algal P and the algal N : P ratio with the indices of hairiness: n = 30. (* = p <0.05; ** = p <0.01; *** = p <0.001)

	% hairiness	% terminal hairs
algal N	-0.287	-0.293
algal P	-0.677***	-0.639***
N : P	0.649***	0.626***

Table 4.11 Correlation between phosphatase activity and data for selected environmental variables, algal N and P compositions and N : P ratio (by weight). Relationship is shown using the full set of samples and also a reduced set based on one sample per site (chosen at random where more than one sample per site). (* = p <0.05; ** = p <0.01; *** = p <0.001)

	phosphatase activity	
	n = 30	n = 13
temperature	-0.286	-0.641
current speed	-0.435*	-0.601
conductivity	-0.267	-0.207
pH	0.131	-0.096
NO_3-N	-0.494**	-0.476
FRP	-0.612***	-0.711*
TFP	-0.622***	-0.729*
algal N	-0.440*	-0.614*
algal P	-0.613***	-0.778*
N : P	0.485**	0.514

Table 4.12 Spearman's rank correlation of phosphatase activity with % hairiness and % terminal hairs. Relationship is shown using the complete set of samples and also a reduced set based on one sample per site (chosen at random where more than one sample per site). (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$)

	phosphatase activity	
	n = 32	n = 13
% hairiness	0.710^{***}	0.942^{***}
% terminal hairs	0.567^{***}	0.865^{***}

only the hairy algae were taken into consideration then the significance of the relationship between phosphatase activity and hairiness drops to the 95% level. The spread of points in the scatter diagram of % hairiness against phosphatase activity (Fig. 4.2) does not show a clear relationship, but this may be due to the assay technique (see below).

4.24 Phosphatase and pH

The pH used for the assay reaction was higher than any found at the sampling sites but studies of the pH response of cultured material had indicated that the assay was most sensitive in this range. Also two populations were sampled before the main study to test the suitability of the assay pH: *Chaetophora incrassata* was collected from Croft Kettle on 27/3/85 and *Draparnaldia glomerata* was collected from 0377-01 on 7/4/85. Both of these showed maximum activity in the pH 10-11 region (Fig. 4.3). Samples of *Stigeoclonium* collected from 0013-12, 0048-01, 0096-01 and 0376-65 on 3/6/85 were also assayed over a range of pH values and these gave

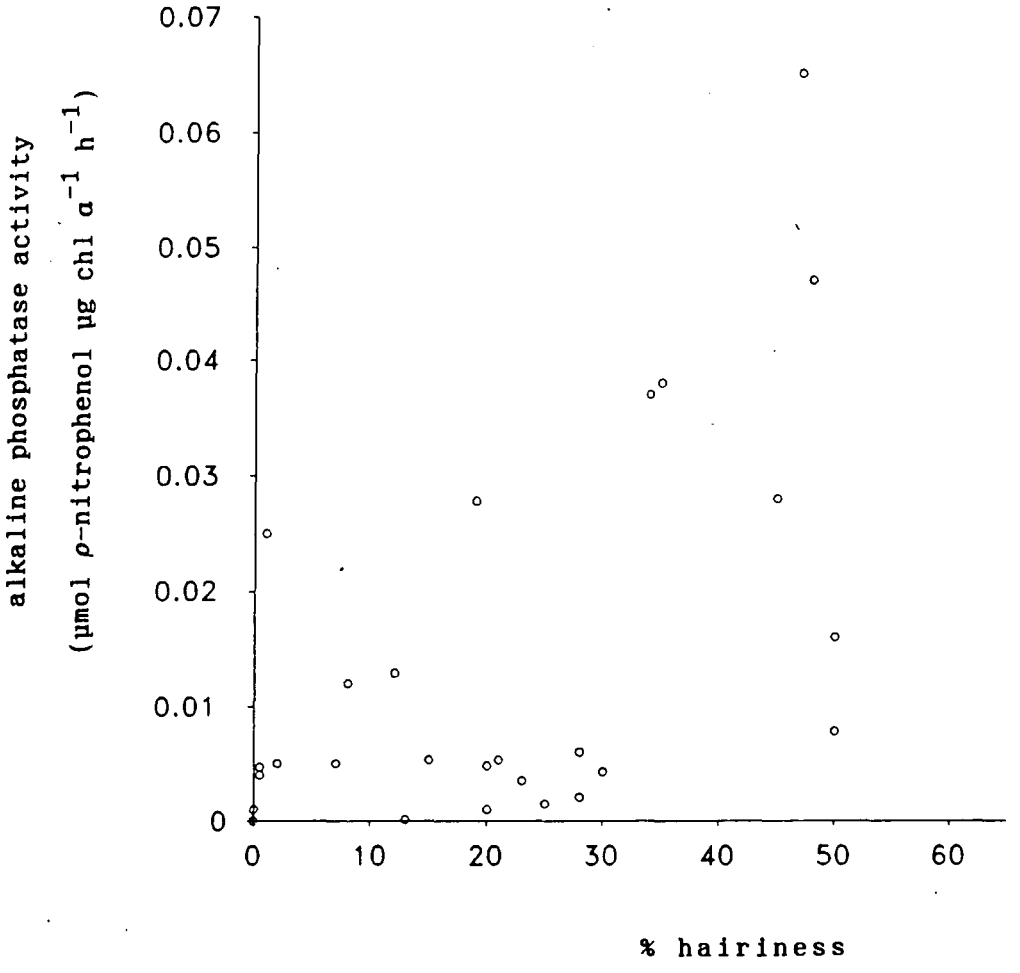
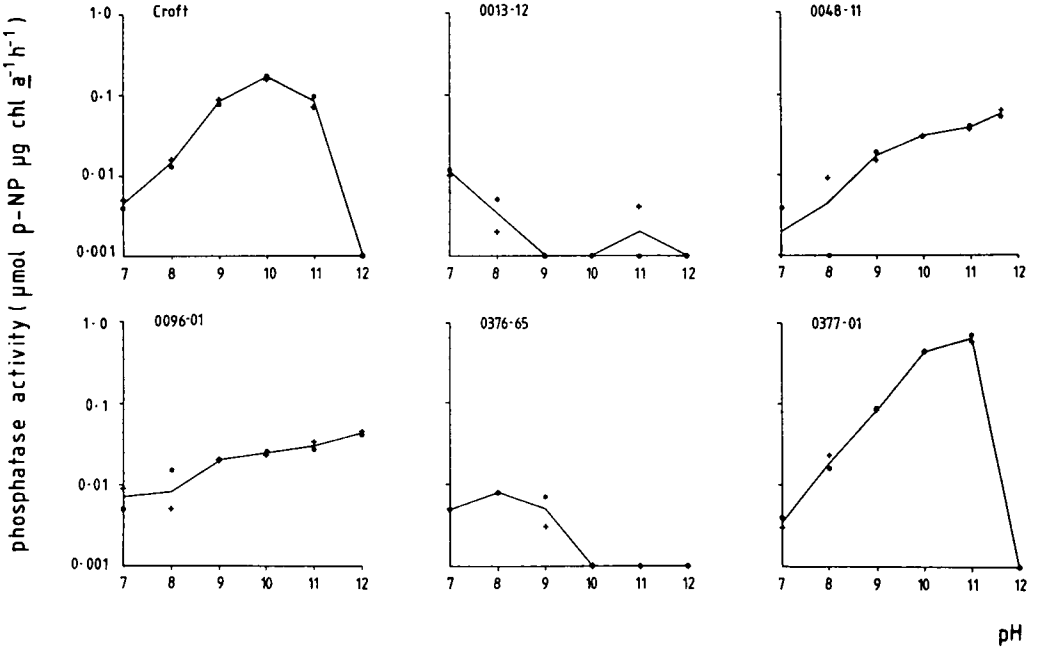


Fig. 4.2 Scattergram showing relationship between alkaline phosphatase activity and % hairiness for the 32 sites listed in Table 4.2 (n=32) for organisms listed in Table 4.5.

Fig. 4.3 Influence of pH on phosphatase activity of six natural populations: + = HEPES, borax or KCl; • DMG or glycine.


phosphatase activity (µmol p-NP µg chl a^{-1}h^{-1})
Croft
0013-12
0048-11
0096-01
0376-65
0377-01
1·0
0·1
0·01
0·001
7
8
9
10
11
12
pH

very different responses. All four showed a peak activity less than those of the *Chaetophora* and *Draparnaldia* and the peaks were not in the same pH range. Algae from 0048-11 and 0096-01 both showed an increase in activity with increasing pH over the range studied. The pH of the water of 0013-12 was typically near 5.5 and the population from this reach had low phosphatase with the highest activity at pH 7. Material from the other reach (0376-65) never had hairs in nature but did have detectable phosphatase activity. Studies of phosphatase localization on some of these algae are presented in Chapter 7.

A population of *Draparnaldia* collected from a stream in Scotland (tirbutary of the Endrick Water, pH 7.0, NS 691868, 19/4/85) was assayed over the same pH range as the organisms above, but phosphatase activity was not detectable.

4.25 Correlations of trait frequency indices

The trait frequency indices for hairiness and % hair terminal cells and total branching were tested for correlations with the environmental variables and the biological variables, as above. The results listed in Table 4.13 show that these correlated with the environmental variables in a pattern similar to the counts of % hairiness and % hair terminal cells; this is also true for the correlations with the biological variables.

4.26 Alkalinity and hot-water-extractable P

The twenty-two samples for which algae were collected in 1985 were also sampled for alkalinity and the hot-water-extractable phosphorus content of the algae. The data from these studies is given in Appendix 2. There was no correlation between any of the calculated carbonate species and any of the morphological or physiological data. The hot water extractable

Table 4.13 Spearman's rank correlation of environmental and physiological variables with trait frequency indices of hairiness and branching. (* = p <0.05; ** = p <0.01; *** = p <0.001).

	trait frequency indices	
	hairiness	branching
temperature	-0.274	-0.024
current speed	-0.626***	-0.008
conductivity	-0.560**	0.164
pH	-0.058	-0.111
NO_3-N	-0.254	0.347
FRP	-0.507**	0.301
TFP	-0.617***	0.468**
algal N	-0.336	0.231
algal P	-0.7440***	-0.386*
N : P	0.7275***	0.316
APA	0.746***	-0.359

fraction of the algal phosphorus, and the ratio of hot water extractable P : total algal P, did not correlate with any environmental, morphological or physiological data.

5. DESCRIPTION OF ORGANISMS

5.1 Introduction

The genus *Stigeoclonium* is found in a wide range of habitats and each species is subject to morphological variation (Section 1.41), so it was not possible to use one strain as a typical representative of the genus. Strains from both high and low nutrient *Stigeoclonium* populations were isolated for culture studies in an attempt to represent the extremes of morphology; strains which had been kept in the Durham Culture Collection for many years were also used. Two strains of *Draparnaldia* and one of *Chaetophora* were also isolated and studied. Details of the strains are listed in Table 5.1.

The taxonomic methods developed by Cox & Bold (1966) for the identification of *Stigeoclonium* have been used by other authors (see for example Franke & ten Cate, 1980; de Vries *et al.*, 1983). Unfortunately, the controlled medium used in this method has a high phosphorus concentration and a low N : P ratio (see Appendix 1); such conditions were not found at any of the sites from which strains for this study were isolated. Preliminary experiments suggested that phosphorus deficiency was important in hair formation in field material so this medium was unsuitable for experimental work. Because of the unsuitability of the medium of Cox & Bold their taxonomic criteria were not used in this study, all specific names given were determined using Printz (1964), however, a comparison between the morphology in Chu 10-F and $BBMPTB_{12}$ is given for some strains.

All of the strains listed in Table 5.1 were used for studies on deficiencies (Section 6.2) but the axenic strains described below were

Table 5.1 List of strains used in experiments, together with details of sites and references to earlier literature on sites and strains. B, Belgium; E, England; G, Germany; Reference I. Harding & Whitton (1976); II Harding and Whitton (1977); III, Whitton & Harding (1978).

taxon	Durham culture	whether axenic	country	site	stream-reach	environment	ref.
Chaetophora incrassata (Huds.) Hazen	652	+	E	Croft Kettle	-	Mangnesian Limestone pond: P mostly organic	
Draparnaldia sp.	651	+	E	Barras End Beck	0212-49	upland stream	
D. plumosa Agardh	653	+	E	Bollihope Burn, below old adit	0015-18	upland stream	
Stigeoclonium tenue Kütz.	565	-	E	Rampgill Level	0096-01	Zn-rich mine drainage	
"	566	-	B	La Gueule	1002-25	calcareous polluted (organic, Zn) river	III
"	567	-	G	Rammelsberg Stollen	4014-01	Zn-rich mine drainage	III
"	575	-	E	R. Wear, Frosterley	0008-30	fast flowing	
"	576	-	E	R. Browney, Stone Bridge	0014-70	moderately calcareous, organic polluted	
"	577	+	E	Hollingside Artificial Stream	0001-01	unpolluted	I II III
"	659	+	G	R. Sülz, nr Lehmbach	4001-60	highly polluted	I
"	699	+	E	R. Nent, above Levels	0048-11	Zn-rich upland river	I
"	702	+	E	Rampgill Level	0096-01	Zn-rich mine drainage	I II III
"	779	+	E	Hollingside Stream, below culvert	0142-50	organic polluted	

used in other experiments. Culture records from the Durham Culture Collection are presented in Appendix 5.

5.12 Notes on terminology

The descriptive terminology is similar to that of Cox & Bold (1966) with 'young' meaning up to about three weeks and 'old' meaning approaching two months growth in $BBMPTB_{12}$; in Chu 10-F the corresponding stages are reached after about two weeks and one month, respectively.

5.2 D577 - *Stigeoclonium tenue* Kütz.

5.21 In nature

This strain was collected from Hollingside Artificial Stream, Durham City, Co. Durham, England. No description of the organism in the field was available but field material from this site was used by Harding & Whitton (1976, 1977) for their studies on zinc toxicity and by Whitton & Harding (1978) in their studies of the effects of nutrient deficiency on hair formation.

5.22 In Chu 10-F

Numerous zoospores formed 2-3 days after inoculation. One or two quadriflagellate zoospores were produced in each cell and released through a pore in the wall; this usually occurred in cells near the end of the filament. Germinating zoospores grew out bilaterally to form a filament which branched several times. A prostrate filament grew from one side of the zoospore before the other, eventually giving bipolar development. The erect filament always developed from the zoospore first, and then often from other basal cells.

The mature basal system was an extensive, irregularly branched prostrate filament; the branches often re-branched. Basal system cells were usually globular near the centre (11 µm radius), becoming more slender towards the ends. The basal system of floating colonies was more compact.

The erect system was always present (Fig 5.1a) and much more extensive than the basal system; the two systems were easy to distinguish. Branching, usually alternate or dichotomous, was rare in young filaments but increased with age. Cells cylindrical, but sometimes barrel shaped near the base in older cultures. Cell length decreased from 24 µm near the base to 13 µm at the tips, width increased from 11 µm to 6 µm. Spherical, thick walled cells were formed near the base of the erect filament in old cultures. Much cell division occurred near the proximal end of the filament, and this was sometimes well defined enough to be termed meristem. Multicellular hairs were formed in old cultures; lengths 63-125 µm and width 3-4 µm.

5.23 In $BBMPTB_{12}$

Zoospore formation and germination, and the morphology of the basal and erect systems was similar to that found in $BBMPTB_{12}$. Multicellular hairs were not observed in this medium.

5.3 D659 - *Stigeoclonium tenue* Kütz.

5.31 In nature

Collected from the River Sulz, a tributary of the River Sieg, Nordheim-Westfalen, West Germany. This strain was included in the study by Harding & Whitton (1976) of zinc resistance in *Stigeoclonium tenue*.

5.32 In Chu 10-F

Numerous zoospores (8 x 5 µm) formed 2-3 days after inoculation. One or two quadriflagellate zoospores were produced in each cell and released through a pore in the wall. Zoospore germination was bipolar, about three cells formed a straight line then one branched at right angles; at least two branches of the basal system were established before a single erect filament formed.

The mature basal system formed by repeated branching of the prostrate cells. The mature erect system consisted of many filaments which had alternate and opposite branches (Fig. 5.1b). Cell decreased in size from 80 x 7 µm at the base to 22 x 5 µm near the end of the filament. Hairs were common in old cultures, size about 65 x 5 µm.

5.33 In $BBMPTB_{12}$

This strain had a very different morphology in $BBMPT_{12}$ than in Chu 10-F; being nearest to the description of *S. farctum* given by Cox & Bold (1966). The basal system consisted of a much branched prostrate filament which eventually gave a pseudoparenchymatous disc. The mature erect system was less extensive than in Chu 10-F and consisted of a filament of about 100 cells, occasionally with a branch of about ten cells.

5.4 D699 *Stigeoclonium tenue* Kütz.

5.41 In nature

This strain was collected from the River Nent about 20 m upstream from the influx of Rampgill Level. Material from this site was studied by Whitton & Harding (1976). The natural material had many branches, both alternate and opposite, and had very long hairs. The method of attachment was difficult to discern but rhizoids were not obvious and a densely

packed, highly branched group of filaments occurred near the base of the colony (Fig. 7.18a shows material from this site).

5.42 In Chu 10-F

Zoospores were released from the erect filament through a pore in the wall. The mature basal system consisted of densely packed, highly branched vegetative cells and colourless rhizoids, usually of about 2 cells: 30 - 45 µm long, 8-9 µm wide. Young cells of the erect filament were cylindrical, 15 - 60 µm long by 8 - 12 µm wide, with the chloroplast covering about 50% of the cell surface (Fig. 5.1c). Branching was rare in young cultures but usually alternately in old cultures. Hairs were present, even in young cultures, and in old cultures these consisted of up to 8 cells ranging in length from about 45 µm to 100 µm but always about 6 µm in diameter.

5.43 In $BBMPTB_{12}$

No zoospores were ever observed in this medium. The basal system was similar to that of material grown in Chu 10-F and similar to that described for *S. helveticum* by Cox & Bold (1966). Young cells of the erect filament were cylindrical, but older cells were often more rounded with constrictions at the partition wall. Cells ranged from 45 x 12 µm near the rhizoids to 18 x 8 µm near the tips. In young cultures occasional alternate branches occurred, but in older cultures branches were more common.. Hairs were never observed in this medium.

5.5 D779 *Stigeoclonium tenue* Kütz.

5.51 In nature

This strain was isolated from Hollingside Beck, Co. Durham just below its emergence from an adit into which treated sewage effluent was released.

The colonies had a highly branched erect system in which the branches often re-branched to give a feathery appearance. The cells of the erect system were slightly bulbous with constrictions at the partition walls in all but the cells nearest the tips. The chloroplast covered the extent of each cell and hairs were never present. The basal system could not be recovered in natural material.

5.52 In Chu 10-F

One zoospore was released from each cell of the erect filament and germinated to give bilateral prostrate branches, almost simultaneously an erect filament was produced from the original zoospore. The mature basal system consisted of rounded cells in a compact disk, with the original prostrate branches still obvious, as they were barrel shaped.

The erect system was less branched than in nature and the chloroplast rarely covered more than about 60 % of the cell surface (Fig 5.1d), cells decreased from about 150 x 16 µm near the base to 20 x 15 µm at the ends of the branches. Branching was frequent, usually alternate. Short hairs, of 1-5 cells occurred in old cultures: hair cell sizes from 75 to 160 µm long by 4 µm.

5.53 In $BBMPTB_{12}$

Zoospores were released as above and the basal system was similar but the erect system was more similar to that of the material in nature and chloroplast usually covered all of the surface of the cell. This resembled the *S. variabile* of Cox & Bold (1966). The cells tended to be barrel shaped, about 24 x 17 µm. Short hairs, 1-3 cells occurred in old cultures. Many akinetes were formed in old cultures.

5.6 D652 - *Chaetophora incrassata* (Huds.) Hazen

5.61 In nature

Isolated from Croft Kettle, near Darlington, Co. Durham on 11/10/82. This population had an obvious mucilage in nature and crystals of calcite were embedded in the colony, long hairs extended out of the mucilage (Fig. 5.2a, 5.2b).

5.62 In Chu 10-F

This strain had erratic yield in culture and very variable morphology. Mucilage production was also variable, but discrete mucilaginous colonies did form occasionally. The cells of the branches always had massive chloroplasts and the pattern of branching often closely resembled that of natural material (Fig. 5.2c).

5.7 D651 - *Draparnaldia* sp.

5.71 In nature

Isolated from the outflow from Race Fell Burn (reach 0212-49) in June 1981. No description of the natural appearance of this material was available.

5.72 In Chu 10-F

Macroscopically the typical symmetry of members of this genus were not present in culture, many loosely attached colonies occurred in the culture flask. The main axis had very occasional large cells (120 x 55 µm) but primarily consisted of cells with banded chloroplasts which gradually decreased in size along the length of the axis (Fig. 5.1e), size ranged from 75 x 27 µm down to 33 x 15 µm. Although no whorls of branches were present some branches off the main axis did rebranch many times. The

cells of the branches, from 18 x 8 µm to 26 x 9 µm, had massive chloroplasts and, when zoospores were released, they were formed from the cells of these branches. Hairs were present at the end of many filaments in older cultures, length in range 57 - 140 µm, width in range 3 - 7 µm. Mucilage was only evident in old cultures and when india ink was used to define its boundaries it appeared to be associated only with main axis cells.

5.8 D653 - *Draparnaldia plumosa* Ag.

5.81 In nature

Isolated from a deep pool in Bollyhope Burn (reach 0015-18) on 7/11/85. The main axis cells were about twice their diameter in length and were slightly constricted at the cross-walls (about 50 x 100 µm). Cells in whorls branched alternate or opposite, with the rachis obvious and extending through and beyond the other branches to give a tapering, plumed effect. Many long hairs extended from the mucilage.

5.82 In Chu 10-F

Macroscopically, this strain formed a large colony loosely attached to the glass, apparently only at one point. The symmetry of the colony when in nature was never seen in culture, but all types of cells did occur (Fig. 5.1f). Mucilage was only obvious in old cultures and appeared to be associated with the cells of the main axis. The size of main axis cells, 110 x 55 µm, decreased rapidly to give cells intermediate in size to those of the main axis and those of the whorls of branches. Branches from the intermediate sized cells consisted of cells similar to those of the whorls of natural material, about 25 x 16 µm, the latter had massive chloroplasts and, when zoospores were formed, (length 12 - 15 µm width 8 - 9 µm) they

appeared to originate from these cells. Hairs often occurred at the end of the branches.

Fig. 5.1 Erect systems of Chaetophorales grown in Chu 10-F for 10 d:

a) D577 - *Stigeoclonium tenue* (see Section 5.22);

b) D659 - *Stigeoclonium tenue* (see Section 5.32);

c) D699 - *Stigeoclonium tenue* (see Section 5.42);

d) D779 - *Stigeoclonium tenue* (see Section 5.52);

e) D651 - *Draparnaldia* sp. (see Section 5.72), ;

f) D653 - *Draparnaldia plumosa* (see Section 5.82).

(Scale bar = 100 µm)

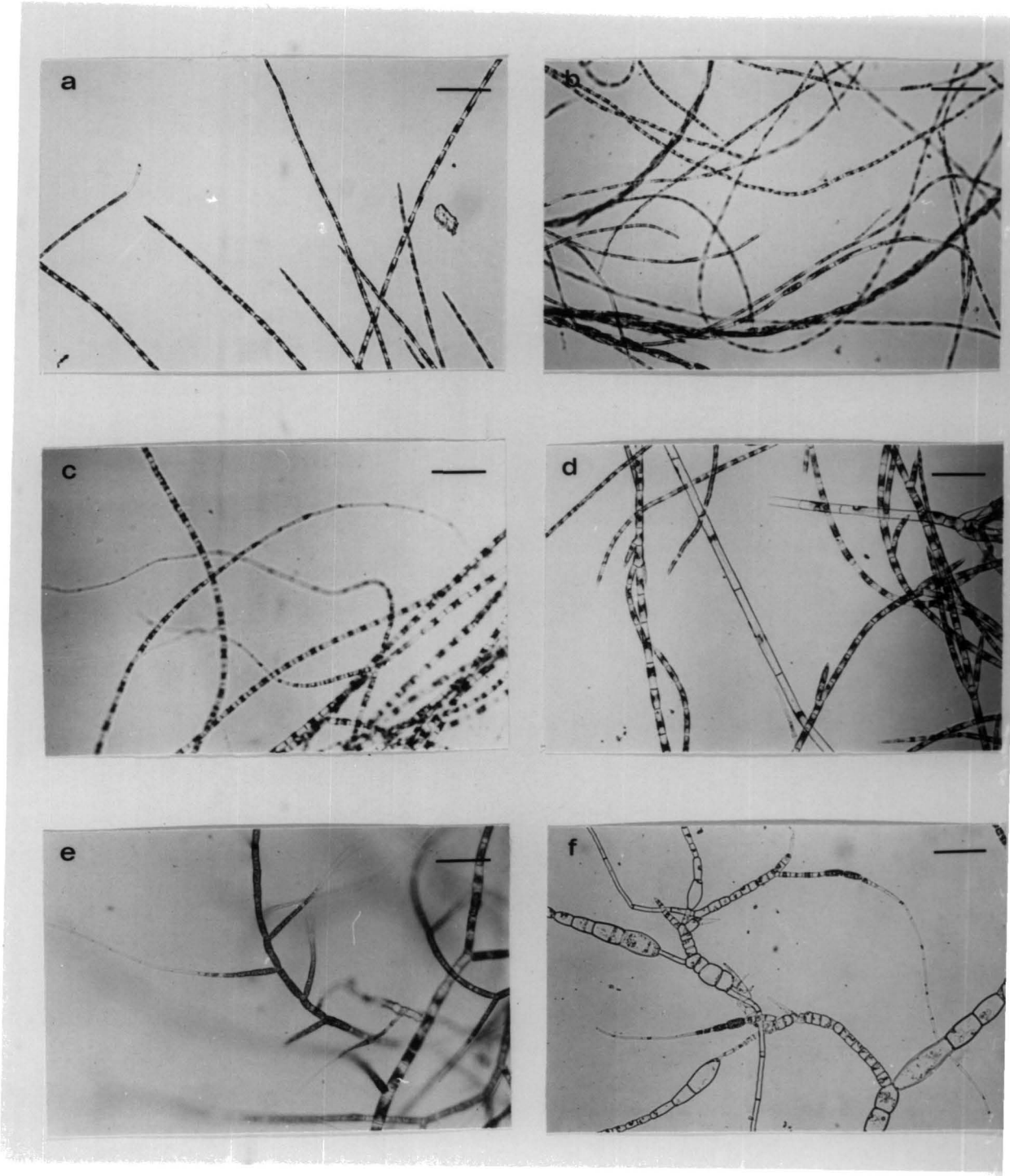
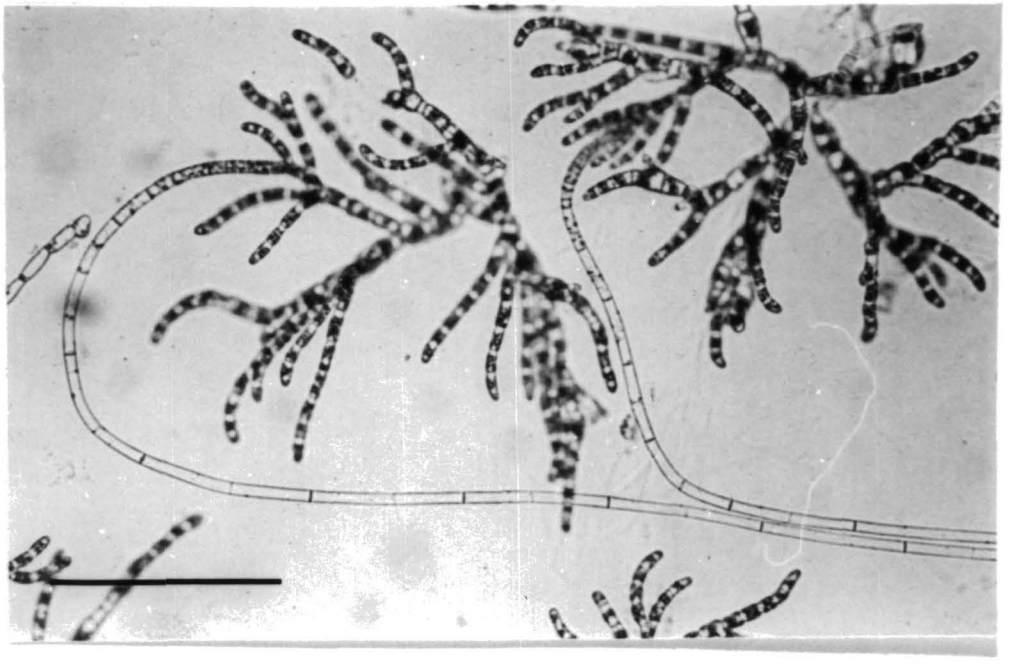

a
b
c
d
e
f

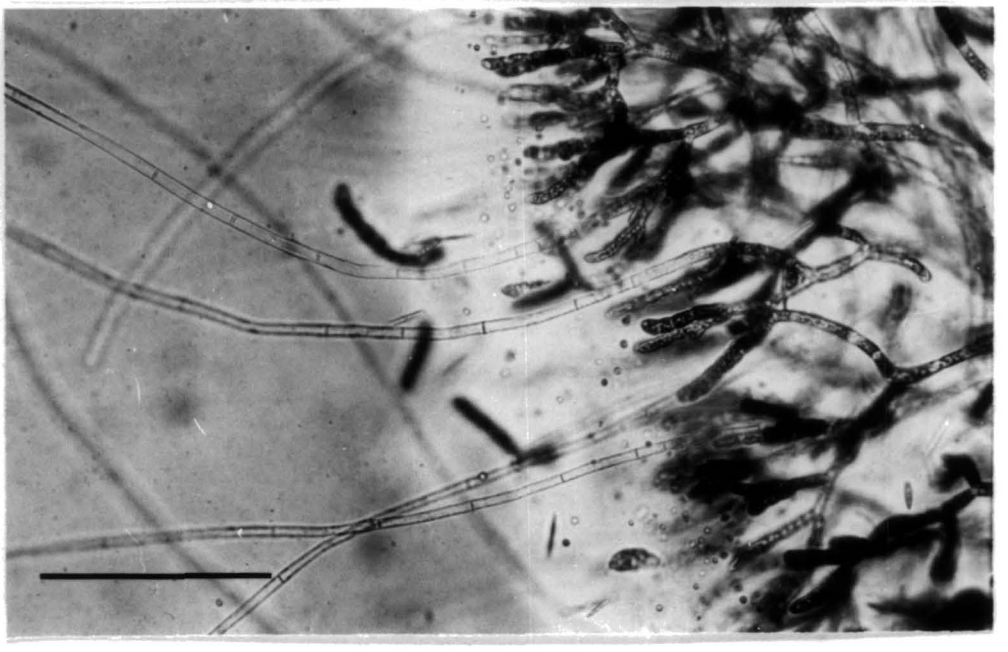
Fig. 5.2 *Chaetophora incrassata*

a) colony of field material (Section 5.61 scale bar = 200 µm),

b) close-up of edge of colony mounted in India ink showing hairs extending from mucilage (scale bar = 200 µm),

c) material grown in Chu 10-F (Section 5.62) for 10 d (scale bar = 200 µm).





6. CULTURE STUDIES

6.1 Introduction

The results of field studies indicated that phosphorus availability was an important factor in hair formation. Studies of cultured material were designed to re-confirm the findings of Whitton & Harding (1978) that certain deficiencies lead to hair formation and to study various aspects of phosphorus in the growth of cultured material.

6.2 Deficiencies in batch cultures

Previous studies have shown that hair formation could be induced in the Chaetophorales by omitting selected elements from the growth medium (Whitton & Harding, 1978). Many of the strains used by the previous workers were available in the Durham Culture Collection.

6.21 Preliminary deficiency studies

Batch cultures of thirteen culture collection strains were prepared with selected elements absent from the Chu 10 medium of Harding & Whitton (1976); this was the media used by Whitton & Harding (1978). The ionic background of each treatment was maintained by the addition of a substitute salt (Section 2.444). Four replicates of each treatment and the control were incubated in boiling tubes under standard experimental conditions (Section 2.462). On day 10 the growth of each strain was ranked visually and duplicate samples were examined microscopically; the samples were scored for hairs according to Section 2.5112. The missing element was added to the remaining replicates and the final yield of each treatment was compared by observation 7 d later.

A summary of the hair production is presented in Fig. 6.1. Many of the deficiencies caused marked morphological changes in the strains tested. Hairs were produced by many deficiencies and the amount of branching was often affected. Only four strains produced hairs when Ca was missing (D567, D652, D699, D702) and all these also produced - Mg hairs; the other strains which produced - Mg hairs were D575, D567, D577, D653 and D659. The control and - P had the fewest branches for all the strains and the basal system was more prominent under - Ca, - Mg and - S. The yield under each deficiency was similar for all the strains:

control > - P > - Fe > - Ca = - S > - Mg > - N.

The - P treatments of all the strains had alkaline phosphatase activity on day 14: the APA assays were carried out using both media and distilled water as the suspension liquid so that any inhibition due to P in the medium could be detected, the possible lack of ions essential for the alkaline phosphatase was discounted as all the assays in which APA was detectable were positive for both assay procedures. All the strains recovered from every treatment to give a yield similar to the control 10 d after the addition of the limiting nutrient. Evidence of massive zoospores settlement could be seen in all the - P cultures within a few days of the additions.

6.22 Deficiencies in axenic strains

The five main study strains where tested after growth in Chu 10-F, which had a higher N : P ratio than the media used in the preliminary studies. Photographs of one strain (D577; Fig. 6.2) are included to illustrate some morphological differences between treatments: - P hairs had up to 14 cells of up to 120 µm in length while - N hairs were only up to 7 cells long but the cells were up to 180 µm in length. Some of the morphological responses

Fig. 6.1 Influence of elemental deficiencies on hair presence and % hairiness in 14-day tests on 13 strains of Chaetophorales. Vertical axis indicates number of strains which formed hairs; horizontal axis indicates average % hairiness for the 13 strains.

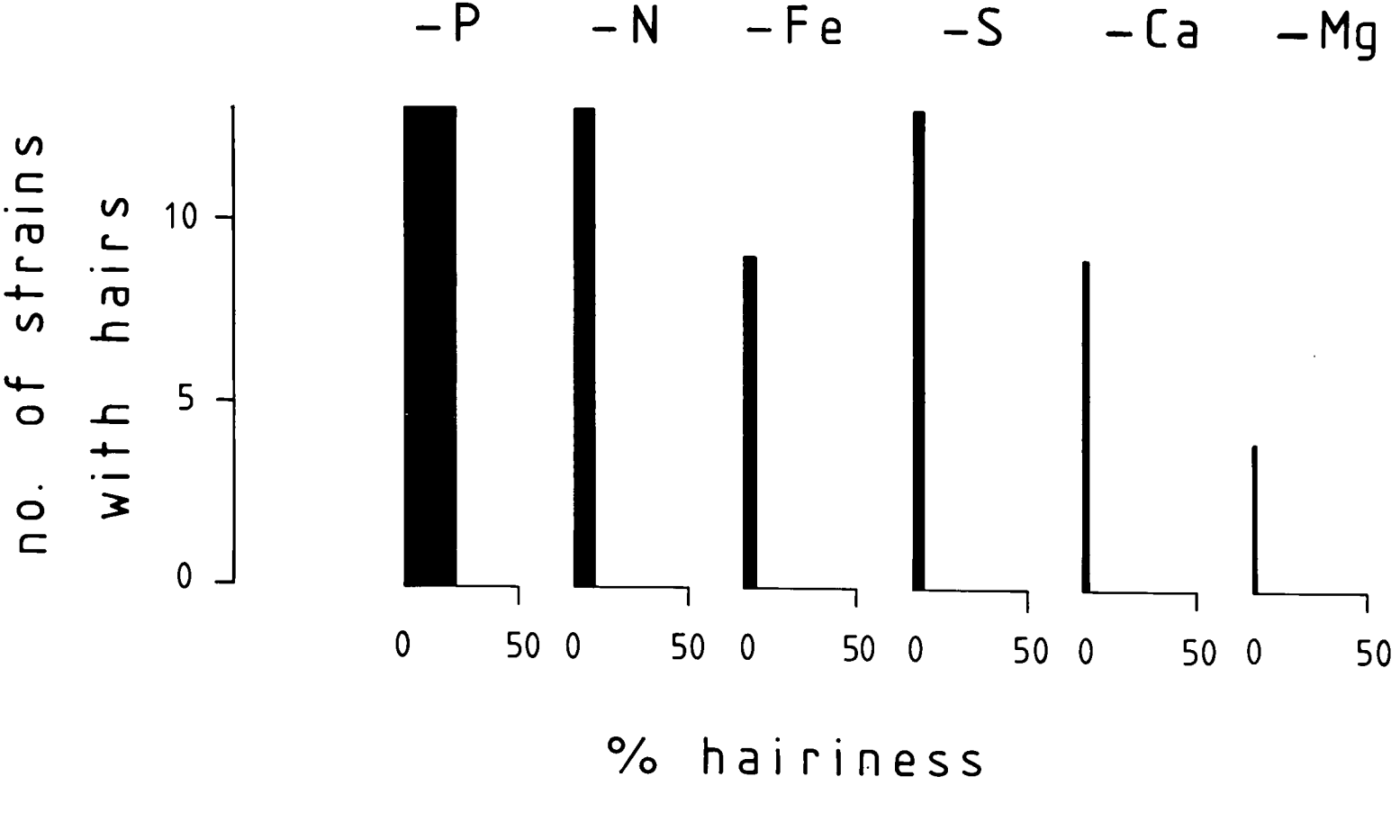
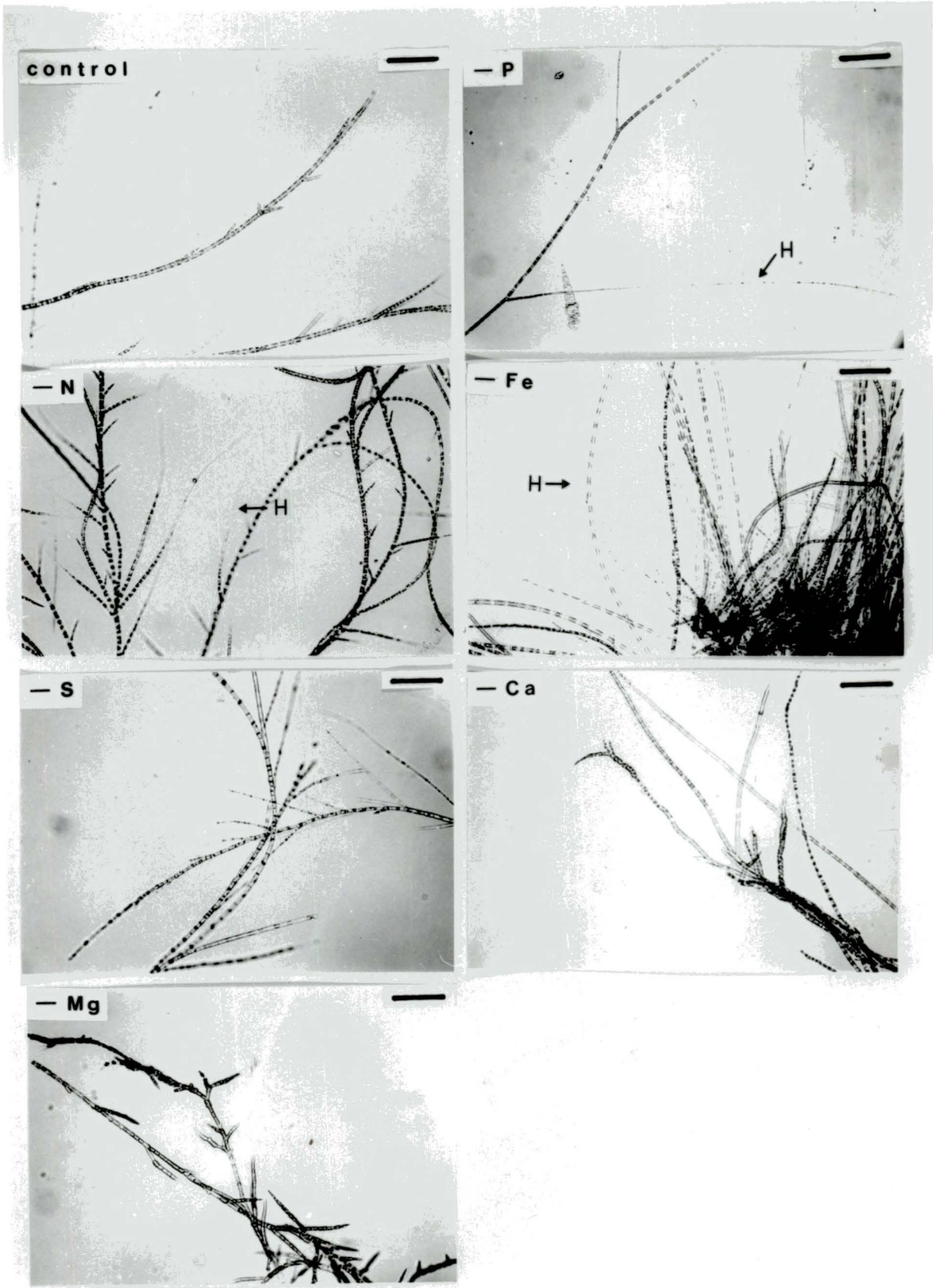

-P
-N
-Fe
-S
-Ca
-Mg
no. of strains with hairs
10
5
0
0
50
% hairiness

Fig. 6.2 Influence of elemental deficiencies on morphology of *Stigeoclonium tenue* D577 (after 14 day incubation). H, hair. Scale bar = 50 µm.


control
— P
H
— N
H
— Fe
H
— S
— Ca
— Mg

differed slightly from those of the preliminary study: no hairs were formed by D653 in - N and hairs were not formed by D577 in - Ca or - Mg. The pattern of yield was also different from the preliminary study but similar for each strain:

control > - P > - Fe > - Ca = - N = - S > - Mg.

Each treatment of D577 was assayed for APA at pH 10.3. All treatments of the other cultures were also assayed for APA and scored on a presence or absence basis; only the cultures lacking P had detectable APA.

6.23 Recovery from deficiencies

The recovery of each strain after addition of the missing element was followed in some detail. A massive release of zoospores always occurred within 24 h of the addition of the limiting nutrient in - P and - N cultures; about 90% of the vegetative cells formed zoospores causing the destruction of most of the erect system in all of the *Stigeoclonium* strains (Fig. 6.3). There was no fixed zone in the branch where zoospore release initiated, it could occur not far from the colourless hairs cells or further down the branch. Further zoospore development took place both distally and proximally from the group of cells where it originated. Fewer zoospores were formed by the two *Draparnaldia* strains under - P and - N, with cells of the main axes never forming zoospores. A moderate number of zoospores were formed by both genera upon addition of Fe but in every case less than 10% of the cells of the erect system were involved, a few zoospores also formed in the other treatments.

The release of zoospores weakened the remaining cell wall material causing the hair and any vegetative cells above the point of release to be lost. Even with the massive extent of zoospore formation in - P and - N

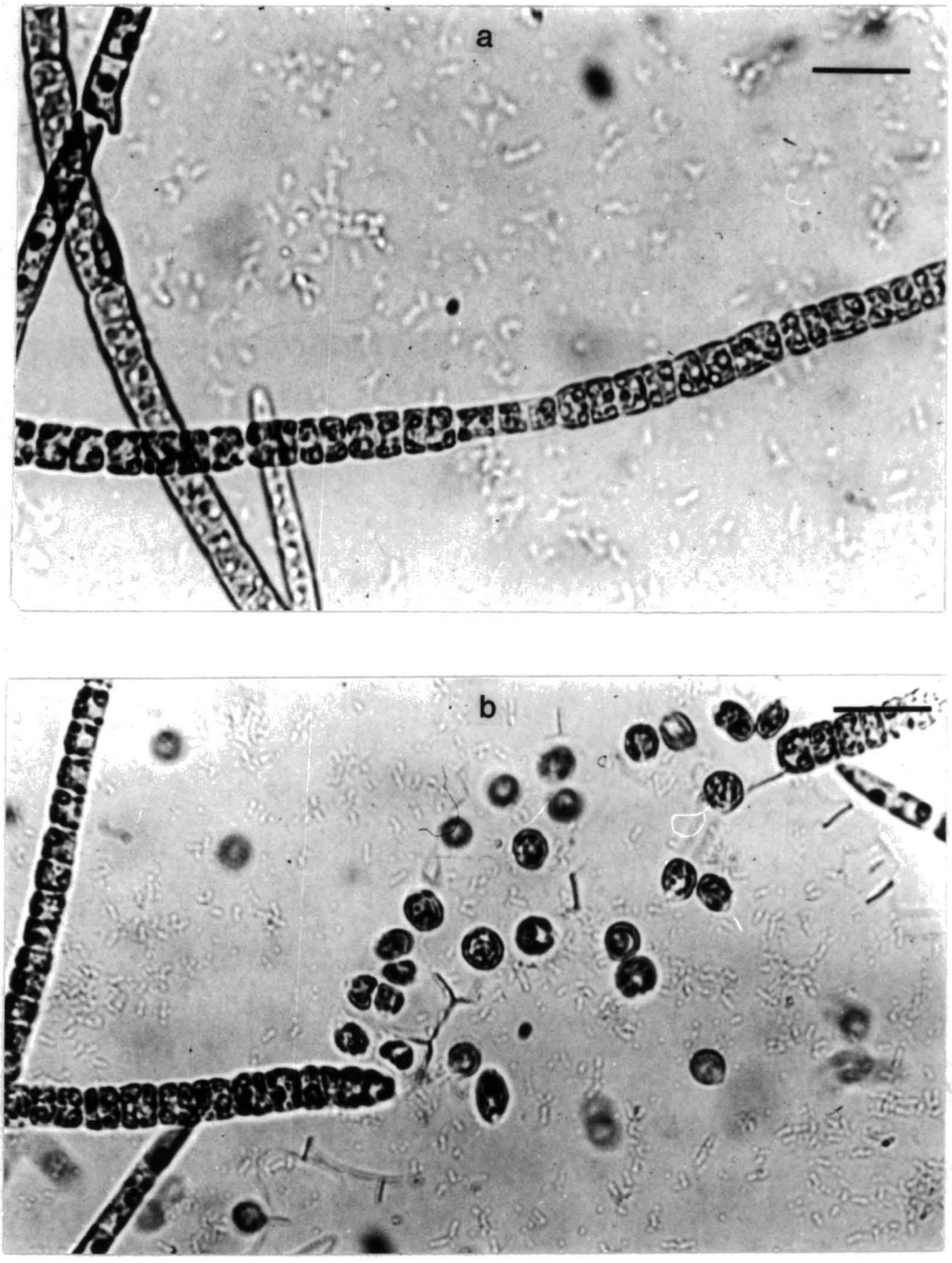


Fig. 6.3 Zoospore formation by *Stigeoclonium tenue* D577:

a) 4 h after addition of P to P-deficient material;

b) 10 min. after a; note loss of integrity of filament.

Stigeoclonium cultures some hairs were still present five days after the addition of the missing element, but this constituted less than 5% of the original number of hair cells. For S, Fe and the remaining deficiencies most of the hair cells remained for some time, but the percentage of hair cells dropped rapidly because normal cells reproduced while the production of hair cells stopped. The hair cells eventually lysed with few remaining five days after the addition of the lacking element.

6.3 Elemental additions to natural populations

The rapid formation of a large number of zoospores, and subsequent loss of hairs, upon the addition of the lacking nutrient in the experiments of Section 6.23 suggested that similar additions to natural populations might form the basis of a rapid assay to determine the growth limiting nutrient of a population. Algal material from a range of sites was collected, washed in distilled water and put into distilled water, Chu 10-F, Chu 10-F (-P) and Chu 10-F (-N) and incubated in the growth rooms (Table 2.5). The cultures were examined after 24 h and then daily up to five days if this was appropriate. The results presented in Table 6.1.

As all the treatments caused zoospore formation in the populations tested the use of this technique as an assay was discounted. Both *Chaetophora* and *Draparnaldia* showed a similar pattern of growth with time- after some initial zoospore formation the colonies gradually lost hairs in Chu 10-F but still had about 10% of their originally number of hair cells after 5 d. Material incubated in the other treatments gradually diminished in size and many cells lysed as bacteria became predominant within 2 d. Growth was only obvious in *Stigeoclonium* in Chu 10-F, young material did not have hairs and the cultures quickly became overgrown with other genera.

Table 6.1 Zoospore formation in natural material placed in media or distilled water upon return to the laboratory. The approximate percentage of cells which formed zoospores after 24 h was judged to the nearest 10%. Details of the sites are listed in Table 4.2.

taxon	site	approximate percentage sporulation after 24 h			
		distilled water	Chu 10-F	(-P)	(-N)
Chaetophora incrassata	Croft Kettle	10	20	20	15
Draparnaldia glomerata	Station Quarry Outflow (reach 0377-01)	10	20	20	20
Stigeoclonium tenue	River Nent (reach 0048-11)	30	80	80	80
Stigeoclonium tenue	Rampgill Level (reach 0096-01)	40	80	80	80

6.4 Utilization of organic phosphorus (continued)

All strains showed moderate to good growth (in comparison with orthophosphate controls) with polyphosphate and pyrophosphate as inorganic sources and β-glycerophosphate and glucose-6-phosphate as organic sources. There were obvious differences in ability to use DNA, ranging from 77% of the yield with orthophosphate in *Stigeoclonium* D577 to hardly detectable with *Stigeoclonium* D699, although the culture did stay green. There was little, if any, evidence of growth with lecithin or phytate and under these conditions the filaments became pale. In general cultures which showed the highest values for % hairiness showed that greatest phosphatase activity and *vice versa*. The most obvious exception was D653 *Draparnaldia* in lecithin.

6.4 Utilization of organic phosphorus

The wide range of natural forms of phosphorus was discussed in Section 1.52. Experiments were carried out to determine whether the main culture strains could utilize a range of phosphorus sources and whether hairs where necessary for any utilization. Batch cultures were incubated in boiling tubes under standard conditions as described in Section 2.462. Material was harvested after 10 d and examined microscopically. Triplicate dry weight measurement was carried out for each treatment. APA was assayed quantitatively for all treatments of D577 and scored on a visual comparison index (Section 2.9) for the other strains. The results are listed in Table 6.2 with the enzymes present listed in Table 6.3.

(See facing page.)

6.5 pH optima of alkaline phosphatase activity

The optimum pH for most alkaline phosphatases is in the range 8 - 11 (Section 1.6) but the studies of natural populations indicated this was not true for some populations of Chaetophorales. The optima of the main cultures were investigated to determine whether this was true of cultured material and whether or not a single pH alkaline phosphatase assay used above could be used for assays of APA of cultured material.

The influence of pH on APA was determined using the assay presented in Section 2.9 and the results are presented in Fig. 6.4. The large pH increments do not allow accurate assessment of the optimum pH of each strain but the three *Stigeoclonium* strains (D577, D702, D724) all had peaks near ten. pH had little influence on the phosphatase of the two *Draparnaldia* strains over the range investigated, but small peaks near pH 8 did occur. As the differences in APA are usually considered in orders of magnitude the inaccuracies due to the assay would not be very great if an

Fig. 6.4 Influence of pH on phosphatase activity of five strains grown to moderate P-deficiency (see Table 2.9).

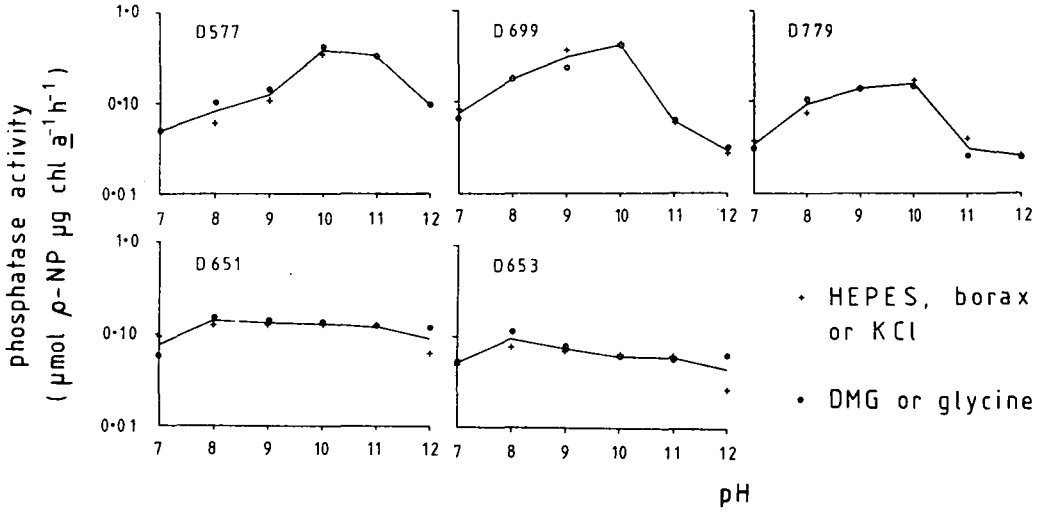

phosphatase activity
(μmol ρ-NP μg chl a⁻¹ h⁻¹)
1·0
0·10
0·01
D577
D699
D779
D651
D653
7
8
9
10
11
12
pH
• HEPES, borax or KCl
• DMG or glycine

Table 6.2 Influence of various P sources (see methods) on yield (mg l^{-1} dry wt), % hairiness (% hair) and phosphatase activity (P-ase: estimated on 0 - V scale, see section 2.9) on day 14. (Initial inoculum in range 0.01 - 0.24 mg l^{-1})

phosphorus source	D577			D651			D653			D699			D779		
	yield	% hair	P-ase	yield	% hair	P-ase	yield	% hair	P-ase	yield	% hair	P-ase	yield	% hair	P-ase
none	3.66	42.5	V	3.64	65	V	2.90	55	V	2.89	60	V	0.98	42.5	V
P_i	160	0	0	97.0	0	II	163	7.5	0	214	0	I	84.2	0	0
poly-P_i	142	7.5	II	75.2	7.5	I	146	5	III	130	5	I	37.8	10	0
pyro-P_i	176	5	II	38.9	0	I	116	2.5	II	51.2	7.5	I	81.6	12.5	II
β-gycero-P_i	200	2.5	0	37.6	32.5	II	136	12.5	II	122	2.5	II	90.7	0	I
glucose-6-P_i	164	0	II	53.8	32.5	II	125	12.5	I	234	50	IV	130	0	I
DNA	124	22.5	V	68.8	42.5	II	66.1	10	II	5.11	47.5	V	30.0	10	III
lecithin	6.44	47.5	V	3.52	62.5	V	4.05	50	0	2.15	37.5	V	1.12	35	V
phytate	4.72	45	IV	5.63	62.5	V	3.70	57.5	V	1.57	50	V	0.98	35	V

Table 6.3 Enzyme classes necessary for utilization of the phosphorus compounds in Table 6.2 and ρ-nitrophenyl phosphate.

substrate	enzyme
β-glycerophosphate	phosphomonoesterase
glucose-6-phosphate	"
ρ-nitrophenyl phosphate	"
phytate	"
DNA	phosphodiesterase
lecithin	"
pyrophosphate	anhydrous hydrolase
metaphosphate	"
polyphosphate	"

assay pH near ten was used for both genera. On the basis of these results a standard assay method using the pH 10.3 buffer recommended by SIGMA (Section 2.9) was used for all APA measurements of cultured material.

6.6 Specific growth rates

The growth of algae can be quantified in many ways, the methods of Fogg (1975) were used in this study with dry weight as the indicator of yield measured. The specific growth rates (K′) of exponentially growing strains used in the laboratory are presented in Table 6.4; these were determined from the slope of the regression line of $\log_{10}$ dry weight values against time for material grown in Chu 10-F in boiling tubes as described in

Table 6.4 - Growth rates of selected algae in Chu 10-F under standard conditions (20°C, 59 ± 9.75 µmol photons m^{-2} s^{-1} (n=24), shaking). K'=specific growth rate, n=number of points in regression, G=doubling time (h). (See Section 6.6 for an explanation of specific growth rate.)

Strain	K'	±	n	G	$K'_{Chl\ a}$	±	n	G
D577	.163	.027	21	44.3	.209	.042	15	34.6
D659	.203	.062	16	35.6	.398	.092	14	18.2
D699	.220	.084	9	32.8				
D779	.136	.081	11	53.1				
D651	.188	.061	14	38.4	.254	.084	11	28.4
D653	.120	.049	11	60.2				

Section 2.462. The 95% confidence limits of the slope and the number of points used for the calculation are also presented, these give a rough guide to the accuracy of the growth rate determination. Mean doubling times corresponding to the specific growth rate are also presented as these use a scale with which everyone is familiar. For three of the strains the specific growth rates calculated from chlorophyll *a* analyses are also given D699 had the shortest doubling time (2.8 h) and D653 the longest (60.2 h). The growth rates calculated from chlorophyll *a* analyses were always greater than those from dry weight. The period of exponential growth was noticeable a day earlier, and ended a day earlier, in the chlorophyll *a* growth curves when compared to the dry weight growth curves.

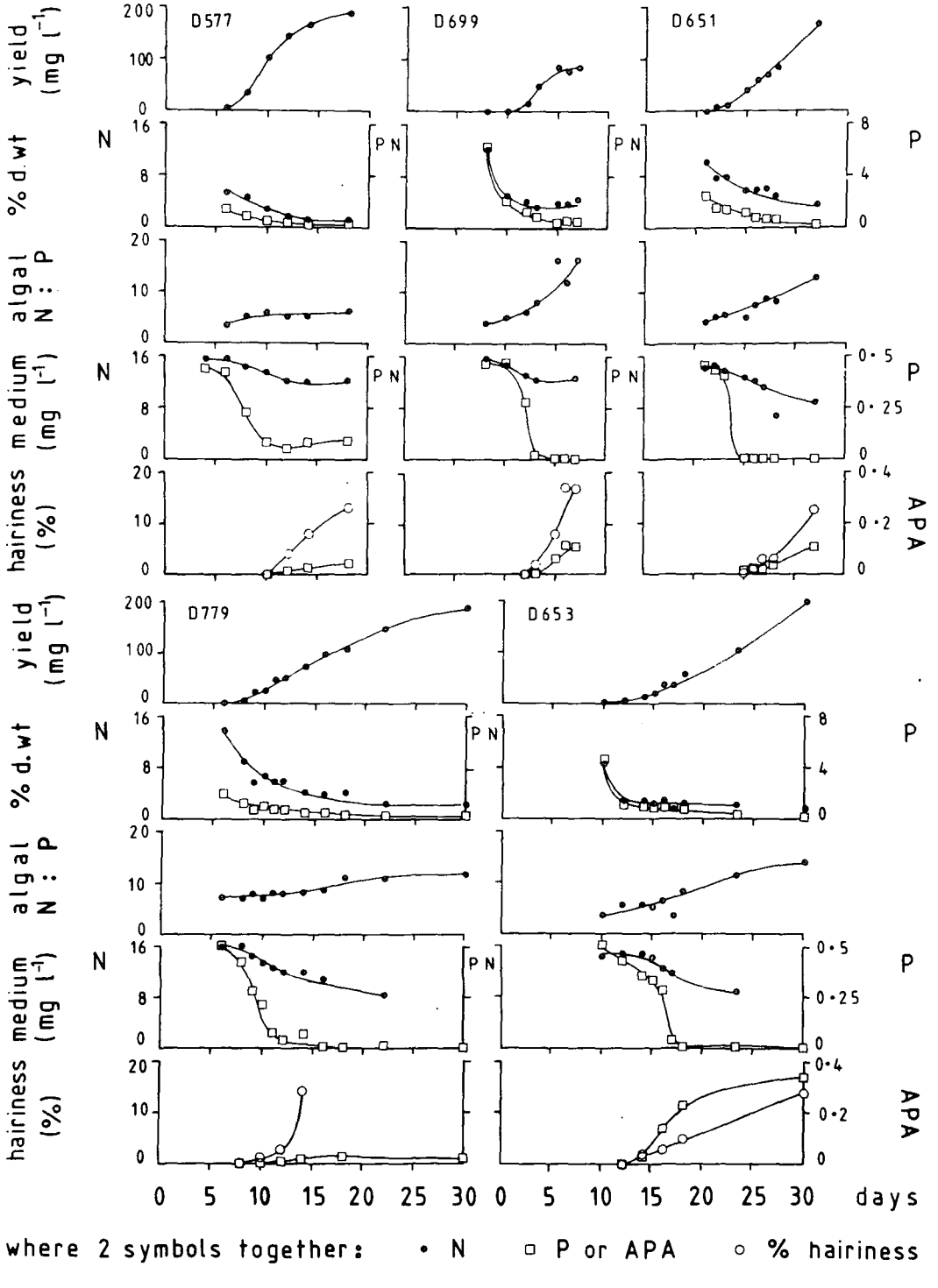
6.7 Growth in batch culture

The growth of five representative strains- three *Stigeoclonium* (D577, D699 and D724) and two *Draparnaldia* (D651 and D653)- was studied in detail for batch cultures in Chu 10-F. Unfortunately cultures of *Chaetophora* did not grow well enough to merit such treatment. A number of variables were followed (Fig. 6.5) for different periods because of the differing growth rates and morphologies of the strains. Three flasks were sampled at each harvest, but if the mass was low the algal material was pooled to obtain an average value for mass and algal nutrient levels. A separate sample was used for both morphological examinations and assays of APA.

6.71 *Stigeoclonium tenue* D577

The shape of the growth curve (Fig 6.5a) of *Stigeoclonium tenue* D577 gave the expected shape for a batch culture going through lag, exponential and early stationary phase but some lysed cells were noticeable by day 14

Fig. 6.5 Changes during growth in batch culture of five strains: yield; algal N and P composition; alga N : P ratio (by weight); % hairiness; alkaline phosphatase activity (APA: µM *p*-nitrophenol µg chl a^{-1} h^{-1}).


D577
D699
D651
D779
D653
yield (mg l⁻¹)
% d.wt
algal N : P
hairiness (%)
medium (mg l⁻¹)
N
P
APA
days
where 2 symbols together: • N □ P or APA ○ % hairiness

and material appeared dead when examined on day 20. Algal N decreased almost as rapidly as algal P, giving only a small rise in the algal N : P ratio. Although the concentration of P in the medium dropped rapidly between days 6 and 10 it was still about 50 µg l^{-1} for the remainder of the growth period. Hairs started to form at the end of the exponential growth stage (day 13) and increased almost linearly; APA followed a very similar pattern to hairiness.

6.72 *Stigeoclonium tenue* D699

The yield of *Stigeoclonium tenue* D699 was very low at the end of the exponential growth phase (Fig 6.5). Both algal N and algal P content dropped but the latter did so more rapidly giving a marked increase in the algal N : P ratio with time. The concentration of P in the medium dropped very rapidly during the early stages of exponential growth but the nitrogen concentration showed a more gradual decline. Hair formation appeared to begin during the algal exponential growth phase (day 12) and the increase in hairs coincided with the increase in APA.

6.73 *Stigeoclonium tenue* D779

Stigeoclonium tenue D779 showed a linear increase in dry weight from day 8 to day 30 (Fig. 6.5) but by day 18 akinete formation was noticeable and by day 22 almost all of the biomass was akinete. Algal N and P content decreased until day 18 with the N : P ratio showing an almost linear increase over the same period. A rapid drop in medium P started on day 7 and continued until day 12. Hairs started to form on day 9 with APA detected on day 10; akinete formation caused the loss of all hairs by day 18 and the alkaline phosphatase activity remained constant from this point until the end of the experiment.

6.74 *Draparnaldia* sp. D651 & *Draparnaldia plumosa* D653

Both of the *Draparnaldia* strains had similar growth patterns (Fig. 6.5, 6.5), with dry weight increasing almost linearly and the algal N decreasing more slowly than algal P giving an almost linear increase in the algal N : P ratio. The medium P concentration had a rapid drop for both strains, but this was about 6 days later for D653, presumably due to the slower growth rate. As with the *Stigeoclonium* strains the patterns of increase in % hairiness and APA were similar. Mucilage was produced from about day 10 in D651 and day 14 in D653 and this made harvesting difficult as the filters clogged.

6.75 Initial hair formation

Table 6.5 lists the algal P content and the algal N : P ratio at the time of initial hair formation. Initial hair formation was assumed to have begun on the day before hairs were first observed.

Table 6.5 P composition and N : P ratio of five strains during growth in batch culture at stage when hairs first start to form.

strain	P (% dry wt.)	N : P
D577	0.70	5.41
D651	0.98	6.25
D653	1.04	5.73
D699	1.31	7.16
D779	0.99	7.21
$\bar{x}$	1.00	6.35

6.8 Hairs in continuous culture

Hairs occurred in the field in seemingly healthy populations, but in batch cultures usually hairs formed towards the end of exponential growth (Section 6.7). The continuous culture method was used to determine whether cultured material could grow indefinitely with hairs. Two strains of *Stigeoclonium tenue* were used in this study (D577, D659). The strains were incubated in one-fifth strength Chu 10-F according to section 2.452. Hairs were present on day 4 of incubation in both cases. The hairiness of the algae was maintained at between 5-10% for 20 days for D577 and 14 days for D659. Incubation was stopped when the cultures became contaminated with bacteria, but the material was still healthy at this point.

6.9 Luxury consumption

The ability of many P-deficient algae to accumulate P rapidly, to a concentration far in excess of their needs, has been widely reported (Section 1.55); in many genera this excess P is stored as polyphosphate granules. The strains of Chaetophorales in Section 6.7 were capable of sustained growth with low internal P concentrations but young culture had P concentrations an order of magnitude higher.

The storage capacity of two axenic strains of *Stigeoclonium tenue* (D577, D659) was investigated to determine whether luxury consumption of P occurred and to find out whether an organic P source could be utilized efficiently by P-deficient algae. The two strains were incubated for ten days in flasks in Chu 10-F and Chu 10-F (0.1P) (Section 2.462); the concentrations used ensured that both exponentially growing and P deficient material was present after the initial incubation period. Aliquots of filter sterilized solutions of KH_2PO_4 or β-glycerophosphate were added to three replicates of each treatment to raise the P concentration by

approximately 1 mg l^{-1}. Three replicates of each treatment were analyzed for algal and soluble phosphorus prior to the addition and five hours after the addition (Fig. 6.6). Although both strains accumulated P the final concentration for the PO_4 treatment was not significantly different from the control for either strains and, as they did not attain an excess, they did not appear to be capable of luxury consumption. The β-glycerophosphate was not utilized by D659 over the time period of the experiment even though other studied indicated this strain was capable of utilizing it for growth (Section 6.2). The rate of β-glycerophosphate uptake was lower than that of orthophosphate for D577. Each algal sample was stained to determine if polyphosphate granules were present, none were detected.

6.10 Site of rapid uptake mechanism

The exponentially growing algae of Section 6.6 had an uptake rate of about 0.044 µg P mg dry wt^{-1} which is much less than the average rate of about 0.1 µg P mg dry wt^{-1} h^{-1} of the deficient algae; this suggests a rapid uptake mechanism (Section 1.521) is in effect in the starved algae. As the starved algae have hairs it is possible that the hairs are the site of the rapid uptake mechanism. To test this hypothesis the same two strains were grown in Chu 10-F and Chu 10-F (0.1P) so that material at two stages of phosphorus deficiency- the onset of hair formation (<1% hairiness) and a point when hairs were abundant (20% hairiness)- could be compared with exponentially growing material. The strains were incubated in flasks under standard conditions (Section 2.462). When the desired hairiness was achieved sterile aliquots of K_2HPO_4 were added to each flask to increase the phosphorus concentration by 1 mg l^{-1}. The P content of the algae was determined from triplicate analyses before, and 5 h after, the

addition of the phosphorus (Fig. 6.7). There was no significant difference in the final algal P concentration of either the slightly-hairy or hairy algae. From this study the initial rate of uptake could not be determined but the apparent uptake rate over the time period (Table 6.6) is similar for both the slightly-hairy and hairy algae, with the slightly greater rate in the hairy algae being due to a lower initial P concentration. These results indicate that the rapid uptake mechanism of these two strains is not confined to the hairs.

Table 6.6 Approximate uptake rates (μg P mg dry wt.$^{-1}$ h^{-1}) of slightly-hairy and very-hairy algae.

treatment	D577	D659
control	0.044	0.042
slightly-hairy	0.88	0.29
very-hairy	1.38	1.44

6.11 Microscopy of hairs

A series of simple microscopic procedures was carried out on some strains to further elucidate the structure of hairs. The techniques were also intended to demonstrate whether or not the hairs of Chaetophorales had similar properties to analogous structures in other algal genera.

Stigeoclonium tenue D577 and *Draparnaldia* sp. D651 were grown under standard culture conditions (in flasks, Section 2.462) in Chu 10-F (0.1 N) and Chu 10-F (0.1 P) for 14 days and then mounted on slides in a drop of medium containing neutral red (Section 2.542). Within 20 s of its administration neutral red did appear in the hairs, but this was followed very quickly by its appearance in other cells and it was generally visible throughout the filament within one minute of application of both strains.

The dye appeared to be concentrated in the vacuole hair cells (Fig 6.8a) with the extent of vacuolation clearly shown. Stain was highly concentrated in vesicles in vegetative cells of the *Draparnaldia* sp. (D651- Fig. 6.8b); this was most noticeable in the N deficient material but occurred to some extent in P deficient material. The stain was more diffuse in the vegetative cells of *S. tenue* D577.

Further elucidation of the structure of hairs can be obtained through the use of fluorochromes which bind specifically to certain chemical groups. Both acridine orange and DAPI were used to demonstrate the presence of a nucleus in natural populations of *Chaetophora incrassata* from Croft Kettle and *Draparnaldia glomerata* from Station Quarry outflow; every hair cell had a nucleus. Cultures of *Stigeoclonium tenue* D577 and *Draparnaldia* sp. D651 grown to P- or N-deficiency were also stained with DAPI and, as in natural the populations above, every hair cell had a distinct nucleus.

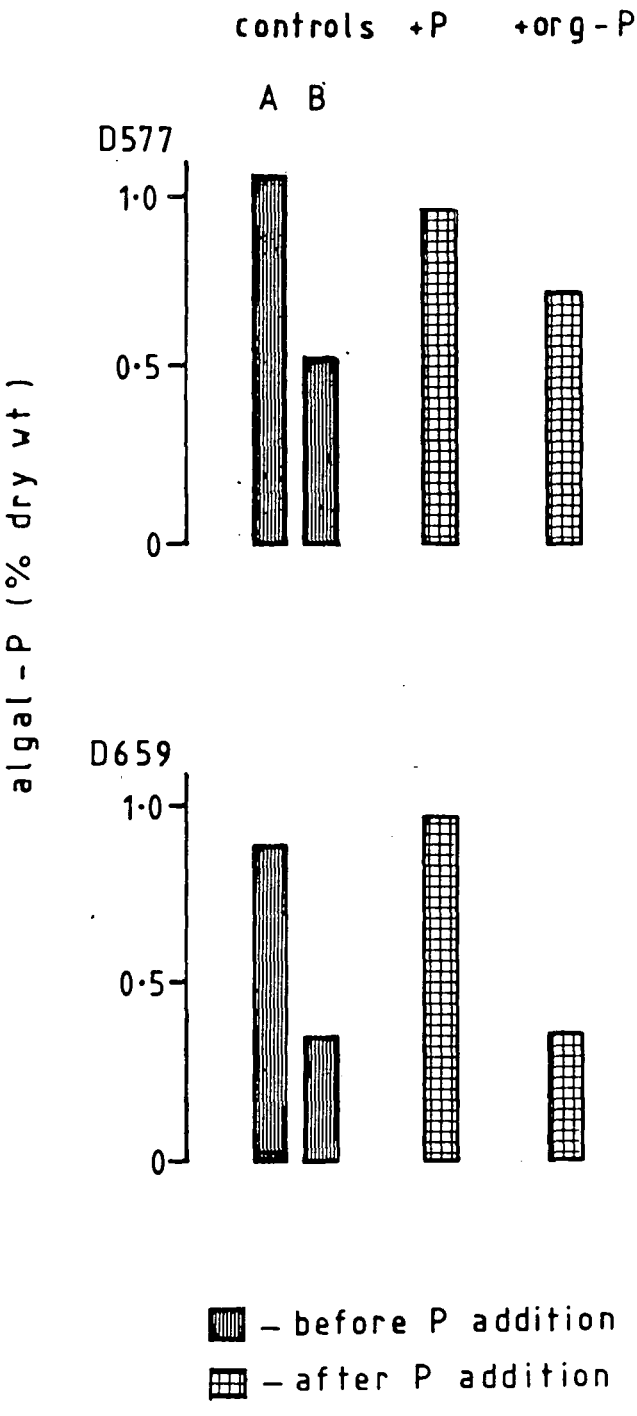


Fig. 6.6 Phosphorus uptake by two moderately P-deficient strains of *Stigeoclonium tenue* (D577 and D659) before, and five hours after, the addition of orthophosphate or β-glycerophosphate.

A) control, grown in Chu 10-F for 10 d;

B) deficient material, grown in Chu 10-F (0.1P) for 10 d.

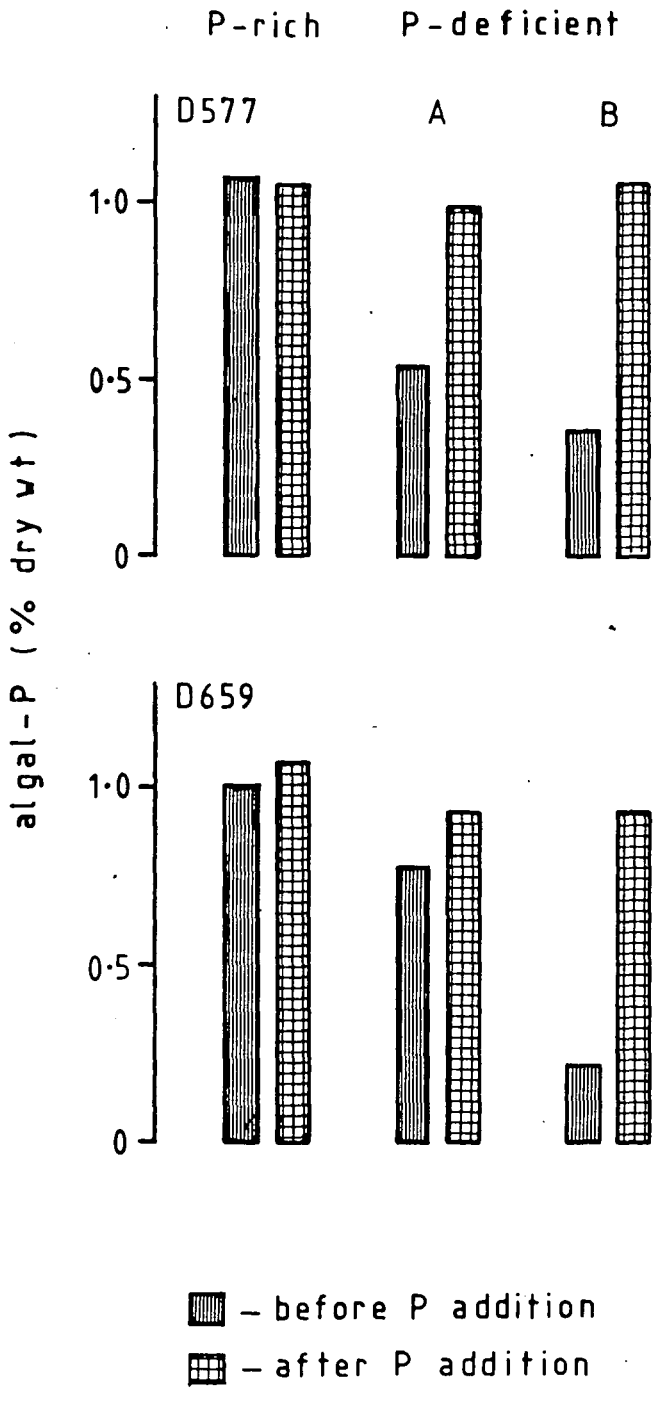


Fig. 6.7 Algal P content of two strains of *Stigeoclonium tenue* (D577, D659) after addition of P to:

A) slightly hairy,

B) very hairy P-deficient cultures.

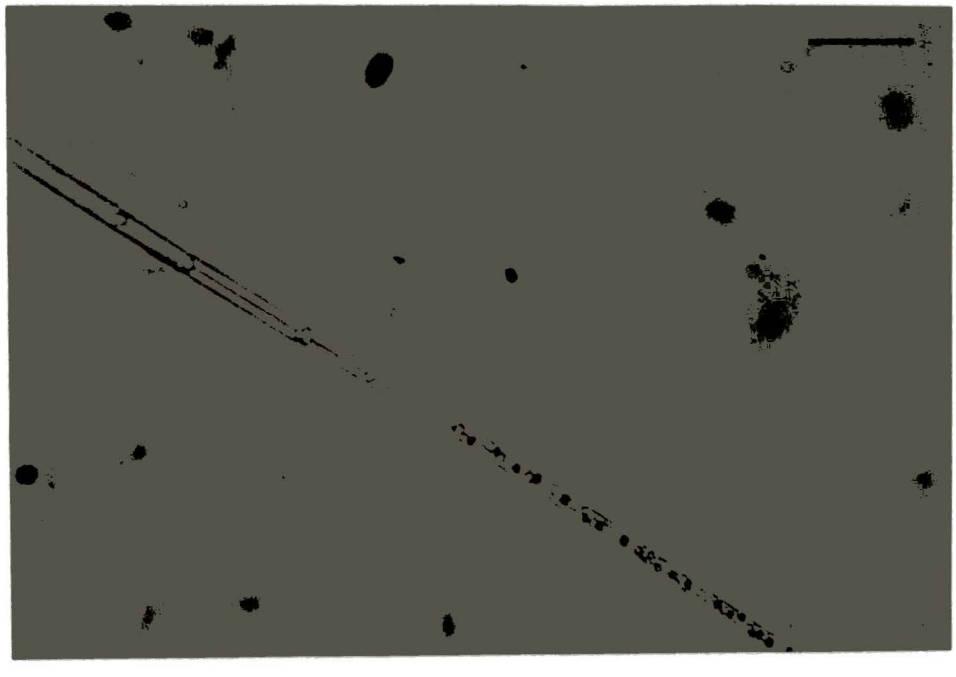
Fig. 6.8 Uptake of neutral red stain by *Draparnaldia* sp. D561:

a) indicating extent of vacuolation in hairs,

Scale bar = 100 µm.

b) showing dense staining globules within vegetative cells.

Scale bar = 20 µm.



7. ULTRASTRUCTURE, PHOSPHORUS UPTAKE PATHWAYS AND LOCALIZATION OF SURFACE PHOSPHATASE ACTIVITY

7.1 Introduction

The large variation in the morphology of the genera studied were produced by only three or four types of cell within each genus. The structure of individual cell types can help to indicate their function as the functions of many intracellular structures are well documented. This chapter presents the results of three lines of study using electron microscopy of field material:

1) comparison of cell types within and between genera.;
2) the use of lead stains to indicate phosphorus uptake pathways;
3) the use of lead capture techniques to indicate the location of alkaline phosphatase activity.

The last method was used in conjunction with light microscopy techniques.

The technical problems of fixation, embedding and sectioning had a marked influence on the results presented. The mucilaginous nature of *Chaetophora* and *Draparnaldia* colonies allowed reasonably large numbers of most cell types to be sectioned and areas of the colony were easily discernible under the microscope even if this was not obvious in electron micrographs. *Stigeoclonium* was much more difficult to study and the chances of obtaining good sections of hair cells was far lower. The dehydration of such highly vacuolated structures as hairs was also a source of concern and the possibility of artifacts was considered when analyzing each section.

7.2 *Chaetophora incrassata*

Samples of *Chaetophora incrassata* collected from Croft Kettle, Darlington (Section 3.31; see Table 4.2) and Sunbiggin Flush 3 (reach 188-01, map reference NY 672077) on 27/4/85 were prepared for electron microscopy to investigate hair structure, alkaline phosphatase localization and phosphorus uptake according to Section 2.5. Photomicrographs are presented for the former population only as the material was very similar. The presence of calcite crystals in the mucilage made sectioning difficult as this material blunted the glass knives and often distorted the section.

7.21 Ultrastructure

Four general zones could be defined within each colony- the centre, intermediate zone and edge of the mucilage and the hairs. The cells attaching the colonies to the substrate were not recovered.

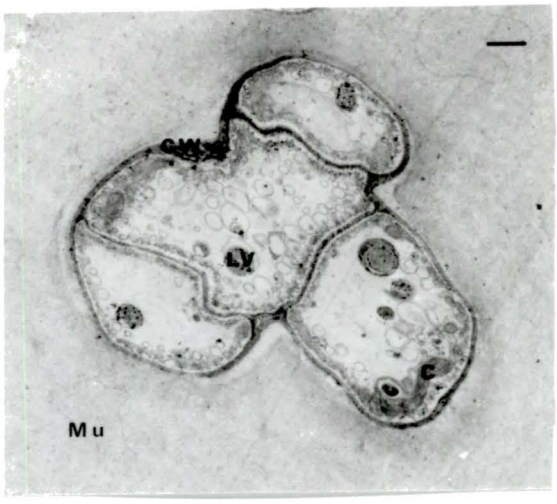
Cells in the central region of the mucilage had very thin plastids around the edges of the cells and numerous membranous vesicles within the central vacuole (Fig. 7.1). Plasmodesmata were abundant between the cells and mitochondria were present near the plastids. Multilayered rounded membranous structures were commom in these cells and will be referred to as lamellar vesicles (the terminology of Gillespie & Hamilton, 1977); these structures will be discussed below (Section 9.6).

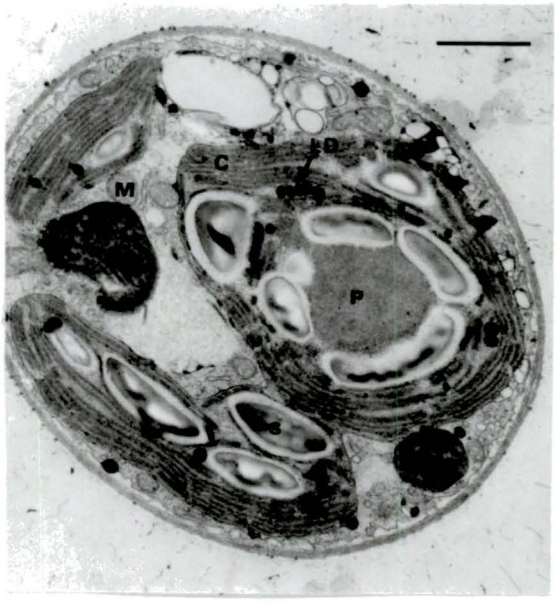
Cells in the middle of the mucilage had little vacuolation and many contained extensive golgi apparatus (Fig. 7.2, 7.3). Sections often contained many vesicles associated with the golgi which appeared to contain mucilage-like material. Starch was abundant within the chloroplasts and was often associated with pyrenoids. Densely staining bodies, which were assumed to be lipid droplets, could also be seen in abundance near the

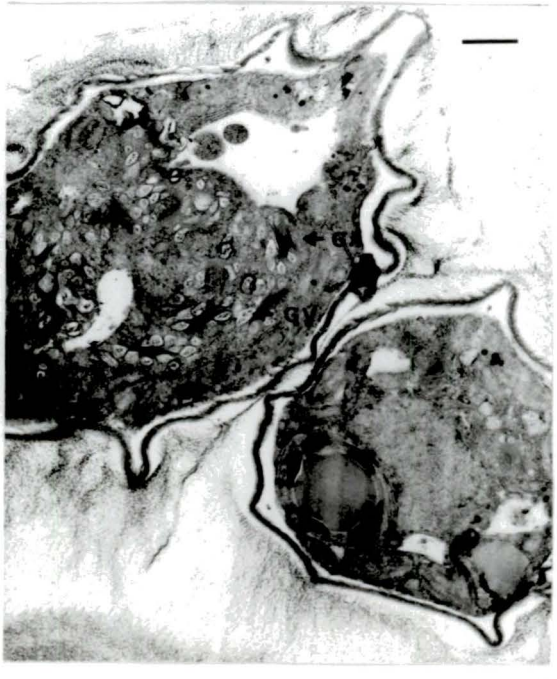
Fig. 7.1 Electron micrograph of cells from centre of colony of *Chaetophora incrassata* showing large central vacuoles containing many vesicles. C, chloroplast; CW, cell wall; LV, lamellar vesicle; M, mitochondrion; Mu, mucilage. Scale bar = 1 µm.

Fig. 7.2 Electron micrograph of cell in intermediate region of mucilage of colony of *Chaetophora incrassata*. Note the large pyrenoid within the chloroplast and the lack of vacuolation in comparison with cells in Fig. 7.1. C, chloroplast; LD, lipid droplet; M, mitochondrion; P, pyrenoid; S, starch. Scale bar = 1 µm.

Fig. 7.3 Electron micrograph of cell from same region of colony as that in Fig. 7.2 but showing numerous golgi apparatus and associated vesicles. GA, golgi apparatus; GV, golgi vesicles. Scale bar = 1 µm.


Mu


M
C
P



chloroplast; whether these were always within the plastid membrane was not clear. Nuclei and nucleoli were often visible in these cells and plasmodesmata were noticeable between cells in transverse sections from this area of the colony.

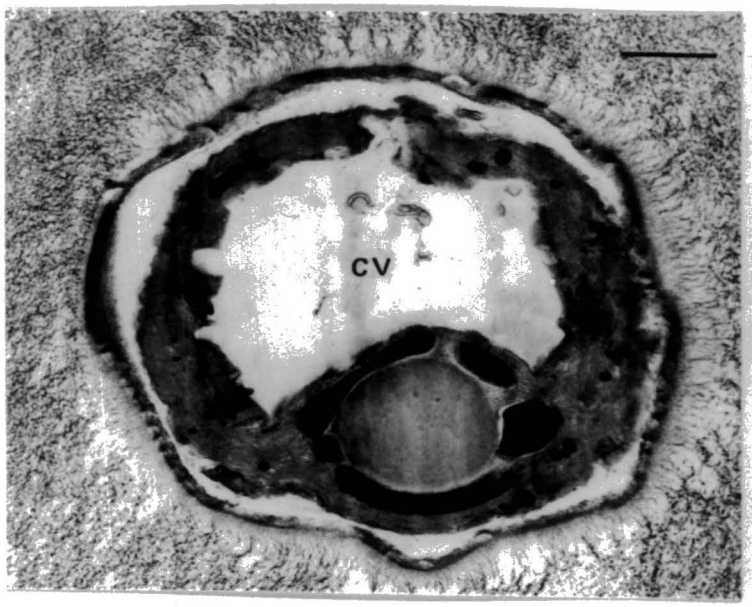
Towards the edges of the colony the cells became progressively more vacuolated with cytoplasmic material restricted to a ring near the cell wall (Fig. 7.4). The cytoplasm had obvious chloroplasts but evidence of golgi apparatus was rarely seen. There was little evidence of mucilage production as vesicles with wall material could not be seen in any of the sections studied. Starch grains were smaller and less frequent in the cells as a whole, but probably just as frequent in relation to chlorophyll present and pyrenoids did occur occasionally. Plasmodesmata were also noted between the cells and mitochondria were common. The lamellar vesicles noted in the central region were also present in this zone.

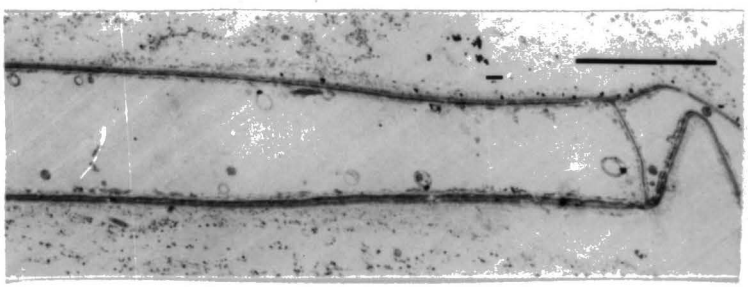
Hair cells were very obviously outside of the mucilaginous matrix but tended to collapse during the later stages of dehydration so often had mucilaginous material in close proximity. Sections of hair cells were usually oblique and very rarely was a whole cell produced. The cells were mostly empty (Fig 7.5) with a ring of membrane-like material near the wall with frequent lamellar and membranous vesicles within the tonoplast which appeared to be concentrated near the cell membranes. The nuclei noticeable under the light microscope were not, unfortunately, ever present in the sections; this was presumably due to their small size in relation to the rest of the cell. Plasmodesmata were abundant between all the hair cells.

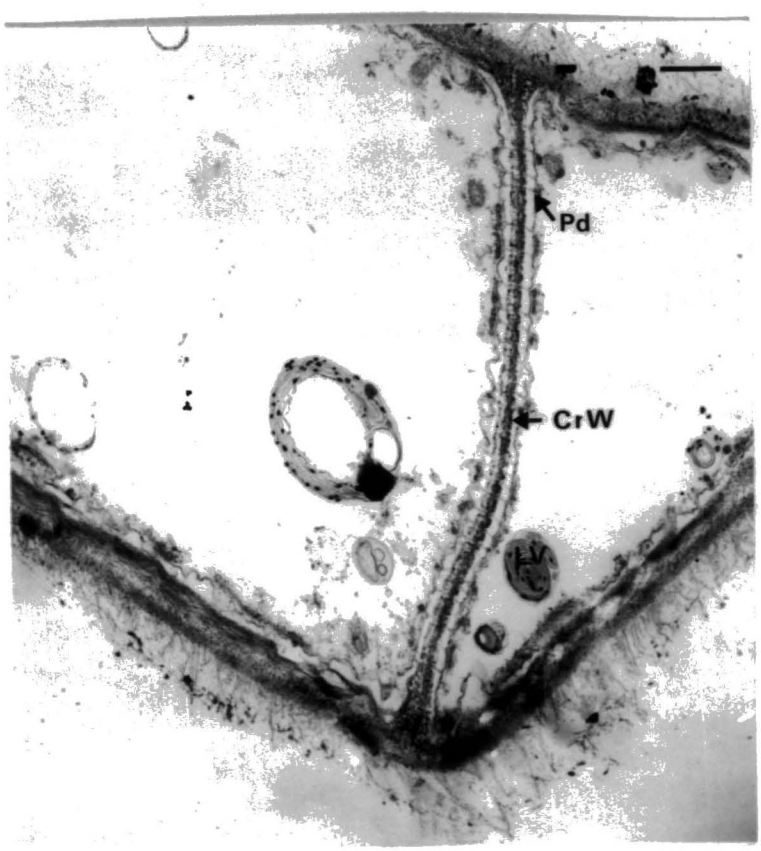
Fig. 7.4 Electron micrograph of a cell with a large central vacuole from near the edge of the colony of *Chaetophora incrassata*; compare this with Fig. 7.3. CV, central vacuole. Scale bar = 1 µm.

Fig. 7.5 Electron micrograph of longitudinal section near the cross-wall between two hair cells of *Chaetophora incrassata*. Scale bar = 10 µm.

Fig. 7.6 Close-up of region near cross wall in Fig. 7.5. Note the lack of plastids and the presence of many lamellar vesicles and numerous plasmodesmata between the cells. CrW, cross-wall; LV, lamellar vesicles; Pd, plasmodesmata. Scale bar = 0.5 µm.


CV




Pd
CrW

7.22 Phosphorus uptake

The studies of phosphorus uptake pathways were conducted on fresh field material from the same sites as above. Material was incubated for 1, 5, 30 or 60 min before plunging into ice-cold fixative as described in Section 2.543. After incubation for one minute the hair cells had a darkening of the cell wall indicative of increased phosphorus concentrations. The outer mucilage also showed darkening in stream-like ridges.

After five minutes a pronounced darkening of the mucilage was noticeable near the edge of the colony (Figs. 7.7), but the matrix within the colony did not change significantly. The apparent darkening of the cell wall of the hair cells had not increased from the one minute stage but the walls between both hair and vegetative cells were very densely stained (Fig. 7.8).

One hour after the addition of P there was a noticeable darkening of much of the mucilage with striking increases in concentration around cells near the edge of the colony (Fig. 7.9). The cross-walls between hair cells were still very densely stained, as were some of the associated vesicles.

7.3 Draparnaldia

The population of *Draparnaldia glomerata* used in this study was isolated from reach 0142-50 (Section 3.31; see also Table 4.2). Cells attaching the material to the substrate were not studied.

7.31 General morphology and ultrastructure

The size of the cells of the main axis made it difficult to obtain whole cells in section but an oblique section is presented in Fig. 7.10. These cells had a thin ring of cytoplasm near the cell wall in which chloroplasts and starch grains were seen. Even at the low magnification used many

Fig. 7.7 Electron micrograph showing longitudinal section through a cell from near the edge of a colony of *Chaetophora incrassata* five minutes after the addition of P. The dark areas are caused by lead phosphate deposits; note the density of stain in the mucilage (compare with Fig. 7.4) and densely stained inclusions within the cell. Scale bar = 1 µm.

Fig. 7.8 Electron micrograph of cross-wall between two hair cells of *Chaetophora incrassata* five minutes after addition of P. The dark areas are caused by lead phosphate deposits. Compare density of stain with Fig. 7.5. Scale bar = 0.5 µm.

Fig. 7.9 Electron micrograph of cell near edge of mucilage of *Chaetophora incrassata* one hour after addition of P. The dark areas are caused by lead phosphate deposits. Note very dense staining of mucilage. Scale bar = 1 µm.

Fig. 7.10 (Overpage) Electron micrograph of main axis cells (oblique section) of *Draparnaldia glomerata*. Note large central vacuole (CV). C, chloroplast. Scale bar = 1 µm.

Fig. 7.11 (Overpage) Electron micrograph of cell from lateral whorl of *D. glomerata*. C, chloroplast; LD, lipid droplet; M, mitochondrion; N, nucleus; P, pyrenoid; S, starch. Scale bar = 1 µm.

Fig. 7.12 (Overpage) Close-up of golgi apparatus (GA) from a cell from a lateral whorl of branches of *D. glomerata*. Scale bar = 0.1 µm.

Fig. 7.7

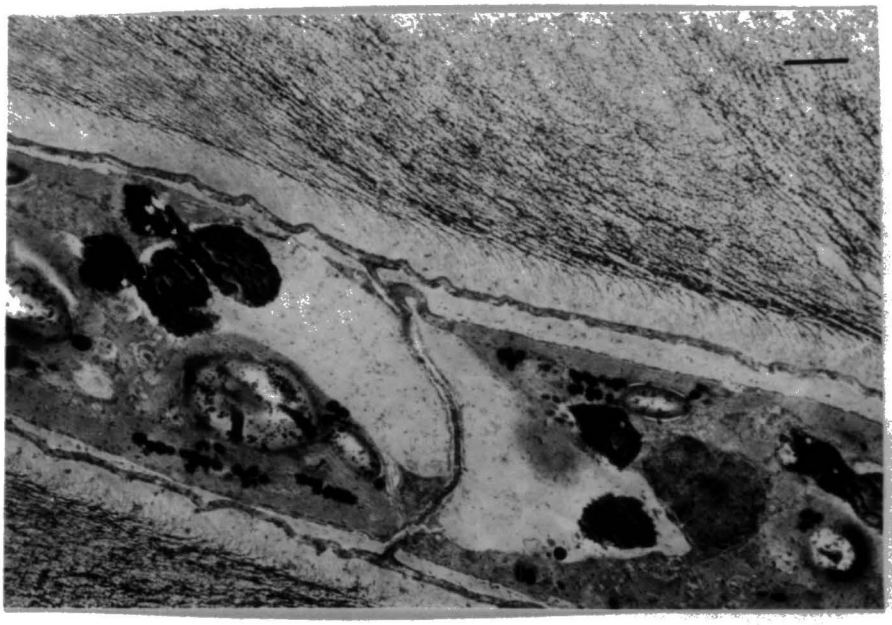


Fig. 7.8

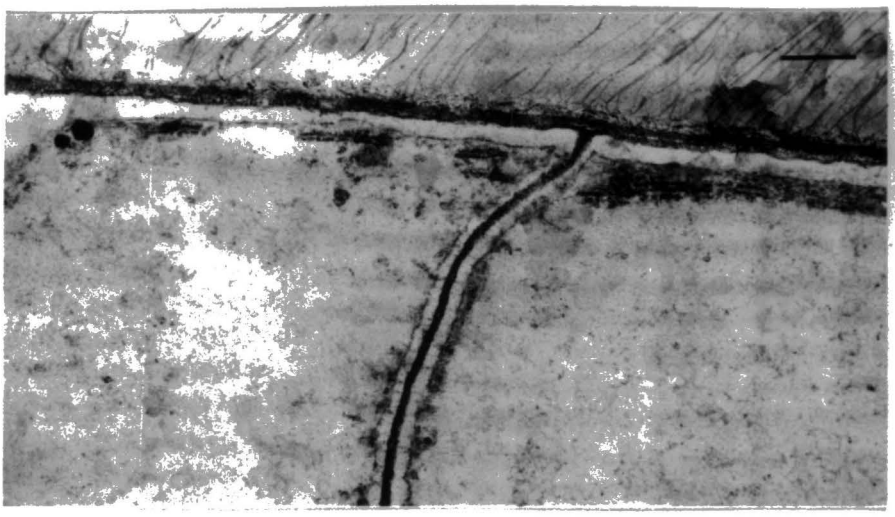


Fig. 7.9

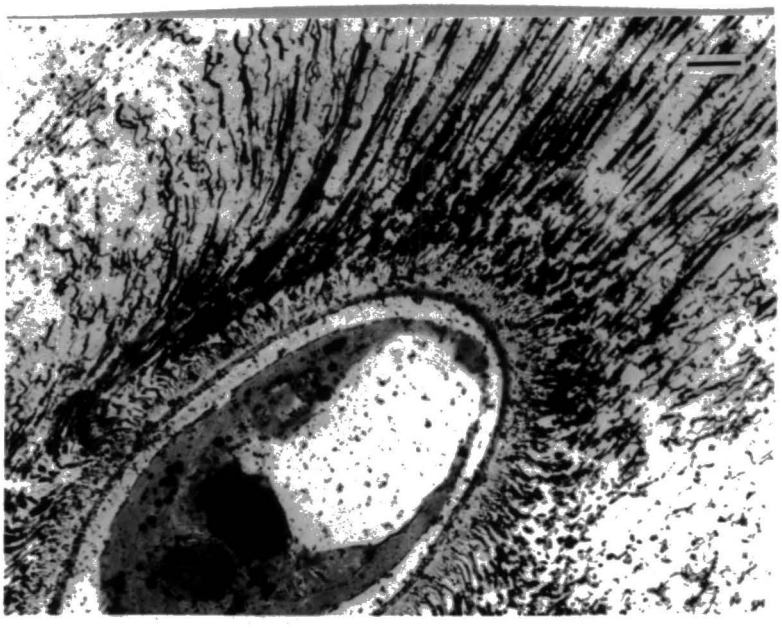


Fig. 7.10

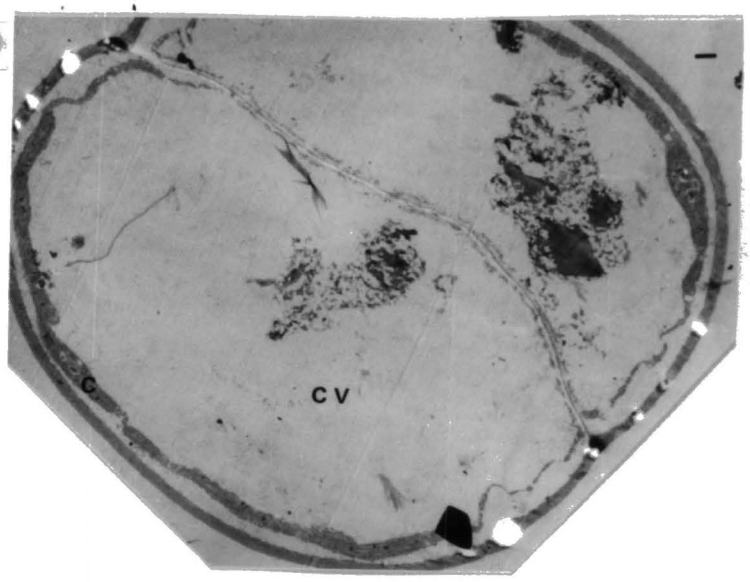


Fig. 7.11

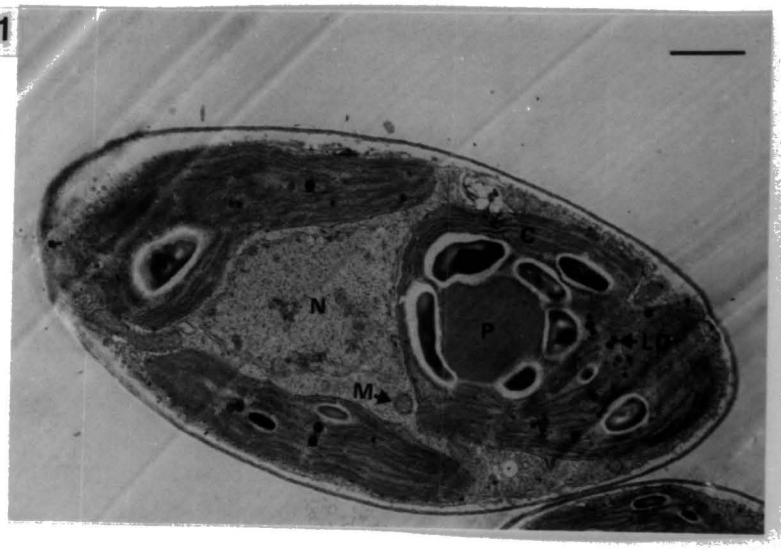
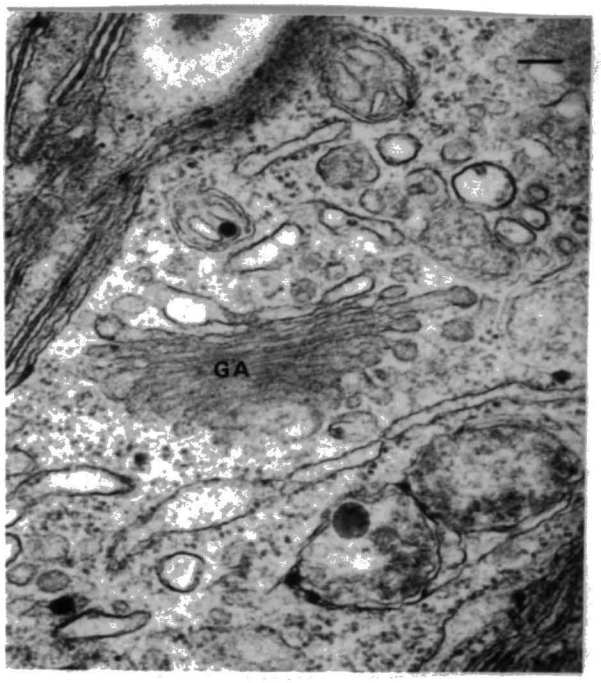


Fig.7.12



vesicles can be seen within the cell and evidence of plasmodesmata is present between the cells. Plasmodesmata do not appear particularly well defined between the cells but this is probably an artefact of the angle of the section.

No evidence of a rachis cell was found in any of the sections studied. A cross section of a cell from one of the lateral whorls of branches is presented in Fig. 7.11; this exhibits typical green algal cell construction (Pickett-Heaps, 1975) with an obvious nucleus, chloroplasts, mitochondria and a complex pyrenoid surrounded by starch grains; many plasmodesmata were present between the cells in this region. Each chloroplast, or lobe of chloroplast, contains starch deposits and lipid droplets similar to those in *Chaetophora incrassata* (above). There was some evidence of vacuolation in cells from this part of the colony and when present they contained many membrane bound vesicles but no lamellar vesicles were seen.

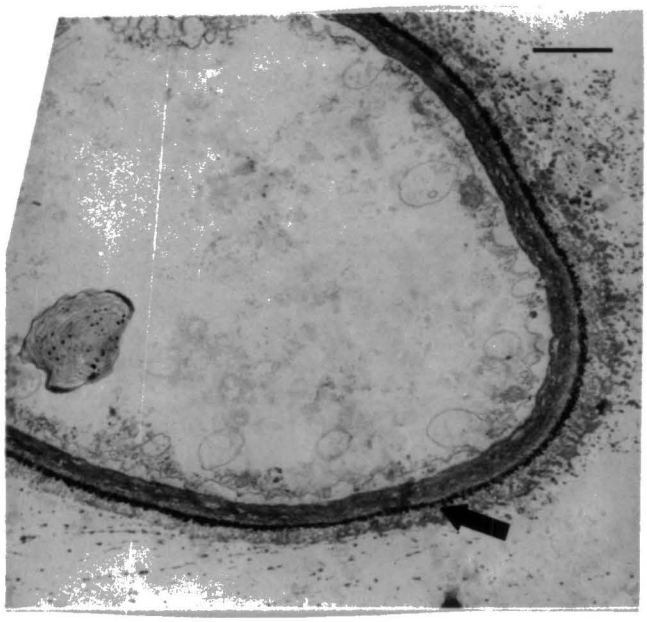
The abundance of golgi apparatus in cells from this region of the colony (Fig. 7.12) suggests these cells produce mucilage. This appears to contradict the observations of old culture material (Section 5.72, 5.82) where mucilage was only in evidence around the cells of the main axis.

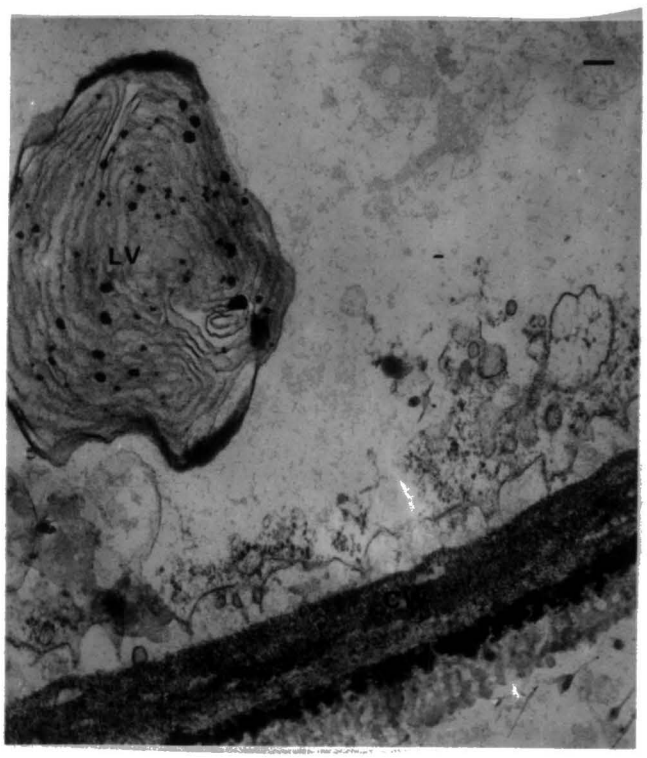
Towards the edge of the mucilage cells had a larger central vacuole and contained lamellar vesicles as well as many membranous vesciles within the central vacuole. Shrinkage of the cytoplasm separated the membranes from the cell wall in most of the hair cells sectioned. Hair cells were very similar to those of *Chaetophora incrassata* (Section 7.22) with very large central vacuoles containing lamellar and other vesicles but, unlike in *C. incrassata*, small chloroplasts were occasionally present in hair cells.

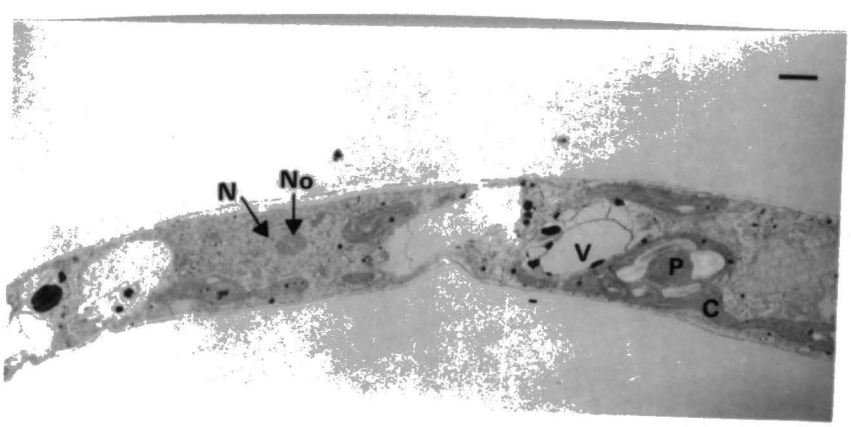
Fig. 7.13 Electron micrograph of cross section of hair cell from *D. glomerata* 5 minutes after addition of P. The dark areas near the cell wall (indicated by arrow) are caused by lead phosphate deposits. Scale bar = 1 µm.

Fig. 7.14 Close-up of wall and lamellar vesicle (LV) from Fig. 7.13. CW, cell wall. Scale bar = 0.1 µm.

Fig 7.15 Electron micrograph showing longitudinal section through cells from a lateral whorl of branches of *D. glomerata* one hour after addition of P. Note densely stained bodies within each cell (compare with Fig. 7.7) and lack of stain at cross-walls. C, chloroplast; P, pyrenoid; N, nucleus; No, nucleolus; V, vacuole. Scale bar = 1 µm.




LV


N
No
V
P
C

7.32 Phosphorus Uptake

After one minutes incubation in phosphorus there was no noticeable increase in the density of staining but after five minutes some small dense spots were noticeable in the cytoplasm of vegetative cells and obvious lead deposits were associated with the walls of hair cells (Fig. 7.13, 7.14). After one hours incubation densely stained areas were common in vegetative cells from the centre of the whorls (Fig. 7.15) but there was little evidence of P at the cross-walls. As in *Chaetophora incrassata* (Section 7.23) the mucilage was densely stained near the edge of the colony and cells in this region had obvious deposits of lead associated with the cell walls, which was also true of hair cells after one hour.

7.4 *Stigeoclonium*

Samples of *Stigeoclonium* were collected from the River Nent just below the influx of Rampgill Level (Section 3.42; see also Table 4.2) and from the sewage works outflow from which D779 was isolated. The results of identical fixation procedures varied considerably between treatments of each sample for no obvious reason. The most disturbing aspect of this study was the infrequent occurrence of hairs in any of the sections; when hairs were present they were usually poor examples and their microstructure was not distinguishable. To investigate the microstructure of hairs cultured material (D577) was prepared for electron microscopy after ten days growth in Chu 10-F (0.1P) (Section 2.444) to ensure hair formation. Unfortunately, the cultured material was counterstained with toluidine blue and borax (Section 2.52) which produces slightly different images than the uranyl acetate/lead citrate stain normally used.

Fig. 7.16 Electron micrograph of vegetative cells of *Stigeoclonium tenue* from:

a) Rampgill Level (reach 0096-01): cross section,;

b) Hollingside Stream (reach 0142-50: D779 was obtained from this site): longitudinal section.

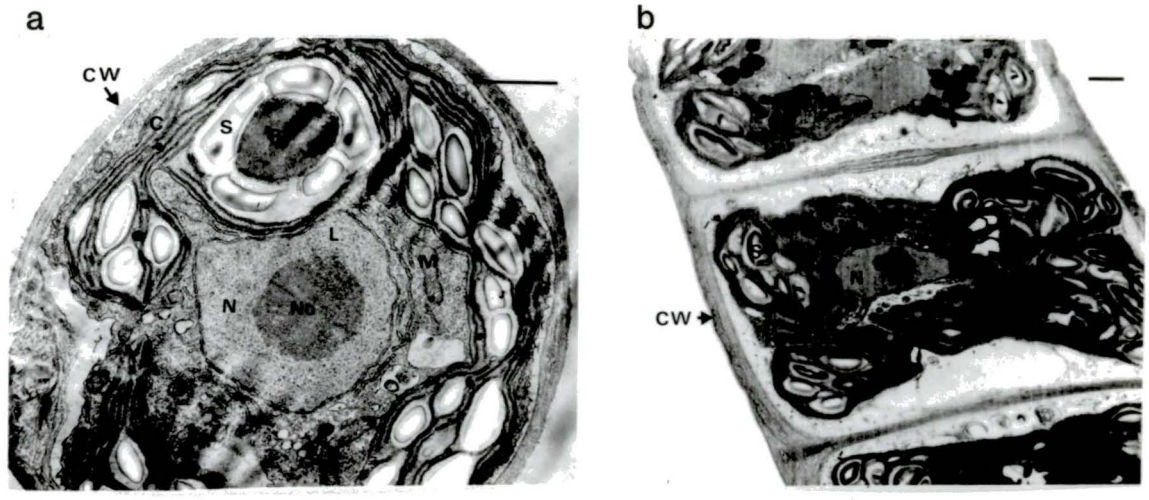
C, chloroplast; CW, cell wall; M, mitochondrion; N, nucleus; No, nucleolus; P, pyrenoid; S, starch. Scale bar = 1 µm.

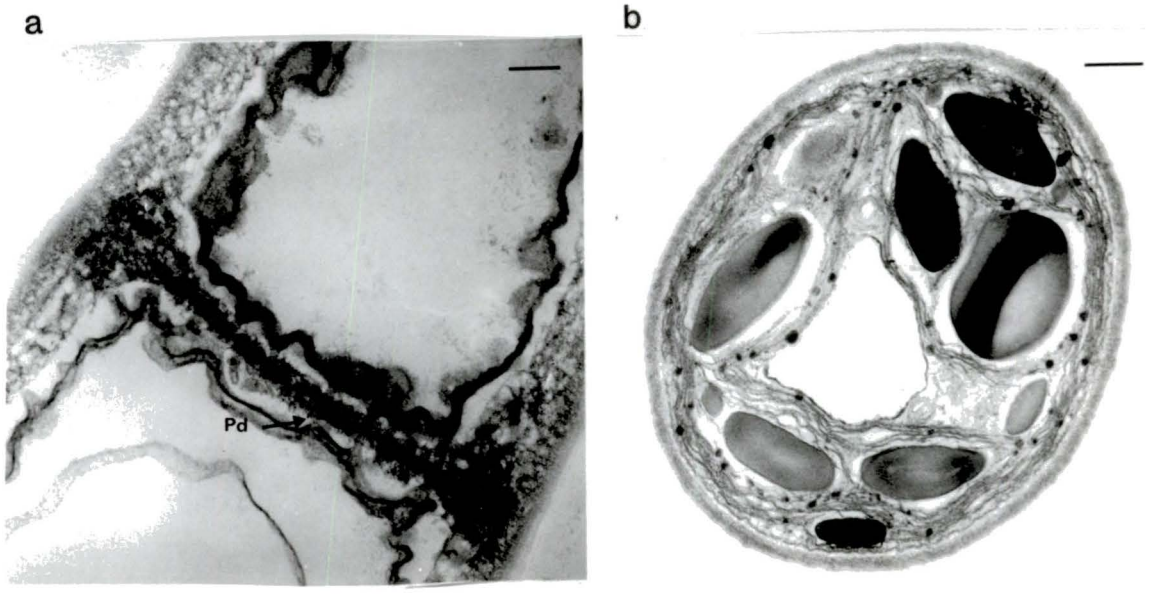
Fig. 7.17 Electron micrograph of *Stigeoclonium tenue* (D577) grown in Chu 10-F (0.1 P) for 14 d. and stained with toluidine blue (Section 2.52):

a) longitudinal section of hair cells with large central vacuoles and many plasmodesmata between the cells;

b) cross section of vegetative cells containing large starch deposits.

P, plasmodesmatum. Scale bar = 0.5 µm.


a
cw
C
S
L
M
N
Nu
Os
b
cw
N


a
Pd
b

7.41 General morphology and ultrastructure

The vegetative cells of both populations display most of the features described for cells of *Chaetophora* and *Draparnaldia*, but no obvious central vacuoles were seen and golgi apparatus was much less abundant. Nuclei with distinct nucleoli were obvious in many cells (Fig. 7.16) and starch and lipid droplets were present within the chloroplasts; numerous lipid droplets were also present in the cytoplasm of the population from reach 0142-50.

No evidence of plasmodesmata was seen in the longitudinal sections of *Stigeoclonium* from the sewage works outflow but they were present between all cell types in the cultured material and can be seen between hair cells in Fig. 7.17a. Little evidence of any plastids was found in any of the hair cells but cells near the hairs had obvious central vacuoles and lamellar vesicles. Cells from near the basal region of the material grown in batch culture had very thick cell walls and a large amount of starch (Fig. 7.17b).

7.5 Localization of alkaline phosphatase

Localization of alkaline phosphatase was investigated by staining both cultured and field material using a lead capture technique (Section 2.541). Phosphatase activity releases PO_4^{3-} from an organic source, this precipitates lead phosphate as an electron dense stain; for viewing under the light microscope the lead phosphate is converted to lead sulphate through the application of ammonium sulphate.

7.51 Culture studies of APA

Continuous cultures of D577 and D659 were grown until hair development was various and the APA was above 0.05 µmol-P µg chl a^{-1} h^{-1}. The material was stained for light microscopy as above (Fig. 7.18a). Stain was rare on the hairs but obvious around many pigmented cells, especially near branches towards the end of filaments.

7.52 Field samples

Samples of *Stigeoclonium*, *Chaetophora* and *Draparnaldia* collected for the studies of microstructure and phosphorus uptake in Sections 7.1, 7.2 & 7.3 were also used for this study. The presence of APA was tested by the single pH assay for all samples, and live material was then treated as above.

7.53 *Stigeoclonium*

No staining was seen on hairs cells from any of the samples, but stain did appear to be associated with the basal system. In many cases this could not be definitely associated with any particular cells and could have been due to foreign material such as bacteria (Fig 7.18b). The material from Rampgill Level (0096-01) has obvious staining associated with basal rhizoids (Fig. 7.18c, 7.18d).

The difficulties encountered in the preparation of samples for electron microscopy have been mentioned above (Section 7.4). The lead control did fix and stain well but no lead could be seen associated with the walls of any types of cell. Although the same preparation procedure was used for the Na-β-glycerophosphate treated samples they did not fix well. No lead was associated with the cell walls of vegetative cells treated with β-

glycerophosphate but the rhizoids of the material from Rampgill level did have obvious lead localization which was in marked contrast to the hairs.

7.54 *Chaetophora* and *Draparnaldia*

Chaetophora and *Draparnaldia* showed a similar pattern of staining under the light microscope which contrasted sharply with that of all the populations of *Stigeoclonium* (compare Fig. 7.18*d* & *e* with 7.18b & c). Lead sulphide was heavily deposited on all the hairs but there was little present on the mucilage. Attempts to remove the mucilage failed so it was not possible to tell whether the lack of staining of cells within the mucilage was due to lead sulphide not penetrating the mucilage.

Under the electron microscope these genera appeared very similar so only the micrographs of *Chaetophora* are presented here. Cells from near the edge of the mucilage had some lead deposits near the cell wall when incubated in β-glycerophosphate but this was not seen in the control (Fig. 7.19); vegetative cells nearer the middle of the colony did not have any noticeable lead deposition. The hairs of the control have some lead deposits around the cell. The hairs of control material did have some deposits of lead near the cell wall (Fig. 7.20a) but these were much less dense than the deposits around the treated material (Fig. 7.20b) indicating phosphatase is very active on the hairs.

Fig. 7.18 Light micrographs showing dense lead stain associated with alkaline phosphatase activity.

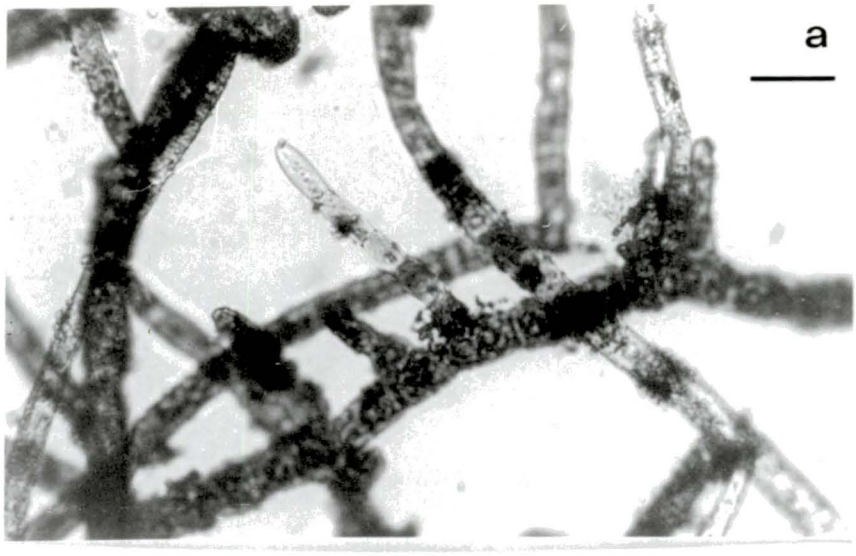
a) *Stigeoclonium tenue* grown in continuous culture (scale bar = 20 µm),

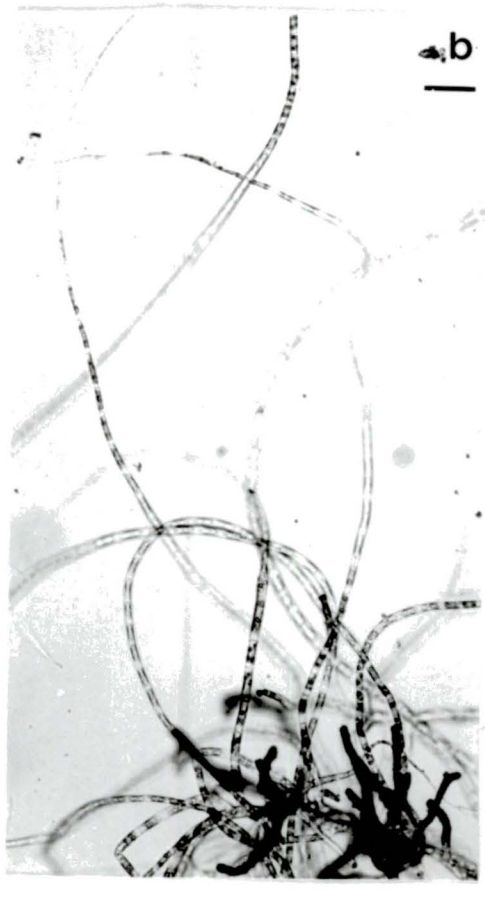
b) *Stigeoclonium tenue* from the River Nent (reach 0048-12: D699 was isolated from this site: scale bar = 50 µm),

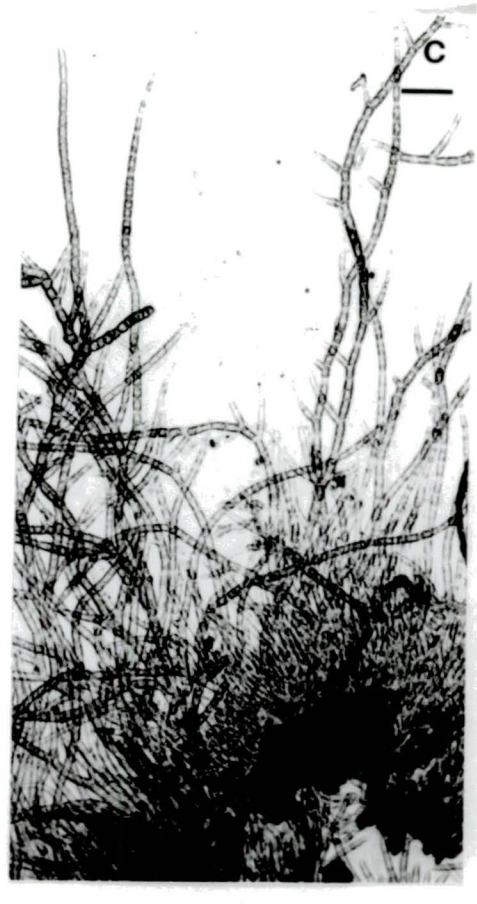
c) *Stigeoclonium tenue* from Rampgill Level (reach 0096-01: scale bar = 50 µm),

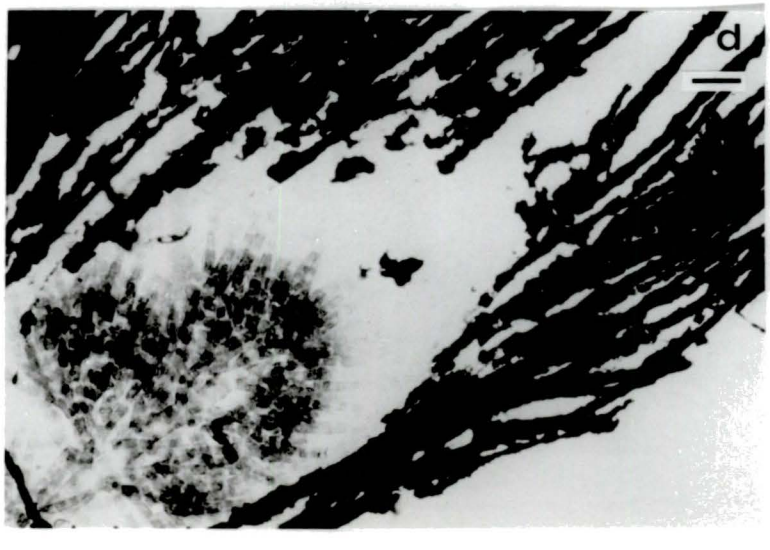
d) *Draparnaldia glomerata* from Station Quarry outflow (reach 0377-01: scale bar = 50 µm),

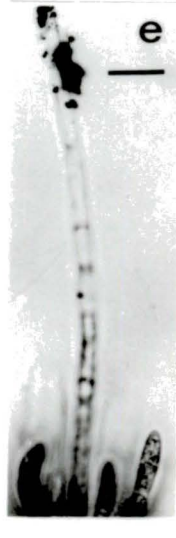
e) *Chaetophora incrassata* from Croft Kettle (D652 was isolated from this site: scale bar = 100 µm).


a


b


c


d

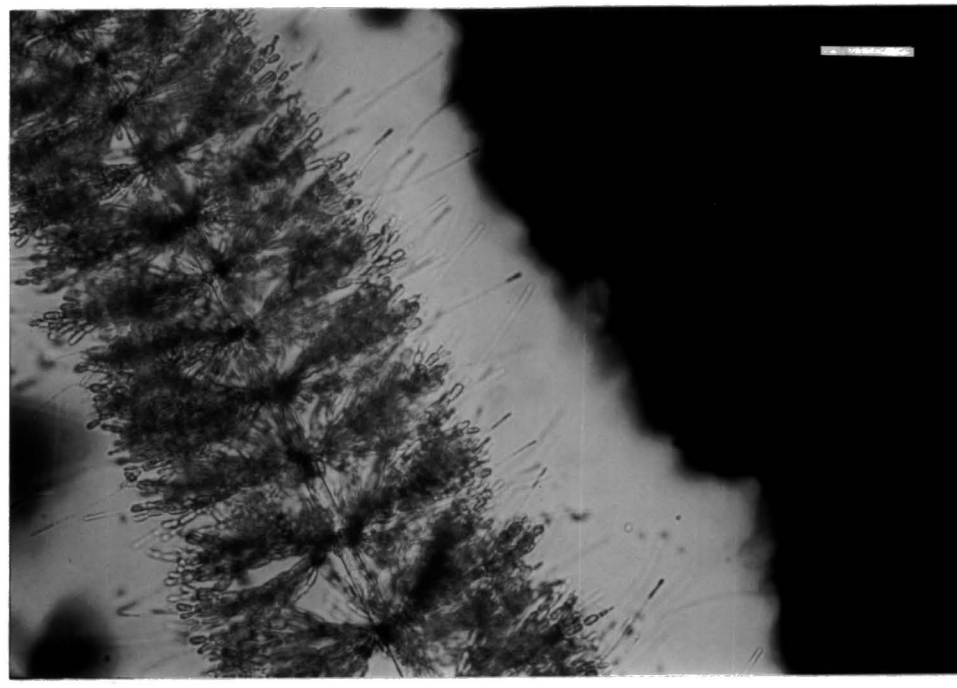

e

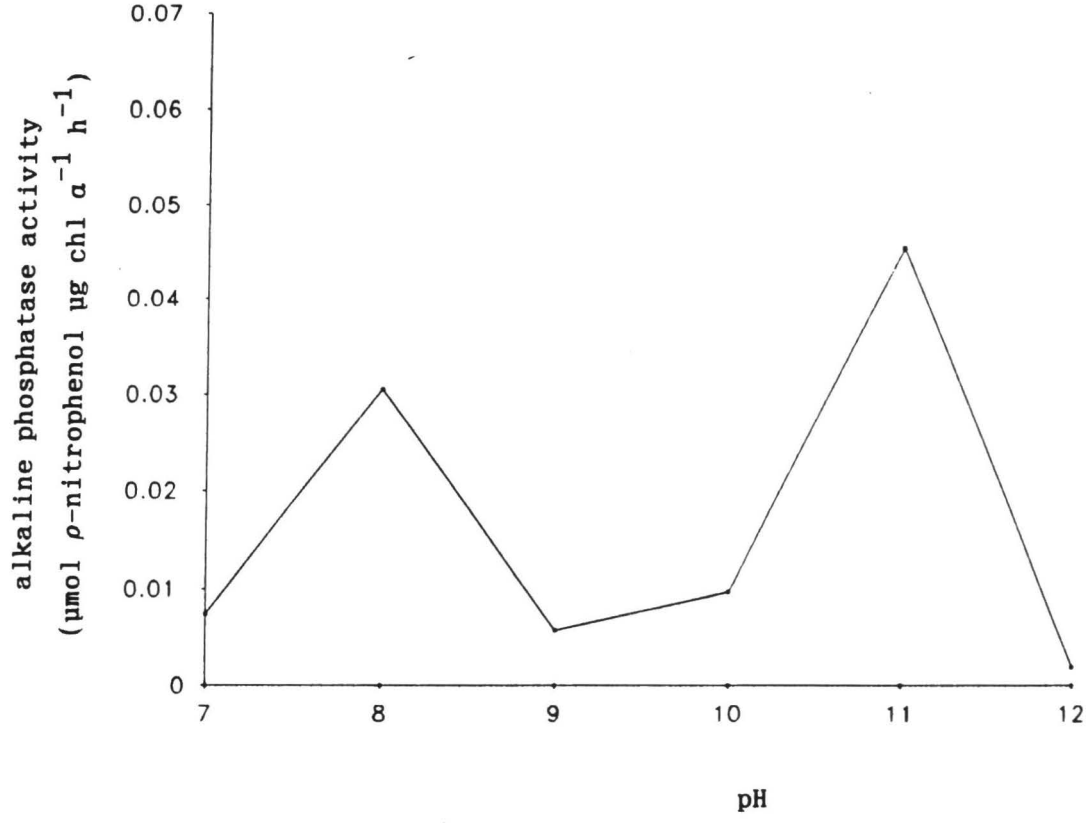
8.3 Alkaline phosphatase activities of other eukaryotes

APA of the *Batrachospermum* and *Lemanea* was assayed over a range of pH (Section 2.9): the response of the former is presented in Fig. 8.2 but in the latter was always below the detection limit. Localization of the APA using the lead capture technique caused some lead precipitation on the surface of the *Lemanea* in both the control and the material treated with β-glycerophosphate; this suggests P may have been present abundant at the surface of the cells, but other ions may have interfered. The alkaline phosphatase activity of the *Ralfsia* was assayed at pH 10.3 (Section 2.9) but could only be estimated as the field spectrophotometer was unreliable. APA was estimated to be in the order of 0.1 µmol *p*-nitrophenol µg Chl a^{-1} h^{-1}.

Fig. 8.1 *Batrachospermum* mounted in India ink to demonstrate extent of hairs. Scale bar = 200 µm.

Fig. 8.2 Influence of pH on APA of a population of *Batrachospermum* (see also Fig. 4.3, 6.4).




alkaline phosphatase activity
(µmol ρ-nitrophenol µg chl α $^{-1}$ h $^{-1}$)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
7
8
9
10
11
12
pH

9. DISCUSSION

9.1 Introduction

Chapters 4 to 8 present the results of investigations into the structure and function of hairs of Chaetophorales which used both natural and cultured material. This chapter discusses the possible functions of hairs.

9.2 Field studies

9.21 General

Environmental data were collected at the same time as algal samples, so statistical relationships between these data and algal morphology could be obscured if the water was subject to marked physical and chemical changes over time periods of less than a few days. All of the sites can encounter very great differences in flow and hence also the concentration of many variables but, as all the samples were collected after periods of relatively constant flow, the values for environmental variables are probably a reasonable indication of the environment in which the alga had been growing.

Strong correlations between the chemical data from the field studies occurred (see Table 4.4), with the two measures of phosphorus (TFP and FRP) particularly closely related. These close intercorrelations made it difficult to separate the nutrient fractions using statistical techniques, such as stepwise linear regression. Indeed, it is not possible to infer any functional relationships between hairs and the environmental variables on the basis of these correlations alone. Although the concentration of FRP is often said to overestimate orthophosphate and the phosphorus available to many algae (Section 1.521) the morphological and physiological variables were better correlated with the concentration of TFP, which was

usually much higher. This suggests the overestimate due to the FRP method may represent a phosphorus fraction different to the fraction released by enzyme hydrolysis.

The strong correlations between hairs and both the algal phosphorus concentration and APA suggest that P availability is the most important factor in hair formation for the populations studied. The algal P concentrations of hairy field populations fall within the ranges found in the laboratory. The ratio of nitrogen to phosphorus within the algae also suggests that P was growth rate-limiting in all the hairy populations; the significance of the algal N : P ratio is discussed in more detail in Section 9.6.

There are indications that hair formation in the field may not always be associated with phosphorus deficiency, such as the lack of phosphatase activity in a population of *Draparnaldia* (Section 4.24). A similar lack of activity was found by B.A. Whitton (pers. comm.) for a hairy *Stigeoclonium* population from a rice field in the Philippines and I. Hawes (pers. comm.) observed a *Stigeoclonium* from Knob Lake, Signy Island, Antarctica which had obvious hairs, but the algal community as a whole was N-limited.

9.22 Trait frequency indices of hairs

The trait frequency indices of hairs (Section 4.25) were much easier to produce than the counts of hairs cells used in the main analyses but showed similar relationships with environmental variables. Such simple indices could be collected and incorporated into an analysis of nutrient status.

9.23 Hot-water-extractable phosphorus and alkalinity

The low number of samples analyzed (Section 4.26, Appendix 2) for hot water extractable phosphorus and alkalinity makes it difficult to draw any

conclusions from the results, but hot-water-extractable phosphorus does not appear to be a good indicator of phosphorus status of the algae sampled. The lack of correlation between hairiness and alkalinity seems to indicate that carbon dioxide was not limiting for the populations studied.

9.3 Morphology

The wide range of variation in morphology of any strain of *Stigeoclonium* was mentioned in Section 1.41. Cultures of members of this genus did show a range of morphological changes over the growth period in batch culture and between cultures in different media (see Chapter 5). The culture medium of Cox & Bold (1966) did not necessarily give an erect system any more like the natural system than material grown in Chu 10-F and the latter medium had nutrient concentrations much closer to the natural systems studied. The morphology of isolates could not really be said to be "normal" for any of the isolates, but for the lowland isolates (D724 & D659) the morphology was not very different in culture to that of the natural populations. The idea of using a defined culture medium and the basal system of *Stigeoclonium* as a basis of classification has its merits, but the medium Cox & Bold (1966) used was not typical of the bulk of fresh waters as it has an N : P ratio of 0.5 (which leads to severe nitrogen limitation) whereas P is more likely to be limiting in most natural waters (Section 1.53).

There were always obvious differences between natural and cultured material but these were not constant. The decrease in branching of *Stigeoclonium tenue* D702 when brought into culture was not seen in *S. tenue* D699, for example. In cultured material traces of pigment were sometimes visible in hairs under the light microscope, but this was very rare for natural material, however fluorescence microscopy indicated that pigmented

material was sometimes present in some hair cells of natural material. Another obvious difference was that the extent of pigmentation was generally greater in natural material. This may have been due to the continuous illumination of cultures. The morphology of colonies of *Chaetophora* and *Draparnaldia* was never like that of the field even though all the cell types of natural material were present in cultured material. Mucilage was only produced near the end of the lag phase in batch culture, when hairs were abundant. The source of mucilage in cultures appeared to be main axis cells, but electron micrographs of natural material indicated that mucilage was produced by cells in the whorls.

The ultrastructure of field material showed obvious differences in cells from different sections of the thalli of *Chaetophora* and *Draparnaldia*. Golgi activity was seen to decrease from the interior to the exterior of the colonies with no golgi apparatus in any of the hairs. The extent of the central vacuole also varied, increasing progressively from the cells of the branches to the first hair cell. The hair cells themselves often had many membranous and lamellar vesicles but lacked plastids, mitochondria and golgi apparatus. Plasmodesmata were always present between cells of the two mucilaginous genera, *Chaetophora* and *Draparnaldia*, but were only present in *Stigeoclonium* with hairs.

Gillespie & Hamilton (1977) reported that lamellar vesicles were often thought to be an artefact of glutaraldehyde fixation, but recent literature indicated that these vesicles were a store of excess membrane material. The excess membrane can be derived either from overproduction or breakdown of material which was no longer required.

9.4 Nutrient depletion

The patterns of hair formation in the deficiency studies were similar to those found by Whitton & Harding (1978) with phosphorus deficiency always leading to the development of multicellular hairs. The hairs produced by nitrogen deficiency were similar to those of P deficient material but lacked the phosphatase activity that was associated with hairs in the field studies. The fact that - N hairs do form to such an extent make the hairs an unreliable indicator of nutrient status without the phosphatase assay. Although hairs were formed under other elemental deficiencies it is unlikely that these would be found in natural environments. Sinclair (1977) pointed out that - Fe hairs in *Calothrix* could be formed as a result of interference with P metabolism, as this treatment gave a low, but detectable surface phosphatase activity.

All the culture strains were capable of continued healthy growth for some while after they started to form hairs in batch culture (Sectionn 6.7) but they did become progressively more unhealthy and eventually died. The decrease in nutrient concentration leading to deficiency in batch culture is unlikely to be found in lotic environments in nature, so hairs must be formed as a response to low nutrient availability, which presumably limits the growth rate. Continuous cultures of D577 and D659 indicated that the hairiness from P-deficiency could be maintained, apparently indefinitely, if the medium had a high N : P composition. Growth under these conditions was relatively rapid but further study will be necessary to determine whether it can be exponential. Technical problems made it difficult to keep the semi-continuous cultures axenic, which limited the duration of the experiments. Unfortunately, standard continuous culture methods are unsuitable for attached algae and until a better system is devised the

growth, uptake and composition of attached algae can not be studied with the same success as that of phytoplankton.

9.41 Addition of nutrients to deficient algae

The influence of adding the missing element to element-deficient cultures requires further investigation, especially when the response is compared to that of field material. All field populations developed zoospores on addition to artificial medium (Section 6.3), though the effect was most pronounced for *Stigeoclonium*. However, as zoospores were also released when field material was put in distilled water it is likely that sporulation could be induced by physical factors alone. These observations are in keeping with those of de Vries *et al.* (1983) for transfer to fresh medium and Agrawal & Sarma (1982) for production by physical factors; the amount of sporulation corresponded very closely to that observed by Agrawal & Sarma. All five culture strains chosen for further study released zoospores on addition of N or P to N- or P-deficient cultures, respectively, and in all cases the response was more marked than under other deficiencies. This, combined with the observations on hair formation, indicates the response to N- and P- limitation can be regarded as a distinct form of differentiation. However a population of *Stigeoclonium* described by Whitton & Harding (1978) did not show a mass release of zoospores, so it is possible that considerable differences exist between populations. Johnstone (1978a) found that calcium-deficient *Draparnaldia* formed zoospores. Whitton & Harding (1978) reported that a hair cell reverted to a vegetative cell upon addition of the missing element, but this phenomenon was never observed in this study.

The term deficiency is often used to describe the condition of algae with hairs, but hairs are formed long before any degeneration occurs in

batch culture. Natural populations with hairs appear healthy and form zoospores when the physical conditions change, whether or not nutrients are supplied (Section 6.3); nutrient deficient populations would show signs of degeneration and would presumably require nutrient additions before reproducing. The close association between hairs and APA in culture and nature indicated that the algal P concentration was sub-optimal when hairs were present.

9.5 External phosphorus concentration

Rosemarin (1982) reported that a population of *Stigeoclonium* was an efficient efficient scavenger of phosphorus at low concentrations. Field populations were often growing in phosphorus concentrations of about 0.005 mg l^{-1} which could be expected from Rosemarin's figures. In the batch cultures grown to P-deficiency in Section 6.7 much of the phosphorus in the medium was depleted before rapid growth started; this pattern was also found by Lean & Nalewajko (1976) for three eukaryotic algae in culture. A rapid drop in the P concentration of the medium occurred in batch cultures of five strains in this study (Section 6.7) but some P was detectable even at the end of the growth period, though in all but one of the five it was only just detectable. One strain (D577) never had an external P concentration below 0.049 mg l^{-1} and the N : P ratio showed only a slight rise with increasing yield. It seems likely that lysis was occurring, as the culture became obviously unhealthy soon after the end of the rapid drop in P. Perhaps some other element, such as Mn, was starting to become limiting. Following their studies on N and P in *Stigeoclonium*, de Vries *et al.* (1985) emphasized the inadequacy of the single limiting nutrient concept. Another explanation of lysis could be viral attack.

9.6 Algal N and P compositions

The N and P composition observed in both the field (Table 4.7) and laboratory studies (Section 6.7, 6.9, 6.10) fell within the range observed by other workers for different algae. This is not surprising as Healey (1982) noted that the P content of all microscopic algae was quite similar. Laboratory values of P content of healthy algae ranged between 6.2% and about 0.2% of dry weight; the lower figure was not always easy to determine as it was difficult to recognize from microscopy an exact stage when a culture could be regarded as unhealthy. Field populations had a narrower and slightly lower P range: 1.78 - 0.097%. The P content at which hairs started to form in culture was between 0.70 - 1.31% and in field materials all populations with P $\leq$ 0.605 had hairs while those with P $\geq$ 0.961 were non-hairy. The slightly higher P at initial hair formation in cultures may have been due to continuous illumination and slightly higher incubation temperatures as these would increase growth rate and photo-oxidation without necessarily increasing the rate of internal P cycling. The N : P ratios in laboratory cultures at the start of hair formation ranged from 5.41 - 7.21 while hairy field populations had N : P ratios of 6.46 or above. The latter is above the critical N : P ratio of 6.2 found by de Vries *et al.* (1983) indicating that hairs in the field populations studied were formed due to P limitation. This agrees with the inferences from the statistical analyses of natural populations (Section 9.21, above).

The algal P content dropped during the growth of strains in the cultures grown to P deficiency. The N content showed a similar trend, although the N : P ratio did increase slightly. This suggests that much of the increase in dry weight was associated with components low in N and P; long hairs are presumably the main such component.

This type of adaptation would fit

the model of Shuter (1979) who postulated that the major chemical constituents of unicellular algae changed in response to nutrient availability (see Section 9.106 also).

9.7 Phosphorus uptake

Although little work was done on uptake in the present study the results of Section 6.10 do indicate that a rapid uptake mechanism exists in phosphorus deficient *Stigeoclonium* and that it is not confined to the hairs. Ultrastructural studies of field *Chaetophora* and *Draparnaldia* (Sections 7.21, 7.31) indicate that hairs can be a route of phosphorus transport but show that mucilage may also have an important role in this. The lack of polyphosphate granules indicates that some other form of P storage may occur, but de Vries & Ouboter (1985) reported the presence of polyphosphate granules in cultured strains of *Stigeoclonium helveticum* but they did not give details of how the material was prepared for examination of the granules.

9.8 Surface phosphatase activity

The field data and the studies of growth to P-deficiency in batch culture both show a marked correlation between the presence of surface phosphatase activity and not only the presence, but also the extent of hairs (Table 4.12, Fig. 6.5). This suggests that hairs themselves may be the main site of phosphatase activity, which fits the observations on field populations of *Chaetophora* and *Draparnaldia* where localized activity is clearly demonstrated (Fig 7.18). However, field materials of *Stigeoclonium* did not show the same marked localization on hairs, so the possibility remains that in at least some *Stigeoclonium* populations it is nothing more that a statistical relationship, with most of the phosphatase activity

on some other part of the thallus. In one population (reach 0096-01) surface phosphatase activity was localized on rhizoid cells (Fig. 7.18).

Electron micrographs of *Chaetophora* and *Draparnaldia* indicated that lead was localized around cells within the mucilage. Such deposits may be an artefact, as mucilage could cause some lead precipitation, but it seems more likely that these deposits indicate phosphatase enzyme produced prior to the final differentiation of branch cells into hair cells.

The response of phosphatase activity of field materials to pH (Fig. 4.2) was quite varied and the interpretation of results must take account of this. Indeed, comparison between sites, and possibly within a site at different times, may not be valid. It is possible that the pH optimum can change with environmental factors as two strains brought into culture had very different optima from algae from the same site, but as the pH optima were not determined at the time of isolation it is not certain that a change occurred. There was little variation in the pH response of all the *Stigeoclonium* strains and both the *Draparnaldia* strains in culture, though the two genera did have different responses: the uniformity may be due to the strong buffering of the medium. Further study will be required to establish whether some sort of adaptation has really taken place in the laboratory and whether it occurs in nature. Anomalous results may be due to shortcomings in the assay procedure.

The utilization of a range of phosphorus sources by three *Stigeoclonium* and two *Draparnaldia* strains (Table 6.2) indicates that some of the organic phosphorus fraction in nature may be available to deficient algae. This fits with the pattern of correlation between algal P and the two phosphorus fractions analyzed in the surrounding water (Table 4.9). The strains studied could not utilize phytate in contrast to *Calothrix parietina* from an upland stream (Livingstone *et al.*, 1983) and all but one of 11 strains

Anabaena isolated from a rice field in Iraq (Al-Mousawi, 1984). Lecithin was also not utilized, again this contrasts with the results of Al-Mousawi (1984) who found that all the strains he tested could utilize this substrate; this could help to explain some differences in the distribution of the these algae. Further study is needed to determine whether more than one enzyme is involved in the hydrolysis.

9.9 Suggested hair functions

Many functions have been suggested for hairs in all algal groups (Section 1.3); the possible functions of hairs of Chaetophorales are discussed in relation to the results of this study.

9.91 Senescence

The lack of apparent pigmentation and occurrence of hairs in old cultures has lead to the suggestion that they are merely dead or dying cells (Section 1.34) In this study hairs were most abundant in old cultures and electron micrographs of hair cells (Section 7.21, 7.31) did not reveal any obvious microstructures which could refute such an argument, indeed, the presence of lamellar vesicles in hairs could be taken as a sign of degeneration as they have in many species of plant (Gillespie & Hamilton, 1977) There is also evidence of resistant stages forming in old *Stigeoclonium* cultures (Section 5.53) and cells the near the basal region had much starch (Fig. 7.17). A number of findings undermine the argument that hairs are simply dying cells. Whitton & Harding (1978) pointed out that the length of hairs of *S. tenue* often greatly exceeded that of the remainder of each branch making it seem unlikely that they arose simply from degeneration. In batch cultures hairs were found to form at an algal P concentration much higher that that at which obvious signs of

degeneration occurred and natural material formed zoospores when placed in distilled water upon arrival in the laboratory. Also hairs did not necessarily occur at the end of every filament in any strain studied and this was especially noticeable in *Chaetophora* and *Draparnaldia*. A functional role is implied for some populations as hairs were found to be a site of localized phosphatase activity in some *Chaetophora* and *Draparnaldia*. Finally, it is obvious that natural populations of Chaetophorales do grow (i.e. increase in biomass) and hairs increase in length due to reproduction of the meristematic cells further down the filament.

9.92 Shielding of light

The suggestion that hairs function as a light shielding mechanism was made as long ago as 1882 (Berthold), arising from observations of increased hairiness in high light intensities. A non-pigmented light shield would not seem ideal but the hairs could be opaque to ultraviolet light; however these wavelengths do not penetrate very far through water. In the study of natural populations two of the lowland sites (0025-85, 0142-50) were quite shallow and these would have had longer periods of illumination than upland sites (Section 3.2). Hairs were never present at either of these sites so if hairs do shield light other factors must also influence their presence. It is possible that U.V. light was screened from the lowland sites by organic pollutants which would not have been present at upland sites.

9.93 Secretion

Few ultrastructural studies of Chaetophorales have been published, but Cook (1970) found high golgi activity in a population of *Draparnaldia* and suggested hairs might exchange materials with the exterior. The hairs do

not appear to secrete mucilage, as most cells of each hair protrude from the mucilage (Fig 5.2, for example). The electron micrographs of *Chaetophora* and *Draparnaldia* do not give any indication of golgi activity in the hairs but do show areas of activity in vegetative cells near the centre of the colonies. It is possible that some secretions could be transported along the filament and this could explain the presence of membranous vesicles in some hair cells and the obvious plasmodesmata between many of the cells. As older, nutrient limited populations of other algae have been shown to release organic substances (Fogg, 1962) the hairs could be performing this function; unfortunately, this possibility was not investigated in the present study.

9.94 Uptake

The apparent suitability of the hairs for uptake and the evidence supporting such a function was mentioned in Section 1.311. Hairs can have a physical advantage due to their aspect in relation to water flow and their increased surface area to volume ratio as compared to vegetative cells. In *Stigeoclonium* the reduction in radius compared to vegetative cells does give a marked increase in SA/V, but the aspect of the cells is similar to the aspect of all the other cells in the filament. In *Chaetophora* and *Draparnaldia* the reverse was often the case as many populations had hairs which tapered only slightly but the mucilage ensured that at least some of the hairs were nearly perpendicular to the flow of the surrounding water. In the mucilaginous genera the surface area of the hairs was probably much greater than that of the surface of the mucilage.

The production of hairs under diminished nutrient conditions (Section 6.6, 1.3) also supports an uptake function as increased uptake capabilities

would bring obvious advantages under such conditions. Specialized cells could allow the most efficient use of limited resources.

There was no evidence of any uptake specific to hairs, for example, the uptake of neutral red was not noticeably greater in hair cells in the present study and certainly did not match the results of Gibor (1973) for *Acetabularia* (Section 1.312). The rapid uptake mechanism present in hairy algae was not confined to the hairs, this is similar to the findings of DeBoer & Whoriskey (1984) for *Ceramium rubrum*. Unfortunately, attempts at removal of the hairs were not successful; this would have made studies of their role much easier.

That hairs can be a route for P uptake is confirmed by the electron microscopic studies (Section 7.22, 7.23) for both *Chaetophora* and *Draparnaldia*, and the mucilage also appears to be very important in such uptake. The high concentrations of external P necessary to allow detection could have given an unnatural result for all the uptake studies; the use of continuous cultures with low nutrient concentrations and radioactive tracers could help elucidate uptake pathways.

9.95 Alkaline phosphatase localization

The advantage gained from the utilization of organic phosphorus was obvious in many of the field sites studied (Section 6.21). In *Chaetophora* and *Draparnaldia* surface phosphatase activity on the hairs could be very advantageous due to their high surface area and favourable aspect to flow and the fact that many organic molecules might not penetrate the mucilage, or be very slow to diffuse through it. In these two genera the hairs were always within a relatively short distance from the main axis but in *Stigeoclonium* that was not always the case.

The striking localization on the hairs of *Chaetophora* and *Draparnaldia* under the light microscope (Fig 7.5) may be an artefact of the staining technique as there was obvious lead deposition around cells near the edge of the mucilage when examined under the electron microscope; this would suggest that the NH_4S does not penetrate the mucilage. The presence of activity in cells near the edge of the mucilage was not unexpected as the cells differentiate into hairs when still within the mucilage. It seems plausible that phosphatase enzymes are produced and extruded prior to the completion of differentiation of a cell into a hair cell and the apparent limitation of phosphatase activity to cells near the edge of the mucilage support this hypothesis.

As fixed material released PO_4^{3-} from organic substrates the reaction could be a source of energy for maintenance of the concentration gradient across the membrane and possibly for other uses. If the energy released were available to the cell it would help to explain how the membranes of hairs could remain intact without any obvious mitochondrial presence. Once inside the cell P could be transported by diffusion along the cells as long as the gradient across the membrane was maintained. Conversion to an ionically inactive form would reduce the energy needed to maintain a gradient accross the membrane; the vesicles seen in many hair cells could be involved in such transport. Brassard & Auclair (1984) discussed the possiblity of coupled transport between alkaline phosphatase and PO_4^{3-} uptake in natural populations of phytoplankton.

9.96 Hairs as an adaptation to deficiencies

There is strong evidence that hairs play an important part in adaptation to low nutrient conditions. The drop in algal N and P content during batch culture (Section 6.7) indicates that hairs may be low in compounds

containing these two elements. This type of adaptation is similar to the model for phytoplankton proposed by Shuter (1979), with the major chemical constituents of cells changing in response to nutrient availability. The high vacuolation and presence of lamellar vesicles may be indicative of such a change in constituents.

9.10 Chaetophorales and Rivulariaceae

There are a number of striking parallels between Chaetophorales and Rivulariaceae with multicellular hairs. The longest and most typical hairs in both cases form under conditions of P-limitation. Changes in the morphology of cultured *Calothrix parietina* (Livingstone & Whitton, 1983) took place at similar P compositions to those of the Chaetophorales reported here. The range of P was from about 5 - 0.25% for the *C. parietina* and about 4 - 0.2% for the Chaetophorales, with hairs forming at about 1% P in both groups. The addition of P to P-deficient cultures lead to the loss of hairs and release of motile reproductive structures. In *Calothrix* the motile spores are produced in a defined region- between the terminal portion of the trichome (filament) at the base of the hair and the separation disc. This is not true in *Stigeoclonium*: the spores can be released initially from almost anywhere along the erect filament, and release spreads both apically and distally. In both groups the hairs drop off but in the *Calothrix* this is before the release of the spore whereas in *Stigeoclonium* it is after zoospore release.

9.11 Conclusions

Section 1.9 outlined the aims of this study and proposed five questions which the research was designed to answer, the extent to which each question was answered is presented here.

Is the presence of hairs in nature correlated with a specific chemical deficiency in nature? Correlations between hairs and both nitrogen and phosphorus fractions in the environment were demonstrated in Section 4.22. It was not possible to demonstrate which factor or factors lead to hair formation on the basis of these correlations alone but other evidence indicated that hairs were caused by P limitation.

Is the formation of hairs related to the internal nutrient concentration of the algae? In natural populations the presence of hairs was negatively correlated with the algal P content but not with algal N content. The algal P content at which hair formation started in cultures grown to P deficiency was similar to the highest algal P content of hairy natural populations. The observations of other workers have suggested this may not be true for all natural populations.

Is the relationship between alkaline phosphatase activity and the hairs of blue-green algae found for members of the Chaetophoraceae? The presence and amount of hair formation was positively correlated with alkaline phosphatase activity in the natural populations studied and P deficiency in culture also leads to both alkaline phosphatase activity and hair formation. Alkaline phosphatase activity was localized on the hairs of *Chaetophora* and *Draparnaldia*, but this was not the case for *Stigeoclonium*. The findings for *Chaetophora* and *Draparnaldia* were similar to those reported for hair-forming members of the *Rivulariaceae* (Section 9.11).

Can the presence or absence of hairs of Chaetophoraceae be used as an indicator of the nutrient status of an environment? On the basis of the

results of the present study it appears that hair presence in some members of the Chaetophoraceae indicates P limitation, but hairs can be formed by N and Fe limitation in culture and there is some evidence that N limitation may lead to hair formation in natural populations (Section 9.21). Further study is necessary to determine whether hair presence can be a reliable indicator of a particular nutrient but such presence does appear to indicate nutrient limitation. The absence of hairs appears to indicate that nutrients are not limiting.

Do hairs have any unique functions? The hairs of *Chaetophora* and *Draparnaldia* were found to be localized sites of surface phosphatase activity and were involved in the transport of phosphorus; no unique function was demonstrated for the hairs of *Stigeoclonium*. The change in algal N and P content and the presence of lamellar vesicles in hairs suggests they are an important adaptation to nutrient limitation allowing continued growth in sub-optimal nutrient conditions.

SUMMARY

A) A study was carried out on the influence of physical and chemical factors on hair formation by three genera of Chaetophorales: *Stigeoclonium*, *Chaetophora*, *Draparnaldia*.

b) Statistical analyses of 32 samples from 15 populations indicated that the extent of hair formation was correlated with both physical and chemical environmental factors, including conductivity, filtrable reactive phosphorus (FRP) and total filtrable phosphorus (TFP); hair formation was also correlated with algal P and the N : P ratio. Algal P and the N : P ratio were correlated with conductivity, NO_3-N , FRP and TFP, but algal N was not. All hairy populations had a P content $\leq$ 0.605% dry weight; all non-hairy populations had a P content of $\geq$0.961% dry weight. The N : P ratio ranged from 6.46 - 35.6 for hairy populations and from 3.65 - 5.58 for non-hairy populations.

c) Alkaline phosphatase activity (APA) was correlated with NO_3-N, FRP and TFP in the water and with algal N, P and the N : P ratio. Alkaline phosphatase was also highly correlated with % hairiness and % terminal hairs. A lead capture technique indicated that APA was localized on the hairs of *Chaetophora* and *Draparnaldia*. APA was localized in the basal region of *Stigeoclonium*, on one occasion being localized on rhizoid cells.

d) The pH optima of phosphatase activity for six natural populations ranged from seven or below to twelve or above. In three strains of *Stigeoclonium* grown in culture at pH 7.5 the pH optimum of phosphatase

activity was always about ten; the influence of pH on the phosphatase activity of two strains of *Draparnaldia* was less pronounced.

e) Elemental deficiencies produced a similar pattern for the extent of hair formation in 13 strains: - P > - N > - Fe = - S > - Ca > - Mg > control. The response when judged by yield was similar for all strains but differed from that for hairs: control > - P > - Fe > - Ca = - S > - Mg > - N. Only N- and P- limited cultures had typical hairs, and hair development was much greater in the former. Only P-limited cultures had detectable APA. Addition of the missing element to three N- and P-limited strains of *Stigeoclonium* gave rise to considerable zoospore release, a few zoospores were produced when the missing element was added to the other deficiencies, with Fe-limited cultures giving the most. Zoospore formation also occurred when the missing element was added to two strains of *Draparnaldia*, again with P- and N-limited cultures producing the most; the production was much less than in *Stigeoclonium* with only the cells of the lateral whorls of branches producing zoospores. Zoospore production destroyed the integrity of the filament causing the hairs to drop off, but some hairs remained in all five strains up to five days after the addition of the missing element.

f) Natural populations of *Stigeoclonium*, *Chaetophora* and *Draparnaldia* all released zoospores when incubated in Chu 10-F medium or distilled water in the laboratory; almost the entire erect system of *Stigeoclonium* disappeared within 24 h of incubation, but *Chaetophora* and *Draparnaldia* remained as intact colonies for at least five days.

g) N and P compositions, % hairiness and surface phosphatase activity were followed in three strains of *Stigeoclonium* (D577, D699, D779) and two

strains of *Draparnaldia* (D651, D653) grown to P-deficiency in batch culture. Hairs started to form when algal P was in the range 0.70 - 1.31 % dry weight, long before the material had ceased to grow or become unhealthy; the corresponding N : P ratio was 5.41 - 7.21 (by weight). The algae died when the P content dropped to about 0.2 % dry wt, but one strain of *Stigeoclonium* formed resistant spores.

h) The morphology of *Stigeoclonium* cultures was not always normal in culture. The structure of *Draparnaldia* in culture was not like that of natural colonies, but all the cell types did occur. Mucilage was only produced in old cultures of *Draparnaldia*.

i) The ability of five strains (D577, D651, D653, D699, D779) to utilize organic phosphorus compounds as the sole source of phosphorus was tested, all were capable of growth with a number of organic-P compounds. β-glycerophosphate, glucose-6-phosphate, polyphosphate, and pyrophosphate were utilized by all five strains; DNA was utilized by four strains, but lecithin and phytate could not be utilized by any of the strains.

j) Ultrastructual studies were carried out on natural populations of *Stigeoclonium*, *Chaetophora* and *Draparnaldia*. The contents of cells was variable *in Chaetophora* and *Draparnaldia*, being dependent on the position within the thallus. Cells of the main axis had a thin ring of cytoplasm containing chloroplasts and other plastids, the vacuole of these cells contained many vesicles. High golgi activity was apparent in cells of the branches near the main axis, but such activity decreased with increasing distance from the main axis; the extent of vacuolation increased near the edge of the colony and lammelar vesicles where present in many cells near

the edge of the mucilage. Hair cells were highly vacuolated and contained many vesicles, including lamellar vesicles. Plasmodesmata were present between cells from all parts of the thallus. The cells of the erect filaments of *Stigeoclonium* had typical green algal structure, but plasmodesmata were only present between the cells of hairy samples.

k) Laboratory studies of two strains of *Stigeoclonium* (D577, D659) indicated that a rapid uptake mechanism was present in P-limited material, but this was not confined to the hairs. There was no evidence of luxury consumption when P was added to P-limited material. Ultrastructural studies indicated that P was taken up through the hairs and mucilage of *Chaetophora* and *Draparnaldia*.

l) The possible functions of hairs and their role in adaptation is discussed. Parallels between hair forming blue-green algae and hair forming Chaetophorales are also discussed.

APPENDIX 1. $BBMPTB_{12}$

Cox & Bold (1966) presented a revision of the genus *Stigeoclonium* based on studies of isolates grown in a standard medium. The medium used was a modification of Bold's Basal Medium which contained vitamin B_{12} and tris-(hydroxymethyl)aminomethane (TRIS) buffer; they used the initials $BBMPTB_{12}$ to describe the medium.

The constituents of $BBMTB_{12}$ are:

salt	concentration mg l^{-1}	element	concentration mg l^{-1}
$NaNO_3$	255.0	B	2.00
KH_2PO_4	136.1	Ca	8.02
K_2HPO_4	104.5	Cl	28.82
$MgSO_4.7H_2O$	73.95	Co	0.099
$CaCl_2.2H_2O$	29.40	Cu	0.0063
NaCl	23.4	EDTA	326.22
TRIS	60.57	Fe	1.00
Na_2EDTA	50.0	K	107.51
KOH	31.0	Mg	7.29
$FeSO_4.7H_2O$	4.98	Mn	0.36
H_2SO_4	1.0	Mo	0.47
H_3BO_3	11.42	N	42.25
$ZnSO_4.7H_2O$	8.82	Na	84.35
$MnCl_2.4H_2O$	1.44	P	49.56
MoO_3	0.71	S	11.71
$CuSO_4.5H_2O$	1.57	Zn	2.01
$Co(NO_3)_2.6H_2O$	0.49		
B_{12}	20 µM		

The N : P ratio of this medium is 0.85; this would lead to severe N-deficiency if nutrients were limiting.

APPENDIX 2. TRAIT FREQUENCY INDICES AND CARBONATE SPECIES

The trait frequency indices of hair formation (Section 2.5112, 4.25), hot-water-extractable phosphorus (Section 2.76, 4.26) and carbonate species (Section 4.26) for the sites in Table 4.2 are listed below. The carbonate species were calculated from alkalinity, conductivity, pH and temperature according to Mackereth *et al.* (1978).

sample number	trait frequency indices		HWEP : algal P	total-CO_2 mmol l^{-1}	free-CO_2 mmol l^{-1}	HCO_3^- mmol l^{-1}	CO_3^{2-} mmol l^{-1}
	hairs	branching					
1	7	7	-	-	-	-	-
2	0	7	0.247	55.0	1.1	54.1	0.31
3	6	5	0.082	2.4	2.11	0.29	<0.001
4	7	7	0.109	5.93	5.57	0.36	<0.001
5	7	6	0.061	51.3	2.41	48.7	0.11
6	0	6	0.87	47.8	1.2	46.5	0.21
7	0	6	0.034	27.2	0.76	26.3	0.10
8	0	6	0.017	23.6	0.52	23.0	0.12
9	10	5	-	-	-	-	-
10	10	6	0.052	13.3	0.79	12.4	0.002
11	10	5	0.039	12.7	0.54	12.2	0.31
12	9	6	0.13	33.0	1.13	31.8	0.10

13	10	6	-	-	-	-	-
14	10	6	-	-	-	-	-
15	5	4	-	-	-	-	-
16	6	5	0.076	29.1	0.36	28.5	0.26
17	5	7	0.019	30.2	0.44	29.5	0.23
18	8	7	0.076	30.1	0.48	29.4	0.21
19	7	5	-	-	-	-	-
20	7	5	0.081	24.8	0.21	24.2	0.33
21	5	8	0.76	18.7	0.24	18.3	0.16
22	7	6	0.45	14.1	1.73	12.4	0.010
23	0	6	-	-	-	-	-
24	0	6	-	-	-	-	-
25	10	5	-	-	-	-	-
26	6	5	0.08	23.9	0.42	23.3	0.15
27	5	6	-	-	-	-	-
28	6	6	0.039	25.7	1.97	33.7	0.066
29	4	6	0.096	31.3	0.73	30.4	0.14
30	5	5	0.087	3.38	3.02	0.36	<0.001
31	0	6	-	21.7	1.24	20.4	0.04
32	8	6	0.26	40.0	0.95	38.9	0.18

APPENDIX 3. GROWTH IN BATCH CULTURE

The data plotted in Fig. 6.5 are presented here in tabular form.

day	yield ($g\ l^{-1}$)	algal N (%dry wt)	algal P (%dry wt)	N : P	media N ($mg\ l^{-1}$)	media P ($mg\ l^{-1}$)	% hairs	phosphatase activity
Stigeoclonium tenue D577								
6	4.8	5.04	1.42	3.54	15.6	0.415		
8	33.6	4.70	0.905	5.19	14.3	0.224		
10	102	2.89	0.498	5.81	13.8	0.076	0	0.002
12	142	1.76	0.350	5.02	12.5	0.049	4	0.014
14	169	1.32	0.262	5.05	12.2	0.077	8	0.021
18	189	1.44	0.234	6.04	12.4	0.086	13	0.043
Stigeoclonium tenue D699								
8	0.54	12.3	6.27	3.94	14.9	0.494		
10	2.4	5.26	2.15	4.88	15.1	0.470		
12	16.6	4.42	1.39	6.36	13.4	0.280	0	<0.001
13	47.4	3.53	0.886	7.96	12.6	0.017	2	0.005
15	84.0	3.99	0.481	16.4	-	-	8	0.065
16	78.6	3.83	0.650	11.8	-	0.004	17	0.012
17	81.0	4.49	0.537	16.7	13.2	0.005	17	0.011
Stigeoclonium tenue D779								
6	1.20	13.88	1.89	7.35	16.4	0.51		
8	4.54	8.70	1.22	7.10	16.8	0.415	0	<0.001
9	21.6	5.70	0.743	7.67	14.7	0.285		
10	25.3	6.80	0.991	6.86	13.5	0.197	1	0.004
11	48.2	5.94	0.760	7.82	12.3	0.076		
12	50.4	5.90	0.757	7.80	12.1	0.039	3	0.010
14	73.4	4.24	0.523	8.10	12.0	0.069	13	0.021
16	98.2	4.12	0.491	8.40	10.8	0.005	-	0.037
18	107	4.12	0.370	11.12	-	0.004		
22	171	2.50	0.233	10.7	10.3	0.006		
30	192	2.36	0.204	11.6	-	0.004	-	0.024
Draparnaldia sp. D651								
6	2.47	10.72	2.29	4.68	14.5	0.454		
7	6.87	7.72	1.49	5.19	14.6	0.430		
8	9.53	7.98	1.42	5.61	13.9	0.401	0	<0.001
10	41.8	5.48	1.17	5.00	12.9	0.015	<1	0.015
11	59.8	6.00	0.801	7.49	12.5	0.005	1	0.025
12	67.3	5.98	0.677	8.83	11.3	0.006	3	0.020
13	81.3	4.74	0.571	8.30	7.3	0.006	3	0.034
17	171	3.66	0.284	12.9	8.6	0.005	10	0.11
Draparnaldia plumosa D653								
10	1.0	8.40	4.64	3.62	14.1	0.505	0	<0.001
12	2.66	3.06	1.09	5.61	14.7	0.420	0	0.010
14	11.8	2.90	0.991	5.85	14.7	0.348	2	0.030
15	17.0	2.24	0.819	5.47	14.2	0.337		
16	36.4	2.80	0.861	6.50	13.2	0.206	3	0.14
17	39.0	1.58	0.903	3.50	12.5	0.038		
18	61.4	2.49	0.606	8.21	-	0.007	5	0.23
22	105	2.13	0.371	11.5	10.7	0.004		
30	207	1.43	0.168	17.0	-	0.005	14	0.34

APPENDIX 4. USE OF THE AZO-DYE TECHNIQUE

In Chapter 7 a lead capture technique was used to indicate localized phosphatase activity; this technique was effective but could obscure the structures being stained (Fig. 7.18e, for example). An azo-dye technique has been used to localize phosphatase activity in *Plectonema boryanum* (Doonan & Jensen, 1977) and was amended by Livingstone *et al.* (1983) to indicate surface phosphatase activity on *Calothrix parietina* D550.

The principle of the technique is simple: phosphate is cleaved from a naphthol -phosphate compound by phosphatase activity, leaving the insoluble naphthol group at the site of enzyme activity, the naphtol then reacts with a diazonium salt producing an insoluble azo-dye. The technique was carried out on the material listed in Table A4.1 using the methods below.

Table A4.1 Algae stained with azo-dye to indicate phosphatase activity.

strain/population	comments
Calothrix parietina D550	used by Livingstone *et al.* (1983), culture grown to P-deficiency
Chaetophora incrassata	natural population
Stigeoclonium tenue D577	axenic culture, grown to P deficiency
Draparnaldia sp. D651	axenic culture, grown to P deficiency

Methods

Fresh material was fixed using one of two protocols:

1) Material was incubated for 1 h in 3% glutaraldehyde buffered with 0.01M cacodylate (pH 7.4). The material was then washed overnight in cacodylate

buffer and rinsed briefly in 5mM HEPES (pH 8.2, 4°C) immediately prior to incubation.

2) Material was incubated in chromic acid - acetic acid for for 1h and washed overnight in 5 mM HEPES (pH 8.2, 4°C). The recipe for the chromic acid was:

chromic acid (1% chromium trioxide)	20 ml
acetic acid 10%	10 ml
formalin	5 ml
water	65 ml.

Two staining procedures were used, one produced the dye in one step, the other in two (post-coupling).

1) 5.0 mg naphtol AS-BI phosphate (SIGMA Chemical Co.) was dissolved in 0.25 ml dimethyl sulphoxide and diluted with 25 ml distilled water. 25 ml of 5 mM HEPES buffer (pH 8.2) was added to the solution, followed by 30 mg *p*-nitrobenzene diazonium tetrafluoroborate. The reaction mixture was stirred until all the components were in solution, 0.1 ml 10% $MnCl_{2\ (aq.)}$ was added as an activator. Algal material was incubated in the solution for 1.5 - 3 h at room temperature.

2) In the post-coupling procedure *p*-nitrobenzene diazonium tetrafluoroborate was omitted from the reaction solution. After incubation the modified solution algal material was incubated in 0.1 M cacodylate buffer (pH 7.0) containing 600 mg l^{-1} *p*-nitrobenzene diazonium tetrafluoroborate.

After incubation material was washed in distilled water and viewed under the light microscope.

Results

The dye was localized around hairs of *Calothrix parietina* D550 (Fig. A4.1); this confirmed the findings of Livingstone *et al.* (1983). Localization of the stain was not apparent around the hairs of any of the Chaetophorales (*Chaetophora incrassata*, *Stigeoclonium tenue* D577 and *Draparnaldia* sp. D651), but stain penetrated the cells and was associated with chloroplasts (Fig. A4.2); the results were similar for material fixed in either glutaraldehyde or chromic acid - acetic acid.

Fig. A4.2 Phosphorus deficient *Calothrix parietina* D550 stained with azo-dye to indicate localization of alkaline phosphatase activity. Note concentration of pink colour around hairs.
Scale bar = 100 µm.

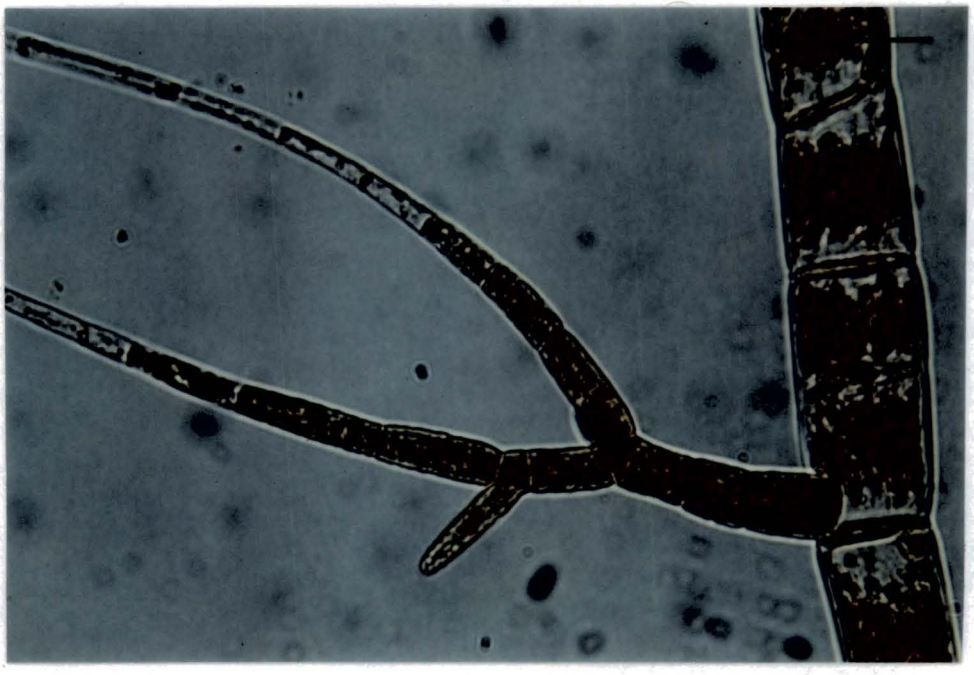


Fig. A4.2 Phosphorus deficient *Draparnaldia* sp. D651 stained with azo-dye as above. Note pink colouration of internal strucutres.
Scale bar = 20 µm.

APPENDIX 5. CULTURE COLLECTION RECORDS

This appendix presents the data recorded for each strain on the Durham Culture Collection database. The records are presented in the standard format for output from the database.

D0565 STIGEOCLONIUM SP.
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
ENGLAND		J.P.C.HARDING	0096-01
Culture source:	Date:	Sender:	Isolated by:

Axenic on: 22/02/83 and clonal on 22/02/83 by M.T.GIBSON
Growth: temp 15 light medium CHU 10D
Notes:

D0566 STIGEOCLONIUM SP.
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
BELGIUM		B.A.WHITTON	1002-25
Culture source:	Date:	Sender:	Isolated by:

Axenic on: not and clonal on not by
Growth: temp 15 light medium CHU 10D
Notes:

D0567 STIGEOCLONIUM SP.
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
GERMANY		B.A.WHITTON	4014-70
Culture source:	Date:	Sender:	Isolated by:

Axenic on: not and clonal on not by
Growth: temp 15 light medium CHU 10D
Notes: Possibly from 0014-70 but more likely as above.

D0575 STIGEOCLONIUM SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
ENGLAND J.P.C.HARDING 0008-30
Culture source: Date: Sender: Isolated by:

Axenic on: not and clonal on not by
Growth: temp 15 light medium CHU 10D
Notes:

D0576 STIGEOCLONIUM SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
ENGLAND J.P.C.HARDING 0014-70
Culture source: Date: Sender: Isolated by:

Axenic on: not and clonal on not by
Growth: temp 15 light medium CHU 10D
Notes:

D0577 STIGEOCLONIUM SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
ENGLAND B.A.WHITTON 0001-01
Culture source: Date: Sender: Isolated by:

Axenic on: 11/11/83 and clonal on 11/11/83 by M.T.GIBSON
Growth: temp 20 light 60 medium CHU 10 D + HEPES pH7.5
Notes:

D0651 DRAPARNALDIA SP.
Strain: Repeat isolates:
Country found: Date: Finder: Details of site:
ENGLAND 00/06/81 C.M.OWEN 0212-49
Culture source: Date: Sender: Isolated by:
M.T.GIBSON
Axenic on: 11/11/83 and clonal on 12/01/83 by M.T.GIBSON
Growth: temp 25 light medium CHU10D + HEPES (7.5)
Notes:

D0652 CHAETOPHORA SP.
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
ENGLAND	11/10/82	M.T.GIBSON	CROFT KETTLE
Culture source:	Date:	Sender:	Isolated by: M.T.GIBSON

Axenic on: not and clonal on 03/11/85 by M.T.GIBSON
Growth: temp 25 light medium NOT KNOWN YET
Notes:

D0653 DRAPARNALDIA GLOMERATA (VAUCH.) C.A.AGARDH
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
ENGLAND	07/11/82	J.D.WEHR	0015-18
Culture source:	Date:	Sender:	Isolated by: M.T.GIBSON

Axenic on: 11/11/83 and clonal on 07/11/82 by M.T.GIBSON
Growth: temp 25 light medium CHU 10 D + HEPES pH7.5
Notes:

D0659 STIGEOCLONIUM SP.
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
GERMANY		B.A.WHITTON	4001-60
Culture source:	Date:	Sender:	Isolated by: J.P.C.HARDING

Axenic on: 11/11/83 and clonal on 11/11/83 by M.T.GIBSON
Growth: temp 25 light medium CHU 10D + HEPES pH7.5
Notes: Harding J.P.C. & Whitton B.A. 1976 Br. phycol. J. 11: 417-426 (25839/56444) pH 7.5; Zn(0.77), Cd(0.002), Pb(0.005).

D0699 STIGEOCLONIUM SP.
Strain: Repeat isolates:

Country found:	Date:	Finder:	Details of site:
ENGLAND	09/08/84	M.T.GIBSON	0048-11
Culture source:	Date:	Sender:	Isolated by: M.T.GIBSON

Axenic on: 06/02/85 and clonal on 03/12/84 by M.T.GIBSON
Growth: temp 20 light 100 medium CHU 10 D + HEPES pH7.5
Notes:

D0779 STIGEOCLONIUM SP.

Strain:		Repeat isolates:	
Country found:	Date:	Finder:	Details of site:
ENGLAND	01/04/85	M.T.GIBSON	0376-65
Culture source:	Date:	Sender:	Isolated by:

Axenic on: 23/04/85 and clonal on 23/04/85 by M.T.GIBSON
Growth: temp 20 light medium CHU10 D + HEPES pH7.5
Notes: No hairs in field

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