Ecological role of surface phosphatase activities of Rivulariaceae

Julia M. Yelloly

Ecological role of surface phosphatase activities of Rivulariaceae

by

Julia M. Yelloly

B.Sc. University of Ulster (Coleraine)

A thesis submitted for the degree of Doctor of Philosophy in the University of Durham, England

Department of Biological Sciences

February 1996

This thesis is entirely my own work and has not previously been submitted for any other degree.

Julia Yelloly

February 1996

Ecological role of surface phosphatase activities of Rivulariaceae

A thesis submitted by Julia Yelloly for the degree of Doctor of Philosophy in the University of Durham, England, February 1996

ABSTRACT

The literature suggests that the cyanobacterium *Rivularia* is found at sites where organic phosphorus (P), at times forms a high proportion of total P in the environment. Its ability to utilise organic P through "surface" phosphatase activity may be important in its success. The aim of this thesis was to investigate this in detail using both field material (from a freshwater stream and from a marine intertidal zone) and axenic isolates of Rivulariaceae.

At both sites inorganic P concentrations peaked: in March/May (1992-4) at the freshwater site, and in June (1992-3) at the marine site (although in 5 of the pools containing *Rivularia*, inorganic P was maximal in February/March 1993). Pools associated with rotting seaweed had higher concentrations of inorganic P (which made up most of the total P) during peaks. It is likely that the high tide resulted in the mixing of weed pool water with *Rivularia* pool water, slightly lower down the eulittoral zone, and also influenced the retreating seawater. Organic P was a greater proportion of total P in the pools containing *Rivularia* and was found to increase in these pools during the tidal cycle, suggesting internal generation. At the freshwater site organic P concentrations were higher in pools associated with peat than in stream water. At the freshwater site phosphorus fractions were often below detection limits, but combined nitrogen was rarely this low; the reverse was the case at the marine site.

At the freshwater site phosphomonoesterase activity of *Rivularia* was generally high, except when hormogonia were present in the colonies. At the marine site, phosphatase activity was usually low, with a peak using p-nitrophenyl phosphate (pNPP) as a substrate in July/August and, using 4-methylumbelliferyl phosphate (4-MUP) as a substrate in September/October, especially in 1992. Phosphatase activity of *Rivularia* at both sites was negatively influenced by inorganic P and positively correlated with the presence of hairs.

K_m (Michaelis-Menten constant) was lower using 4-MUP than pNPP in all organisms assayed. Apparent negative cooperativity was found in 7-day cultures of *Calothrix parietina* D550 using 4-MUP and in whole colonies of freshwater *Rivularia* using pNPP.

These results were discussed with reference to the relationship between nitrogen (N) and P at the sites and the enzyme kinetics of field organisms and axenic isolates. *Rivularia* is successful in these apparently different environments probably because organic P can be utilised when phosphorus is limiting and colonies are able to fix N_2 when N is limiting. Seasonal peaks in inorganic P probably allow regeneration of the *Rivularia* population.

ACKNOWLEDGEMENTS

Thanks to my supervisor, Dr Brian Whitton, for thinking of the project, interesting discussions, encouragement and algal identification. Thanks also to Drs Martyn Kelly and Ignacio Hernández for help with statistics, advice and support. I am grateful to Prof. P. R. Evans for providing research facilities; to Drs Marta Evans, Amit Gupta, Abdulrahman Al-Shehri and Alan Donaldson for teaching culture and assay techniques; to Catherine Casey for companionship at Upper Teesdale and in learning chemical techniques. Thanks also to Drs Alan Pearson, Arthur Marker and John Gatehouse for helpful comments.

Warm thanks to all the people in the laboratory: Dr Gul Baloch, Dr Joy Langston, Paul Stephenson, Heather Luff and Eileen Bresnan, especially. I am most grateful for the advice and experience of Bill Simon and John Gilroy. Thanks to Gillian Storey for help with culturing difficult cyanobacteria and Dr Iain Downie for help with computing and general advice.

Eric Henderson and Michael Bone provided trustworthy vehicles and banter; and David Hutchinson and Paul Sidney provided photographs and slides, sometimes at very short notice. Thanks to Gordon Bainbridge and Jack Warner and also to George Redshaw for introducing me to running.

English Nature gave access to the Upper Teesdale site. Thanks to Ian Findlay for sharing his knowledge of the site and use of meteorological data from Widdybank Fell and to both himself and Mrs Findlay for their friendly chats on cold mornings. East Lothian District Council allowed access to Tyne Sands. Thanks to Bobbie Anderson for sharing his knowledge of the site and to the people who sometimes accompanied me. Funding was provided by a NERC studentship.

During writing up thanks to Melchie for her company. Thanks, during the whole process, to my parents, Tony and Bryony; to my friends, especially Jane Lewis, Rachel Pardoe, Al Downie, Gibbo Gibson, Naziha El-Hashani, Pat Thompson, Jackie Fairweather, Martin Christmas, Kirsty McMorran, the Kellys (Martyn, Heather, Rosie and Edward), Paloma Santos, Ignacio Hernández and Janice Drew; and to Iain Downie who was a source of love and encouragement for which I am especially grateful.

LIST OF ABBREVIATIONS

°C	degrees Celsius	
g	gramme	
mg	milligramme	
µg	microgramme	
l	litre	
ml	millilitre	
µl	microlitre	
m	metre	
cm	centimetre	
mm	millimetre	
µm	micrometre	
nm	nanometre	
M	molar	
mM	millimolar	
µM	micromolar	
µmol	micromole	
meq l^{-1}	milliequivalents per litre	
mS cm^{-1}	millisiemens per centimetre	
µS cm^{-1}	microsiemens per centimetre	
chl a	chlorophyll *a*	
d. wt	dry weight	
d	day	
h	hour	
min	minute	
s	second	
w/v	weight / volume	
GMT	Greenwich mean time	
BST	British summer time	
TFP	total filtrable phosphorus	total P
FRP	filtrable reactive phosphorus	inorganic P
FOP	filtrable organic phosphorus	organic P
pNPP	p-nitrophenyl phosphate	
pNP	p-nitrophenol	
4-MUP	4-methylumbelliferyl phosphate	
4-MU	4-methylumbelliferone	
BCIP	5-bromo-4-chloro-3-indolyl phosphate	
CAPS	3-(cyclohexylamino)-1-propanesulphonic acid	
DMG	dimethylglutaric acid	
EDTA	ethylenediaminetetra-acetic acid	
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid	
HEPPS	N-2-hydroxyethylpiperazine-N'-2-propanesulphonic acid	
K_m	Michaelis - Menten constant (µM)	
V_{max}	maximum velocity (µmol product µg chl a^{-1} h^{-1})	
[S]	substrate concentration (µM)	
v	activity (µmol product µg chl a^{-1} h^{-1})	
n_H	Hill coefficient	
n	number of replicates	
s.d.	standard deviation of mean	
p	probability	
df	degrees of freedom	
NS	non significant	

CONTENTS

LIST OF TABLES

Tables con.

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 General comments

The literature suggests that Rivulariaceae are found in both freshwater and marine environments where organic P forms a large proportion of total P, at times (Livingstone & Whitton, 1984; Khoja *et al.*, 1984). These organisms are able to utilise organic P through "surface" phosphatase activity. An axenic strain of Rivulariaceae, *Calothrix parietina* D550 (Livingstone *et al.*, 1983; Grainger *et al.*, 1989), as well as field samples of *Rivularia* colonies from two sites (Livingstone & Whitton, 1984; Khoja *et al.*, 1984) had high "surface" phosphatase activity. An investigation into phosphorus concentrations during a year at sites with Rivulariaceae, together with phosphatase assays would be important in determining the role of phosphatase activity (organic P utilisation) in the ecology of Rivulariaceae. Morphology and observations on populations would also be important since the morphology, as well as the physiology, of Rivulariaceae is influenced by phosphorus (Livingstone & Whitton, 1983).

1.2 Phosphorus

1.21 Forms in the aquatic environment

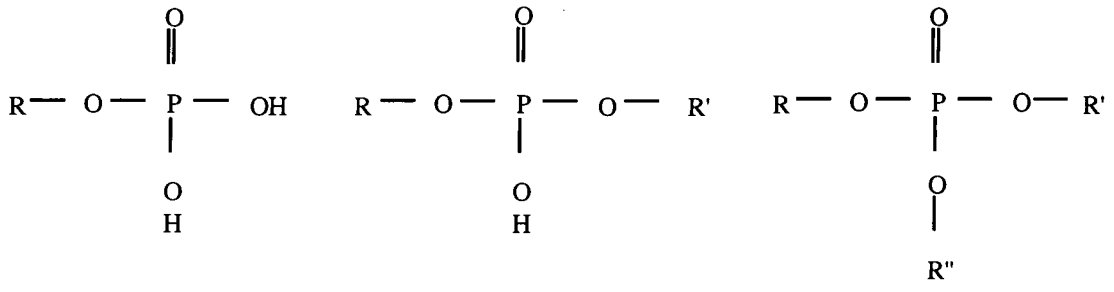
Phosphorus is required by all organisms yet its concentrations in some waters may fluctuate; being as little as 1 $\mu g\ l^{-1}$ at some times of year (Livingstone & Whitton, 1984). The direct source of phosphorus is erosion of rocks and soils (Ruttner, 1953). Phosphorus forms < 0.1% by weight, of the earth's crust. It is classed as a trace element geochemically because it is so scarce, and as a key nutrient ion biologically because of its scarcity in relation to biological demand (Moss, 1988).

Orthophosphate, a form of inorganic P, is made up of one PO_4^{3-} group. It is the most biologically available form of phosphorus (Boström *et al.*, 1988) but is probably a transient state and is therefore being rapidly cycled between phosphorus pools, mostly organic which are not biologically available without alteration. Organic P sources

include excretion from living material, as well as living material itself, and from dead and decaying matter. Consequently unmodified organic P compounds are likely to be high molecular weight compounds such as nucleic acids. Phosphatase enzymes are involved in catalysing the hydrolysis of organic P into more utilisable inorganic P by detachment from the organic part of the molecule. Phosphorus in the environment is dynamic with turnover times of a few minutes to several hours (Rigler, 1956).

Phosphates can be individual, orthophosphate (orthophosphoric acid), or condensed with another, pyrophosphate (pyrophosphoric acid), or many which may combine to form chains or rings (Broberg & Persson, 1988). One or more of the oxygen atoms may be replaced with another compound: orthophosphate may undergo esterification with alcohol or phenols which form one or more esters, monoester, diester, triester etc. (Fig. 1.1); forming an organic P compound (McComb *et al.*, 1979).

Phosphorus is present in water bodies in the form of orthophosphate; mostly $H_2PO_4^-$ and HPO_4^{2-}, depending on pH; inorganic polymers; organic compounds and in living organisms and detritus (Moss, 1988). Phosphorus is not particularly soluble; solubility being related to calcium, iron and aluminium ions (Golterman, 1975).



R represents the organic part of the molecule

Fig. 1.1 General formulae of phosphate esters

Total P is the total amount of phosphorus in the environment. In the aquatic environment this includes particulate and dissolved fractions (nominal porosity of filter 0.45 μm giving a physical cut off that causes a fraction of colloids to be included in dissolved phosphorus (Broberg & Persson, 1988)). These, in turn, can be split up into reactive and unreactive fractions.

After filtration, phosphorus that reacts with molybdate to form heteropoly blue without digestion is usually termed filtrable reactive phosphorus, and is assumed to be largely inorganic but it may also contain reactive organic P which, as high molecular weight phosphorus compounds, may be utilisable by some algae whereas some reactive fractions may not be (Broberg & Persson, 1988). Total filtrable phosphorus forms heteropoly blue after digestion, incorporating reactive as well as unreactive phosphorus. Therefore total P minus reactive phosphorus gives unreactive phosphorus, which is assumed to be mostly organic P, though it may also contain some unreactive inorganic P (Livingstone & Whitton, 1984; Khoja *et al.*, 1984).

1.22 Freshwater

In freshwater environments, concentrations of phosphorus may fluctuate due to natural causes: storms, droughts, natural hydrological and microbiological process or human causes: point sources including sewage and detergent discharges and non-point sources such as agricultural inputs and atmospheric loading. Schindler and Nighswander (1970) found 17-18 μg l^{-1} P in rainwater in June / July and 9-12 μg l^{-1} P in January / February.

Phosphorus is an essential nutrient and, unlike nitrogen, there is no alternative natural atmospheric source. For this reason fertility of water bodies is often characterised according to mean annual phosphorus concentrations. The OECD (Organisation for Economic Co-operation and Development) classifies lake waters accordingly (Newman, 1988):

oligotrophic	<	0.010	mg l^{-1} total P
mesotrophic	≥	0.010 < 0.035	
eutrophic	≥	0.035 < 0.100	
hypertrophic	≥	0.100	

However, this classification is not always appropriate. Phosphorus concentrations may fluctuate markedly or phosphorus inputs may cause a change in the trophic status of a water body (eutrophication). The biological effect of increased inputs may be far greater in some areas than in others. In Ireland, McGarrigle (1993) used a combination of biological and phosphorus data in order to classify water with signs of eutrophication. He found that waters were eutrophic with an annual median phosphorus concentration above 30 $\mu g\ l^{-1}$, 5 $\mu g\ l^{-1}$ lower than that specified above. Changes in the natural chemical composition of water bodies alters the survival of species native to them. Organism survival in low trophic environments is important in understanding the possible effects of nutrient enrichment and enabling proper conservation measures to be adopted to ensure their survival.

In a largely oligotrophic stream (Red Sike in Upper Teesdale, England), with very low total filtrable phosphorus concentrations (1-3 $\mu g\ l^{-1}$) for most of the year Livingstone and Whitton (1984) found extremely high levels of organic P in spring, up to 1 $mg\ l^{-1}$. Although the organic P concentration peaked at least 100-fold, the inorganic P concentration was only four times higher than the background level. Other studies have found elevations in P concentrations, some in late spring (Mulholland, 1992; Devito & Dillon, 1993a, b). Sæbo (1968) found that a major part of the total P found in *Sphagnum* peat was organic and slow decomposition rates in upland peat allowed nutrients to accumulate so that the peat could act like a sink (Heath, 1992). Livingstone and Whitton (1984) suggested the ideal circumstances for the release of this type of phosphorus would be when daytime temperatures are relatively high but below freezing at night. Devito and Dillon (1993b) found this to be the case: phosphorus and nitrate were flushed out of a *Sphagnum* coniferous swamp system headwaters due to snow melt. They suggested that the drainage pattern of the surrounding area may also affect the phosphorus concentrations in the environment. Two of the tributaries of Red Sike in Upper Teesdale (England) studied by Livingstone and Whitton (1984), drained from different sources:

1. Limestone springs
2. Peat

The peat-drained tributaries generally formed a larger proportion of water in the main stream during high flows. At the time of high organic P concentrations, flow was also high. Livingstone and Whitton (1984) measured phosphorus concentrations during other times of year when flow was high and found that peat drainage did contribute to a larger proportion of the stream water but the water showed only slightly higher than background concentrations of phosphorus.

It seems that the height of the water table as well as the weather conditions maybe important in determining the type and amount of peat drainage. However, when the stream is peat drained, the pH is likely to fall (Grainger *et al.*, 1989) which may alter the solubility of inorganic P. This raises the questions: are organic P pulses seasonal and if so, why and is organic P important in the success of the *Rivularia* population at this site (see 1.5).

1.23 Seawater

Phosphorus is not generally considered to be a limiting nutrient in the marine environment (Goldman *et al.*, 1979). High concentrations of phosphorus, especially organic P, were found in the upper intertidal region of the East Lothian coastline at Tyne Sands, Scotland (Khoja *et al.*, 1984). It was suggested by the authors that these levels of phosphorus, more than two orders of magnitude higher than seawater, were the result of cast seaweed on the strand line.

Seaweeds are important for nutrient cycling in coastal and estuarine environments as they can act as nutrient sources at times of low nutrient levels (especially on decomposition) and sinks at times when nutrients are above levels required for growth (Hanisak, 1990; 1993; Lavery & McComb, 1991). Hanisak (1993) suggested that decomposing seaweeds, especially when in large quantities, were likely to be ecologically important in providing nutrients. In South Africa, McLachlan (1983) found that stranded seaweed provided a source of food for the supralittoral fauna and detritivores as well as nutrients which could nourish the interstitial meiofauna of the whole beach. Studies on energy flow through cast algal systems have shown that cast algae and detritus produce nutrients which return to the sea

(Griffiths *et al.*, 1983; Koop & Lucas, 1983; Robertson & Lenanton, 1984; Bradford, 1989).

Plant litter is generally considered to be an important component of terrestrial (Minderman, 1968; Satchell, 1974), freshwater (Kaushik & Hynes, 1968) and marine (Field *et al.*, 1977; Velimirov *et al.*, 1977; McLachlan *et al.*, 1981) environments. Marine algae are especially important because they:

1. Decompose between 2 - 10 times faster than vascular plants, perhaps because they lack the tough fractile structural polysaccharides of vascular plants (Tenore, 1977; 1983; Godshalk & Wetzel, 1978).
2. Unlike vascular plants, they do not require fauna to decompose (Little, 1953; Bedford & Moore, 1984), although the shredding of material by detritivores may encourage bacterial growth, accelerating decomposition (Hargrave, 1970; Fenchel, 1972; Mann, 1972; Byren & Davies, 1986; Carpenter *et al.*, 1988).
3. They are adapted to rapid accumulation of nitrogen and phosphorus from pulses in the environment (Rhee, 1980; Ryther *et al.*, 1981; Hanisak, 1990).

Fucus species have been found to have hairs associated with rapid uptake of phosphorus (Hurd *et al.*, 1993). Parts of the *Fucus* thallus with hairs have been found to have higher phosphatase activity than those without (I. Hernández, pers. comm.) and Whitton *et al.* (1991) suggested that hairs may be associated with environments where pulses of organic P compounds occur.

Marine algae are still used as natural fertilisers because during their lives they concentrate nutrients in their tissues. *Laminaria* can accumulate nitrate to 28,000 times the concentration in the surrounding water (Chapman & Craigie, 1977). In salt marshes decomposing plant material has been found to encourage growth of the green alga *Ulva fenestrata* (Harrison, 1978).

The natural loss of fronds may cause cast algae. *Laminaria hyperborea* loses old fronds in spring (Kain, 1971); or due to storm events (Kuhnemann, 1970). However, the amount of algae cast on the strand line is not solely related to weather conditions. Zobell (1971) noticed that late summer storms deposited more algae than

early spring storms. Algae lose buoyancy when they decompose (Zobell, 1971; Edgar, 1987) which may relate to whether they remain on the strand line, as well as the sequence of high tide heights.

Khoja *et al.* (1984) suggested that the rotting seaweed played an important role, perhaps through drainage, in the concentrations of the phosphorus fractions in the pools of the upper eulittoral at Tyne Sands. The upper eulittoral area under study, is described by Dring (1992) as one of the most variable environments on earth, combining fluctuating salinity and temperature with fluctuating irradiance and nutrient supply. Khoja *et al.* (1984) found changing concentrations of the phosphorus fractions within the *Rivularia* pools on different days and within one tidal cycle.

Although the phosphorus concentrations in this system are likely to be higher than in the freshwater system, the results of previous studies have raised similar questions. Is the organic P produced seasonally, as a result of cast seaweed and is this important for the *Rivularia* population (see 1.5).

1.24 Nitrogen : phosphorus ratio

Changes in the N : P ratio from an optimum requirement for most organisms of 7:1 by weight (Redfield, 1934), alters nitrogen or phosphorus to deficiency, depending on which element is increased. Redfield (1934) suggested that cells were not under carbon, nitrogen or phosphorus stress if these nutrients were available in the ratio 42:7:1, by weight. If there was a deviation from this ratio deficiencies in one or more of the nutrients would prevent potential maximum growth. For example an N : P ratio below 5 will probably favour nitrogen fixing species (Schindler, 1975; 1977). The consequences of a shift in the ratio the other way may favour organisms that are able to survive phosphorus limiting conditions. Gibson and Whitton (1987) found that hairs formed when the N : P ratio in the cultures of Chaetophorales ranged between 5.4 - 7.2:1. Hairs are generally accepted to be associated with phosphorus deficiency (Livingstone *et al.*, 1983; Grainger *et al.*, 1989, Whitton *et al.*, 1991). These ratios may be lower than those expected for phosphorus deficiency because cyanobacteria often start to hydrolyse organic P before the condition of the organism deteriorates

(Livingstone *et al.*, 1983) or because organic P is the only source of phosphorus. The ratio N : inorganic P is therefore important.

Nitrogen fixation and/or the ability to utilise organic P are likely to be competitive advantages especially when the N : P ratio is not optimal. However, there is a great deal of interrelationship between phosphorus and nitrogen. Although nitrogen fixing organisms may primarily be influenced by phosphorus deficiency, for example under N_2 fixing conditions, P storage capacity may be reduced e.g. *Anabaena flos-aquae* (Thompson *et al.*, 1994).

1.25 Importance in biological systems

Organisms require inorganic P for metabolism. Because phosphorus is often the limiting nutrient in aquatic systems, and most obviously in N_2 fixing organisms, strategies for adapting to phosphorus limitation have evolved. In cyanobacteria fast uptake of phosphorus, large phosphorus storage capacity (polyphosphate bodies) and utilisation of organic P (producing phosphatase enzymes) are strategies for maximising phosphorus in the environment. These strategies are also appropriate if levels of phosphorus or nitrogen are low or subject to fluctuation in the environment (Rhee 1980; Ryther *et al.*, 1981; Hanisak, 1990; Whitton *et al.*, 1991). Organisms that can utilise organic P are at an advantage in an environment where much of the phosphorus is in this form or where inorganic P is rapidly turned over or subject to fluctuation or where N : P > 7. When inorganic P becomes limiting or is variable there may be an increased species turnover, with organic P forming a larger proportion of the total P, originating from dead and decaying material from organisms that are unable to survive.

1.3 Phosphatase

Phosphatases are enzymes which catalyse the hydrolysis of esters and anhydrides of phosphoric acid (Feder, 1973). Phosphatases promote the degradation of a variety of organic P compounds resulting in orthophosphate and an organic moiety in water solutions or in metabolic processes: transport of substances across membranes or synthesis of new organic P compounds (transphosphorylation) (McComb *et al.*, 1979).

Phosphatase enzymes which may or may not be substrate specific, are bond specific and often categorised according to the type of organic P substrate for which they catalyse the reaction (Feder, 1973). A variety of phosphomonoesters are hydrolysed by phosphomonoesterhydrolases (monoesterases); phosphodiesters by diesterases, including nucleases; phosphotriesters by triesterases (Fig 1.1). Also phosphatase enzymes include enzymes acting on phosphoryl-containing anhydrides and enzymes which act upon P-N bonds such as phosphoamidases. For simplification phosphatase, unless otherwise stated, is used in this thesis to refer to phosphomonoesterases.

1.31 Occurrence of phosphatase enzymes

Phosphatases are widely distributed. Different phosphatase enzymes can occur in the same cell as well as in different organisms, from bacteria to vertebrates (McComb *et al.*, 1979).

Enzymes can be intracellular, where the organic P molecule is transported into the cell and the product is either utilised inside the cell or transported out, or the enzyme can be extracellular. If the latter is the case, the enzyme is located outside the cell where it comes into contact with substrate. The product may be transported inside the cell. Extracellular phosphatases may be bound to the cell wall and are termed "surface" phosphatase (Whitton, 1990).

The relationship between phosphatase activity and phosphorus was observed by Horiuchi *et al.*, (1959) and Torriani, (1960) when they found an inverse relationship between phosphatase activity of *E. coli* and phosphorus in the growth medium. Garen (1960) showed that mutants without phosphatase activity were unable to grow in media lacking inorganic P.

Phosphatase activity has been found in many algae and cyanobacteria. A 10 to 25 fold increase in phosphatase activity has been found by growing some species of algae in phosphate-poor media (Kuenzler & Perras, 1965). Many studies have indicated that phosphatase activity can be correlated with algae and algal blooms (Heath & Cooke, 1975; Jansson, 1975; Pettersson, 1980; Olsson, 1983) indicating that the algae are responsible for the phosphatase activity rather than bacteria. Pettersson

(1980) concluded that phytoplankton were the source of phosphatase activity since bacterial biomass was negligible and spatial and temporal changes in phosphatase activity correlated well with changes in chlorophyll concentration.

Most cyanobacteria are able to utilise organic P using "surface" phosphatase enzymes which are usually inducible, when inorganic P is low and when the concentration of substrate in the environment increases (Healey, 1982). These enzymes are often repressed by orthophosphate (Kuenzler & Perras, 1965; Wynne, 1977).

Some cyanobacteria may be able to utilise different types of organic P. All cyanobacteria tested by Whitton *et al.* (1991) showing phosphatase activity, were able to hydrolyse phosphomonoesters, most were able to hydrolyse phosphodiesters, but very few were able to utilise phytic acid. Most strains released monoesterase extracellularly but none released diesterase. In the same study different cyanobacteria were compared. *Calothrix parietina* D550 and *Nostoc commune* UTEX 584 both showed monoesterase and diesterase activity when cellular P fell below 0.8 % dry weight. However, the two species showed different pH optima and the effect of age on phosphatase activity was different. These differences may indicate that the two species had different phosphatase enzymes and that the enzymes showed different characteristics, perhaps relating to their environmental conditions.

Water may contain phosphatase enzymes originating from sources such as excretion from bacteria, plants and animals (Jansson *et al.*, 1988), extracellular enzymes or from lysis of dead or dying material (Berman, 1970).

1.32 Phosphatase activity as an indicator of phosphorus deficiency

Alkaline phosphatase activity is an enzyme induced response to phosphorus deficiency (Shapiro, 1988), therefore an apparently ideal indicator of phosphorus deficiency. Kuenzler and Perras (1965) found many species of marine algae produced phosphatase enzymes when phosphorus deficient. They found that when algae were phosphorus sufficient, production of alkaline phosphatase was repressed. Fitzgerald

and Nelson (1966) found similar results with freshwater algae and suggested using phosphatase activity to determine the physiologic state of algae.

K_m, the Michaelis constant (substrate concentration which corresponds to half maximum activity i.e. 1/2 V_{max}), values can be used as phosphorus deficiency indicators. Pettersson (1980) found that the K_m of the phosphatases in Lake Erken varied by one order of magnitude during a year, with the lowest values in situations with pronounced phosphorus deficiency. He concluded that phytoplankton adapted themselves to low phosphorus supply not only by increasing their enzyme production but also by producing enzymes with improved ability to use low substrate concentrations.

Because phosphatase activity recycles organic P in the aquatic environment it has the potential for regenerating inorganic P. But many studies failed to find an increase in inorganic P concentrations during high levels of phosphatase activity (Rigler, 1961; Berman & Moses, 1972; Pettersson 1980). However Berman (1970) and Kobori and Taga (1979) found that inorganic P was produced when unfiltered water had been subjected to chloroform, which kills the algae but does not affect the phosphatase enzymes. Berman (1970) calculated that the inorganic P released as a result of phosphatase activity would meet the demand of phytoplankton in Lake Kinneret. Presumably, as fast as inorganic P was being released as a result of phosphatase activity, it was being taken up by organisms. However, the rate of phosphatase activity may be greater than that at which it is utilised.

Phosphatase activity is one important way in which organic P is turned over in the environment and is likely to be especially important in environments where phosphorus is predominately in the organic form (Livingstone & Whitton, 1984). Phosphatase specific activity, activity divided by a biomass indicator (d. wt or chl a), has been suggested as a phosphorus deficiency indicator (Pettersson, 1980; Gage & Gorham, 1985).

Kuenzler and Perras (1965) found that algal phosphatase activity hydrolysed glucose-6-phosphate and although the inorganic P was taken in by the cell, the glucose remained in solution. This has been the basis for phosphatase assays. Known

concentrations of organic P substrate are added to a phosphorus-free medium and the enzyme assayed catalyses the hydrolysis of the substrate into a product. In a phosphatase assay the products formed are inorganic P and an organic moiety which may be detectable. In the case of phosphomonoesters, the moiety is released on a 1:1 ratio with the inorganic P, in the case of diesters the ratio is 2:1 and with a triester the ratio is 3:1. Phosphomonoesterases have broad specificity against different substrates; their activity is only restricted to the P-O bond of phosphomonoester (Jansson *et al.*, 1988). Metal ions, usually Zn^{2+}, are incorporated into the active site of the enzyme (McComb *et al.*, 1979), therefore the concentration of divalent cations is important in any study of phosphatase activity.

Phosphatase activity can be measured either by measuring the inorganic P, which may already have been taken up by the cells, or by measuring the organic moiety. The hydrolysis of the monoester pNPP, forms a yellow organic moiety, pNP, which can be measured in a spectrophotometer. Many studies have used this method (e.g. Livingstone *et al.*, 1983; Livingstone & Whitton, 1984; Hernández *et al.*, 1992), but the detection limit of this product is high in comparison to substrates such as 4-MUP which produces the fluorescent product, 4-MU. This method has been used by Chróst and Krambeck (1986), Jansson *et al.*, (1988) and Chappell and Goulder (1992) amongst others. Very few studies have compared calorimetric and fluorimetric methods (e.g. Berman *et al.*, 1990; DeBoever *et al.*, 1995).

Interference with phosphatase activity as a deficiency indicator, especially in phytoplankton, may be caused by the following factors:

1. Dissolved phosphatase enzymes are not traceable and may be active for several days or weeks
2. Constitutive phosphatase activity in microorganisms (i.e. that which does not vary depending on phosphorus deficiency) may vary between species
3. Concentration and supply of substances other than phosphorus for example pyrimidines or guanine (Wilkins, 1972), particulate cAMP (Franko & Wetzel, 1982) and dissolved nucleotides (Franko, 1984), which may affect phosphatase activity
4. Diurnal variations in phosphatase activity

5. Organisms used in the assay: phytoplankton composition and/or associated bacterial phosphatase activity (Jansson *et al.*, 1988)

Phosphatase activity in the environment is different to that measured under assay conditions because:

1. Substrate concentrations are often much lower in the natural environment than in a phosphatase assay
2. Substrates used in assays are artificial and always of one type, not a mixture, as might be the case in the natural system. The affinity of the enzyme(s) to the artificial substrate is likely to be different to that of the natural substrate(s)
3. External temperature and pH are often not sufficient to cause suitable reaction times in assays, so are often increased in assays (Jansson *et al.*, 1988).

Phosphatase assays may be especially useful in long-term monitoring programmes, highlighting periods of phosphorus deficiency and sufficiency. The presence of hairs has been positively correlated with phosphatase activity in Chaetophoraceae (Gibson & Whitton, 1987). It is likely that organisms with the potential to grow hairs would be sensitive to phosphorus concentrations in the environment. Whitton (1988) reviewed hairs in eukaryotic algae and their possible functions which include phosphorus uptake through increased surface area and phosphatase activity. Morphological features, such as the presence or absence of hairs, can be used as a supplement to phosphatase assays as an indicator of the phosphorus status of an environment.

1.33 Types of phosphatase enzyme

There are several ways phosphatase enzymes are classified. One way is by the type of molecule, for example protein phosphatases; or the type of environment they work in, acid or alkaline phosphatases; or the types of substrates they work on; phosphomonoesterase or phosphodiesterase (Fig. 1.1).

Reaction optima for alkaline phosphatase activity are well above neutral pH (Ross *et al.*, 1951). Olsson (1990) found that, after liming, an acid lake had a more

diverse phytoplankton community and he suggests that liming the lake affected the dominant species so that those with activity at a higher pH optima were favoured. In this way it seems that environmental pH may affect the composition of a community such that the pH optima is changed in the same direction as the change in environmental pH. It is interesting then, that so many species producing alkaline phosphatase activity show pH optima well above their environmental levels (Whitton *et al.*, 1991), though species with more calcium environments do have significantly higher pH optima than those without (Livingstone & Whitton, 1984).

Unlike alkaline phosphatases, acid phosphatases are generally not repressed by orthophosphate (Kuenzler & Perras, 1965; Wynne, 1977). It seems, therefore, that acid phosphatases may be constitutive, being synthesised most of the time (Jansson *et al.*, 1988). Mahasneh *et al.* (1993) reported that synthesis of alkaline phosphatase was related to ambient phosphorus concentrations: on the addition of 10 mg l^{-1} inorganic P, phosphatase activity decreased and cellular P increased. Acid phosphatase enzymes may serve internal phosphorus metabolism, while alkaline phosphatases may have external functions, with synthesis beginning in the presence of suitable substrates (Jansson *et al.*, 1988).

1.34 Phosphatase activity

1.341 Substrate affinity

The affinity of phosphatase enzymes for substrate can be estimated by carrying out kinetics experiments. These involve measurement of activity at different substrate concentrations. A double reciprocal plot of activity (v) against substrate concentration ([S]) enables the estimation of K_m and V_{max} (Fig. 1.2). V_{max}, also known as the maximum velocity, is the maximum rate of activity. The substrate concentration at which half the activity of V_{max} is produced, is known as the K_m, the Michaelis-Menten Constant or apparent half saturation constant. The values of these are important in comparing the ability of enzymes to catalyse the hydrolysis of substrates. A high affinity for substrate is indicated by a low K_m and vice versa. K_m values of phosphatases from eutrophic lakes can be 100 times higher than phosphatases from

oligotrophic and mesotrophic lakes (Pettersson, 1980; Chróst & Overbeck, 1987). Affinity for substrate may be different when comparing different levels of phosphorus deficiency, as above; different substrates or different organisms.

Phosphatase activity was stimulated by the cations: Mg, K, Zn, Co and also Ca (Grainger *et al.*, 1989) and inhibited by EDTA, especially at alkaline pH (Cembella *et al.*, 1984, Grainger *et al.*, 1989). Inorganic P, above 0.1 mM had a marked inhibitory effect on phosphatase activity on *Calothrix parietina* (Grainger *et al.*, 1989). This is common for inducible phosphatase enzymes (Torriani, 1960; Ihlenfeldt & Gibson, 1975; Chróst & Overbeck, 1987). Inorganic P is the product of phosphatase activity and has been shown, in many studies, to bind to the active site of alkaline phosphatase enzymes (Engström, 1964; Hull *et al.*, 1976), acting as a competitive inhibitor and interfering with the ability of the enzyme to bind with the substrate. Under these conditions higher substrate concentrations are needed to produce the same activity, resulting in a higher K_m but the same V_{max} (Fig. 1.2).

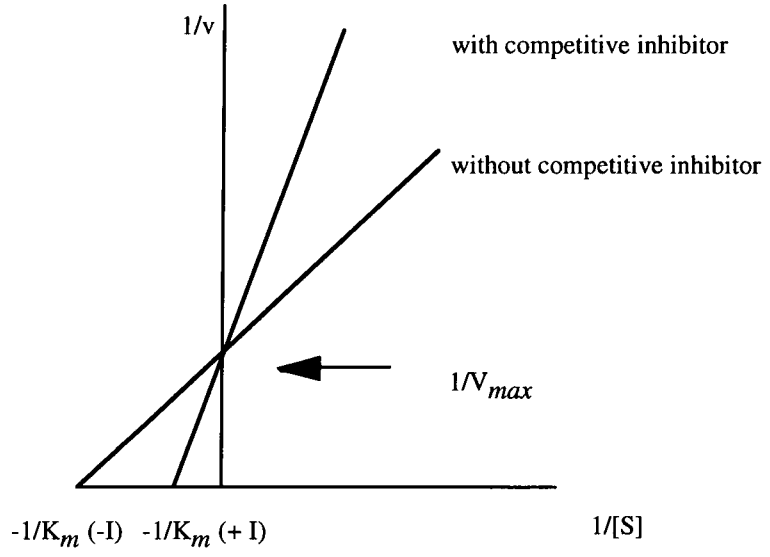


Fig 1.2 Lineweaver - Burk plot to show the effect of competitive inhibition on enzyme affinity for substrate

Phosphatase assays of field organisms may posses more than one phosphatase enzyme. In this instance the Michaelis-Menten equation is not theoretically correct since the values obtained for K_m and V_{max} will be a mixture of the enzymes present.

However, this method is useful in characterising a group of enzymes for comparison (Jansson *et al.*, 1988). The K_m value for a specific enzyme will vary with pH, temperature and ions present in the media. At optimum pH and temperature, a higher affinity is achieved.

1.342 Reaction mechanism

The reaction mechanism of hydrolysis of phosphorus esters, catalysed by phosphatase enzymes is shown in Fig. 1.3.

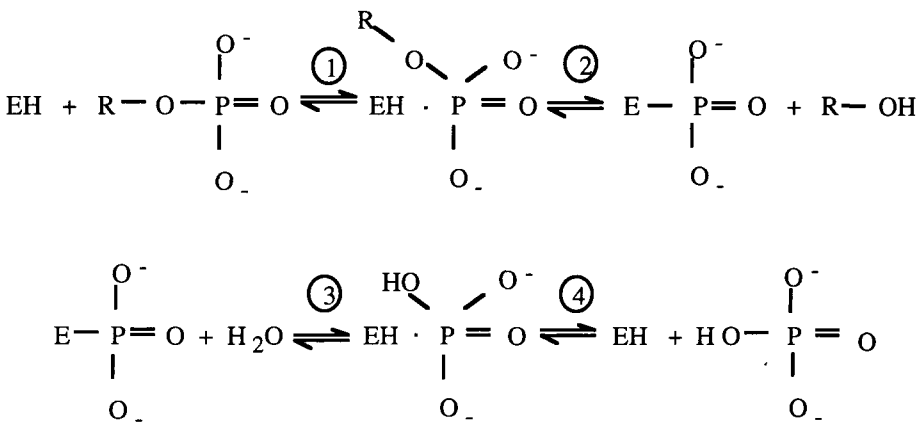


Fig. 1.3 Hydrolysis of phosphate esters catalysed by alkaline phosphatase enzymes (McComb *et al.*, 1979)

1. Non-covalent binding of the substrate to the enzyme - Michaelis Complex
2. Alcohol release from the complex and orthophosphate becomes covalently bound to the enzyme forming a phosphoryl-enzyme compound
3. Conversion of the phosphoryl-enzyme compound, through uptake of water, to a non-covalent complex
4. The release of orthophosphate and regeneration of free enzyme

Any of the steps 2-3 can be rate limiting for the overall reaction (McComb *et al.*, 1979).

1.4 Rivulariaceae

Rivulariaceae are a family of cyanobacteria, the largest and most widespread group of photosynthetic prokaryotes which are only absent at very low pH values, and

have one of the longest geological records (Schopf & Walter, 1982). Cyanobacteria are most successful in environments possessing one or more of the following features: high temperatures, UV irradiation, low light levels, tendency to desiccation, free sulphide, low CO_2 concentration and low combined nitrogen concentrations (Whitton, 1992) and intertidal regions (Whitton & Potts, 1982).

Rivulariaceae are noted for trichomes which form hormogonia (Fig. 1.4): small reproductive bodies which develop into trichomes (Geitler, 1932). Hormogonia, in heterocystous forms of cyanobacteria, are 5 - 15 cell chains with a diameter less than that of the vegetative trichome. They are distinguishable from the parent trichome by size, shape and, in *Calothrix parietina* D550, they are gas-vacuolate. Hormogonia are often motile and do not posses a heterocyst, even in the absence of combined nitrogen (Rippka *et al.*, 1979). In Rivulariaceae, hormogonia develop from a specific area of the trichome below the hair (Whitton, 1987a). Kirkby and Whitton (1976) found that Rivulariaceae, which include *Rivularia*, *Dichothrix* and *Calothrix*, were found largely in calcareous areas with low nutrient levels.

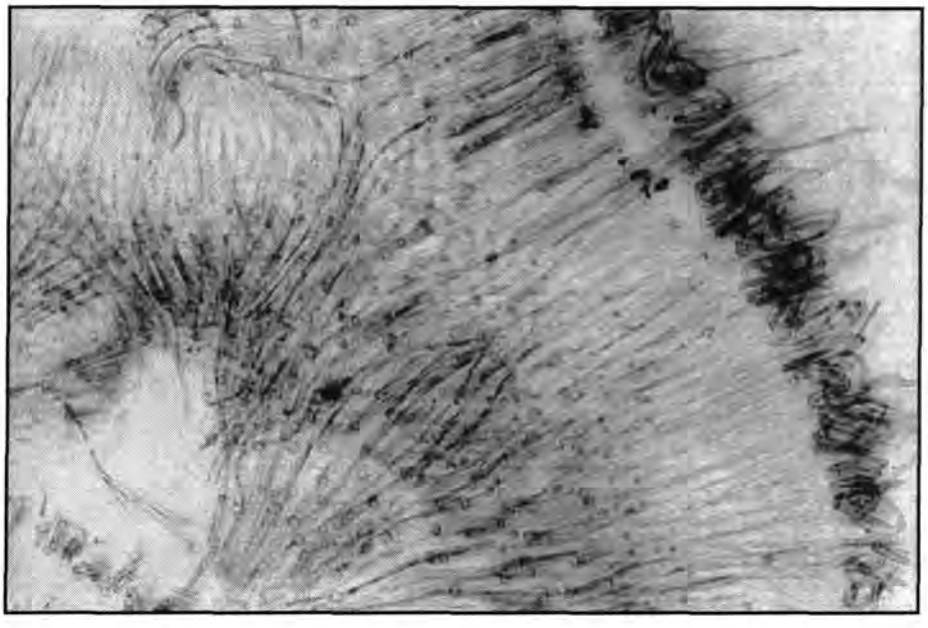


Fig. 1.4 Photomicrograph of hormogonia bands around the edge of a *Rivularia atra* colony (D. Livingstone, 1981). Approximate diameter of whole colony 1.5 mm

1.41 Ecophysiology of Rivulariaceae

Kirkby and Whitton (1976) noticed changes in the morphology of Rivulariaceae trichomes associated with nutrient deficiency; notably long terminal portions with no chlorophyll. Sinclair and Whitton (1977) associated these parts of the trichome to N_2 fixing organisms, usually grown without nitrogen which, in the presence of nitrogen, dropped off. They classified these as hairs: regions of the trichome where the cells are narrow, elongated, highly vacuolate and apparently colourless (Fig. 1.5). Being mostly intrathylakoid space, hairs probably take little energy to produce and maintain (Livingstone *et al.*, 1983).

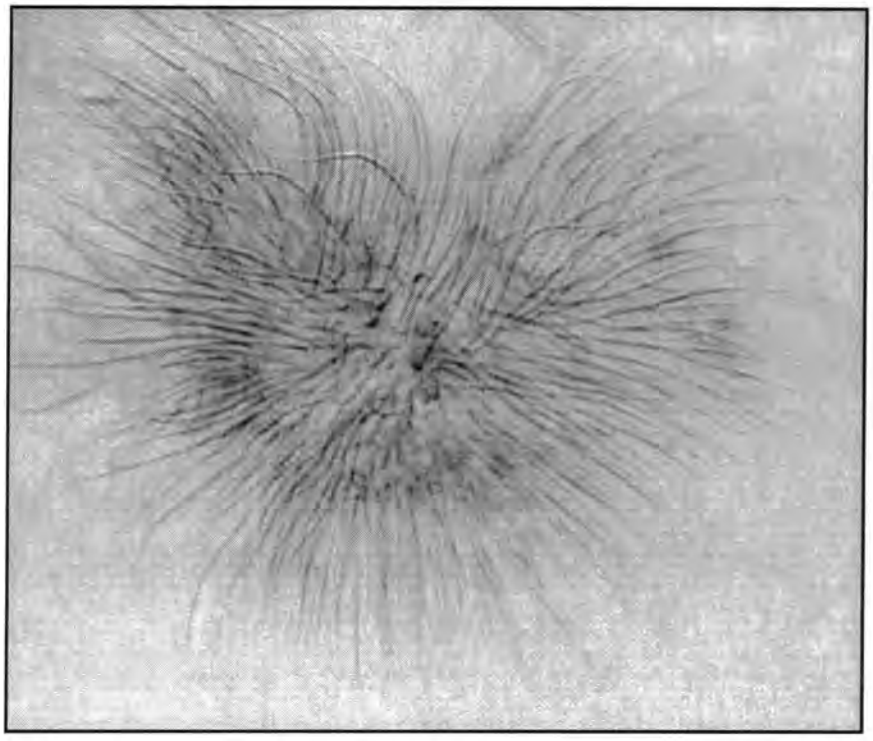


Fig. 1.5 Photomicrograph of hairs around the edge of a new *R. atra* colony (14.7.1993). Approximate diameter of colony 250 µm

Experiments on *Calothrix parietina* D550 showed that it was a deficiency of inorganic P, rather than the absence of nitrogen, which caused the growth of hairs (Livingstone & Whitton, 1983). In the presence of inorganic P the same study showed that the hairs fell off the end of the trichomes and hormogonia were formed. On

staining for phosphorus, polyphosphate bodies were shown in the trichomes very soon after the addition of inorganic P (Sinclair & Whitton, 1977).

Castenholz and Waterbury (1989) suggest that hormogonia formation is associated with reoccurrence of phosphorus in the environment. Islam and Whitton (1992) found cyanophycin and polyphosphate granules in hormogonia from *Calothrix* D764, pointing out that this is likely to be the result of the reappearance of phosphorus in the medium.

In his review of hairs in eukaryotic algae, Whitton (1988) suggests that they may have many roles: e.g. to prevent problems caused by desiccation, as secretory structures and to aid nutrient uptake. Presumably the role of hairs in Rivulariaceae is similar. Hairs form at the same time as phosphatase activity begins, < 10 μg P mg d. wt^{-1} and staining showed phosphatase activity on the walls of hair cells and adjacent chlorophyll containing cells (Livingstone *et al.*, 1983). Whitton (1987b) and Mahasneh *et al.* (1990) concluded that the hair was the site of "surface" phosphatase activity in the Rivulariaceae they studied. DeBoer and Whoriskey (1983), Livingstone and Whitton (1984) and Whitton (1988) suggest that hairs may be an adaptation to pulses of nutrients. Rhee (1980) and Cembella *et al.* (1984) suggest that a large storage capacity for phosphorus, in polyphosphate bodies, would be especially important to organisms when phosphorus in the environment is likely to be subject to pulses. Whitton (1988) also suggests that hair-forming species would be more successful in unpolluted calcareous freshwater environments where most of the phosphorus would be in the organic from because the inorganic P is likely to be deposited with oxidised Fe II. J.W.G. Lund noticed the abundance of *Rivularia* colonies in the nutrient poor habitat of Upper Teesdale (Livingstone & Whitton, 1984).

An evolutionary parallel is suggested by Whitton (1987a) between the hairs of Chaetophoraceae and those of Rivulariaceae. The longest hairs grow in response to phosphorus limitation in both species (*Stigeoclonium*: Gibson & Whitton, 1987; *Calothrix parietina*: Livingstone & Whitton, 1983). Both cultures formed hairs in response to Fe deficiency, but these were less well developed and did not have phosphatase activity.

In cyanobacteria, as in other microorganisms, orthophosphate uptake is hyperbolic with respect to concentration (Whitton, 1992). Yet many unpolluted freshwater environments are phosphorus limited. Under phosphate limited conditions, phosphorus uptake is only linear below 10 mM while in organisms preconditioned to high phosphate linearity of phosphorus uptake extends to higher levels.

Calothrix, Dichothrix and *Rivularia* are able to fix N_2 in heterocysts at the base of each filament. Rivulariaceae inhabiting intertidal environments have been found to have reduced levels of nitrogenase activity during periods of high salinity (*Calothrix scopulorum*: Jones & Stewart, 1969; *Rivularia atra*: Reed & Stewart, 1983). It has been suggested (Whitton, 1992) that increased photosynthesis and the relocation of energy resources resulting in a decrease in nitrogenase activity would be initiated in response to high salinity.

Studies in Upper Teesdale (Livingstone & Whitton, 1984) and at Tyne Sands (Khoja *et al.* 1984), both sites where *Rivularia* were abundant, showed that these organisms had high phosphatase activity, with hairs that stained positively. These two sites had unusually large concentrations of organic P. It was speculated that the high levels of organic P and the high levels of phosphatase activity in these organisms were connected to their presence at these sites.

1.42 *Rivularia*

Rivularia is one of the more complex genera of prokaryotes. It forms hemispherical or spherical colonies consisting of trichomes with a basal heterocyst and, in phosphorus deficient conditions, is likely to possess a hair at the other end of each trichome. Trichomes radiate out from a central ring, each one having a heterocyst at its basal end. Mucilaginous sheaths, around the trichomes, contain the pigment scytonemin (Pentecost, 1987), probably as a protection from UV radiation, causing the dark brown colouration of the colonies.

Rivularia colonies are probably formed by the association of trichomes (Whitton, 1987b) which make it very difficult to culture axenic strains. Many isolates of colony

forming cyanobacteria have lost their ability to form colonies (Whitton, 1992) as bacteria may be essential in colony formation (Schwabe & Mollenhauer, 1967).

The trichomes are tapered. Growth occurs at the basal end, near the heterocyst. It is in the heterocyst that nitrogenase enzymes are found. At Tyne Sands, *Rivularia atra* colonies have marked nitrogenase activity (Khoja *et al.*, 1984), however Livingstone *et al.* (1984) found that there was a 400 : 1 difference in the CO_2 fixed to N_2 fixed during daylight hours in *R. biasolettiana* from Red Sike. They suggest that, at this site, much of the nitrogen requirements of the colony come from combined nitrogen in the environment.

The genus is fairly widespread, growing in fast-flowing streams, salt marshes, intertidal regions, lake plankton and as an epiphyte on submerged plants. It usually grows on calcareous substrata where the water is almost always calcareous (Jaag, 1945; Pentecost, 1978) and where it does grow, it grows in large numbers and may be dominant. It has been postulated that there are features common to all the habitats where this genus occurs, particularly a large proportion of total P being organic (Khoja *et al.*, 1984; Livingstone & Whitton, 1984).

Rivularia colonies are individual and easily recognisable which makes it a suitable organism for experimentation. Livingstone and Whitton (1984) found the features of *Rivularia* at Red Sike to lie somewhere between *R. biasolettiana* and *R. haematites* and referred to it as *R. biasolettiana.* *Rivularia* from Tyne Sands is *R. atra* (Khoja *et al.*, 1984).

1.43 *Calothrix*

The trichomes of *Calothrix* are very similar to those of *Rivularia* and *Dichothrix* having a heterocyst at the basal end and may have a hair in phosphorus deficient conditions (Livingstone *et al.*, 1983). Another similarity to *Rivularia* is the formation of hormogonia which form one after another, and give rise to new filaments (Geitler, 1932). *Calothrix* and *Rivularia* differ in that although the trichomes of *Calothrix* may aggregate, they do not form distinct colonies growing as sheets on the substrate

surface. *Dichothrix* is very similar to *Calothrix*, the main difference being that *Dichothrix* filaments show more false branching (Geitler, 1932).

Studies have been carried out on the relationship of phosphorus to morphology (Sinclair & Whitton, 1977; Livingstone & Whitton, 1983), physiology (Livingstone *et al.*, 1983) and phosphatase activity (Grainger *et al.*, 1989; Whitton *et al.*, 1991) of batch cultures of *Calothrix parietina* D550. This strain was isolated from an Upper Teesdale stream, Sand Sike in 1979. The culture medium, at the point of subculture, contained 1 mg l^{-1} phosphorus, as an attempt to reflect the low phosphorus concentrations of its original habitat. The morphological study (Livingstone & Whitton, 1983) found 4 stages of trichome development in relation to phosphorus levels in the media. The first stage was the introduction of hormogonia to fresh media containing 1 mg l^{-1} phosphorus. Stage II started with the differentiation of a heterocyst . Hormogonia formed from these trichomes, but the extent of hormogonia formation depended on the phosphorus concentration of the medium. Stage III was marked by the development of the hair. Hairs formed when the cellular phosphorus concentration dropped to a mean of 10 μg P mg d. wt^{-1}, although cultures still appeared healthy until a minimum of 2.5 μg P mg d. wt^{-1}. Phosphatase activity began on the wall of the hair cells connected to chlorophyll-containing cells and the part of the sheath furthest from the heterocyst. Stage IV began after the addition of 1 mg l^{-1} inorganic P. Hormogonia could be seen within the apical end of the chlorophyll containing part of the trichome within 8 h. Normally 5 hormogonia were released after the hair dropped off during this stage, compared to one in stage II.

Livingstone *et al.* (1983) suggested that the presence of hairs in this strain, enables the organism to use organic P when inorganic P in the environment is low which is the case in Upper Teesdale, England, from where this strain was isolated (Livingstone & Whitton, 1984).

1.5 Aims

Previous studies showed high phosphatase activity in *Rivularia* from two very different environments: a freshwater stream; Red Sike (Livingstone & Whitton, 1984) and a marine intertidal zone; Tyne Sands (Khoja *et al.*, 1984). For a description of these sites see 3.1 and 3.2. The hypothesis being tested was that environments where Rivulariaceae are present often have intermittent pulses of organic P, and that an ability to utilise organic P, through "surface" phosphatase activity, is an important feature of their ecology. The apparent contrast between the two sites would provide the best solution to test this, using phosphatase assays and morphological features of Rivulariaceae, especially *Rivularia*, and chemical features, particularly phosphorus.

Long-term field studies

1. To relate phosphorus and nitrogen fractions at a freshwater and a marine site to phosphatase activity (using pNPP and 4-MUP) of *Rivularia*, *Ralfsia verrucosa* (later in study) and water (0.7 μM filtered) over at least one year of monthly sampling
2. Record changes in morphology of *Rivularia* which may be associated with change in environmental phosphorus fractions and N : P ratio
3. Record changes in the population of *Rivularia* which may be related to organic P in the environment

Intensive field studies

Establish the source of phosphorus fluctuations found in the long-term field studies at both sites

Experimental studies

Examine the mechanics of phosphatase activity in field and axenic organisms using two different substrates: pNPP and 4-MUP

CHAPTER 2

MATERIALS AND METHODS

2.1 Environment

2.11 Physical Parameters

On each sample date physical parameters were measured. These were slightly different at each of the two sites, outlined in Chapter 3.

A Wissenschaftliche-Technische Werkstäten (WTW) meter (model pH91), calibrated with standard buffers before use, was used to measure water pH. Total alkalinity was measured by titrating 0.02 M HCl into 50 ml of settled water sample until the pH reached 4.2, calculated according to Golterman *et al.* (1978):

$$\text{Total alkalinity} \quad (\text{meq } l^{-1}) \quad = \quad v \times N \times 50000 \times 0.0499 / V \qquad (1)$$

where v is the volume of acid used, N is the normality of acid, V is the volume of sample.

Current was measured using a calibrated Ott meter, positioned perpendicular to the flow of water at the fastest flowing part of the stream. The blade was placed 1/3 the depth of the water. Revolutions measured per minute were converted to m s^{-1} (Patterson, 1983). Absorbance of filtered water samples was measured at 420 nm on a Shimadzu dual beam spectrophotometer (UV 150-02) in a 1 - cm cell.

Water conductivity and temperature were measured using a WTW meter (model FC 910), rotating the submerged probe in the water at the collection point. Salinity was measured in a salinity refractometer (Atago, Japan). Salinity was expressed in ‰ (practical salinity units). Weather conditions, cloud cover, wind speed and direction were also noted.

2.12 Standard laboratory techniques

All glassware, plastic pipette tips and other material was washed in 10 % H_2SO_4 for standard nutrient analysis (HNO_3-washed for cations) or washed in 2 % decon (Decon Laboratories Ltd, England), for routine phosphatase assays and culture work.

Bungs and cuvettes were washed in distilled water only. Equipment was left to soak in the cleaning solution for no less than 20 min, soaked in distilled water then rinsed six times in distilled water. Glassware was dried in an oven at 105 °C, plastics were dried in an oven at 40 °C.

AnalaR chemicals were used to prepare stocks. These were weighed out on a Sartorius 1474 balance and dissolved in MilliQ water. Stocks were generally stable but were discarded and remade if the solution changed in any way (became cloudy, showed precipitation or organism growth).

All blanks and standards for nutrient analysis were made up in MilliQ water and treated in the same way as the samples unless otherwise stated (e.g. some seawater analysis). AnalaR grade inorganic chemicals were supplied by BDH and organic chemicals were supplied by Sigma.

Water was collected from the site in a 2 l plastic beaker. It was allowed to settle for 5 min and then poured into polyethylene bottles, put on ice and transported back to the laboratory. Immediately on return, 1 - 4 h after collection, the water was filtered. All filtration was through Whatman GF/F filters (approximately 0.7 μm porosity), unless otherwise stated.

Samples of water were analysed for phosphorus on return to the laboratory or refrigerated (< 5 °C) for phosphatase assays and for cation analysis (see 2.16 for filtration); or frozen (-20 °C) for nitrate, nitrite and ammonium analysis (Parsons *et al.*, 1984).

Detection limits were based on Sutcliffe (1979), unless cited otherwise.

2.13 Phosphorus

Phosphorus fractions were analysed the same day they were collected. The analysis used was the molybdenum blue technique (Murphy & Riley, 1962) as modified by Eisenreich *et al.* (1975).

TFP was analysed by digesting 25 ml of sample with 5 ml of Digestion Reagent (Table 2.1) at 1 bar for 20 min before addition of 5 ml Mixed Reagent 1 (Table 2.1). Digestion in persulphate and acid causes all the phosphorus, reactive and unreactive, to

form "molybdate blue" after addition of Mixed Reagent 1. The colour has an optical density of 882 nm which is proportional to the total phosphorus present. The colour was stable between 10 min - 2 h.

FRP was measured similarly but the solution was not digested in acid, so that the only phosphorus producing molybdate blue was reactive after addition of 5 ml of Mixed Reagent 2 (Table 2.1) to samples, blanks and standards.

FOP was taken as the difference between TFP and FRP. TFP is referred to as total P, FRP is referred to as inorganic P and FOP is referred to as organic P in the text. For low phosphorus concentrations (< 20 $\mu g\ l^{-1}$), 10 cm cells (Helma, UK) were used. Detection limits were 1 $\mu g\ l^{-1}$ PO_4-P for FRP and TFP, and 3 $\mu g\ l^{-1}$ PO_4-P for FOP (Livingstone & Whitton, 1984).

Table 2.1 Stock solutions and reagents for phosphorus analysis

Reagent stocks (stable)	Reagents (made daily)
Digestion 100 ml 18 M H_2SO_4 mixed with 500 ml distilled water. Make up to 1000 ml, when cool.	Digestion Reagent (TFP) 6 g potassium persulphate mixed with approx. 60 ml distilled water and 25 ml digestion stock (heated and stirred). Make up to 100 ml when cool.
Molybdate Dissolve 10.84 g of $Na_2MoO_4\ 2H_2O$ in 500 ml distilled water. Make up to 1000 ml.	Mixed Reagent 1 (TFP) Dissolve 0.2 g ascorbic acid in a little distilled water. Add 25 ml antimony stock, 25 ml molybdate stock and make up to 100 ml with distilled water.
Antimony Dilute 53.3 ml of conc. H_2SO_4 with 500 ml distilled water, allow to cool then dissolve 0.728 g K(SbO) $C_4H_4O_6$ in this solution. Make up to 1000 ml with distilled water and filter if a precipitate appears.	Mixed Reagent 2 (FRP) Dissolve 0.2 g ascorbic acid in a little distilled water. Add 25 ml antimony stock, 25 ml molybdate stock and 10 ml digestion stock, then dilute to 100 ml with distilled water.

Calibration curves of phosphorus standards were made up from 1000 mg l^{-1} PO_4-P. Standards were made as 50 ml and split between analysis for FRP and TFP. Phosphorus analysis was the same for freshwater and seawater.

There are various problems associated with the molybdate method, such as in differentiating the fractions, but it is quick, cheap and effective at low concentrations in comparison to other methods (ion & gel chromatography, NMR, gel filtration, radiobioassay, enzyme assay, chemical etc.). It is widely used and problems may be overcome, for example humic acids may interfere with the detection of inorganic P using the molybdate method, causing an overestimate (Jones, 1992; McGarrigle & Kilmartin, 1992) but absorbance of water at 420 nm, the brown region of the spectrum, can be used as an estimation of the humic acid content and therefore indicates any possible level of interference. In order to test the effect of humic acid on phosphorus analysis, a sample from Red Sike with high absorbance at 420 nm, 0.035, was spiked with 50 $\mu g\ l^{-1}$ PO_4-P. There was no change in FRP concentration.

Replicates were always taken at Red Sike, but due to the large number of samples at Tyne Sands, only seawater replicates were taken for routine analysis, to check the accuracy of sampling. The highest standard deviations (n=4) were, for total P when the mean was 130.2 ± 8.44 $\mu g\ l^{-1}$, inorganic P when the mean was 114.0 ± 8.58 $\mu g\ l^{-1}$ and organic P when the mean was 16.2 ± 6.30 $\mu g\ l^{-1}$ all taken on 15 June 1992.

2.14 Nitrate

2.141 Freshwater

Defrosted water was analysed for nitrate using the method of Bendschneider and Robinson (1952), outlined by Stainton *et al.* (1977). Nitrate, in an alkaline buffered solution, was reduced to nitrite in a cadmium-copper reduction column. The column was adjusted so that 50 ml of solution ran through it in 4 - 6 min.

50 ml of sample was treated with 2.5 ml alkaline buffer and passed down the cadmium column. To avoid contamination, blanks were run through the cadmium column first and only the last 25 ml of each sample was collected for analysis. 0.5 ml

of sulphanilamide was then added and 0.5 ml N-1-naphthylethylenediamine-dihydrochloride (NNED) was added 5 min afterwards to yield a pink azo dye. The absorbance of the dye at 543 nm was proportional to the nitrate concentration of the sample. The colour was stable 10 min - 2 h. Readings were taken in 4 cm quartz cuvettes. The detection limit was 1 $\mu g\ l^{-1}$ $N0_3$-N (Parsons *et al.*, 1984).

2.142 Seawater

The analysis of nitrate from seawater is very similar to freshwater, though there are some differences. The procedure is outlined by Parsons *et al.* (1984). 1 ml of concentrated ammonium chloride was added to the samples before being reduced by the cadmium column.

Due to the effect of salt on nitrate concentration, standards were made up in synthetic seawater and the blanks were dilute ammonium chloride solution (Parsons *et al.*, 1984). The detection limit for analysis in seawater were also 1 $\mu g\ l^{-1}$ NO_3-N (Parsons *et al.*, 1984).

2.15 Nitrite

Because the method for nitrate analysis converted nitrate to nitrite in the cadmium column, any original nitrite in the sample would be encompassed in the nitrate fraction. Therefore nitrite analysis, similar to nitrate analysis but without the reduction step, was carried out on a few samples to check there was no significant concentration of nitrite present. The detection limit for nitrite was 1 $\mu g\ l^{-1}$ NO_2-N (Parsons *et al.*, 1984).

2.16 Ammonium

2.161 Freshwater

Ammonium analysis was carried out at the same time as the nitrate and nitrite analysis of the defrosted samples and was based on the method of Solorzano (1969). 25 ml of samples were treated with 1 ml phenol solution (100 g phenol dissolved in 95 % ethanol, made upto 1.0 l), then 1 ml sodium nitroprusside solution (1.0 g sodium

nitroprusside dissolved in MilliQ water and made upto 200 ml), then 2.5 ml oxidising solution (4 parts alkaline solution: 100 g sodium citrate and 5 g sodium hydroxide dissolved in 500 ml MilliQ water, and 1 part hypochlorite stock, made fresh daily). Indophenol blue was formed. The blue colour, with an absorbance of 640 nm, was proportional to the ammonium concentration. The solution was stable between 1 - 24 h. Readings were taken in 10 cm quartz cuvettes. The detection limit was 2 $\mu g\ l^{-1}$ NH_4-N (Parsons *et al.*, 1984).

2.162 Seawater

The procedure for seawater was similar to that of freshwater except that the blank was made using seawater where the ammonium content had been reduced: 1 l of seawater was boiled, after the addition of 5 ml 1M NaOH, until it had reduced to 0.7 l. The seawater was then neutralised with HCl; made up to 1.0 l with MilliQ water then filtered through a Whatman GF/C filter (Solorzano, 1969). The detection limit was also 2 $\mu g\ l^{-1}$ NH_4-N (Parsons *et al.*, 1984).

2.17 Cation analysis

Nuclepore filtered (0.2 μm) and unfiltered water samples were preserved with 0.2 ml of concentrated HNO_3. Samples were stored below 5 °C until they were analysed on a flame atomic absorption spectrophotometer for Fe, Mn, Ca (total only), Mg (total only) and K (total only). Detection limits for cations were: Fe, 0.030; Mn, 0.004; Ca, 0.050; Mg, 0.050 and K, 0.050, all in $mg\ l^{-1}$ (Livingstone & Whitton, 1984).

2.2 Biological analysis

2.21 Estimation of abundance

At Red Sike, *Rivularia biasolettiana* was the dominant organism, so percentage cover of the *Rivularia* colonies on the stream floor was estimated. At Tyne Sands, however, *R. atra* was more infrequent and *Ralfsia verrucosa* was also present in the pools so a scale of 1 - 5 of *Rivularia* abundance was more appropriate:

5 = very abundant
4 = abundant
3 = frequent
2 = occasional
1 = rare

2.22 Collection and storage of cyanobacteria and algae

Rivularia colonies (5 - 7 mm at Red Sike and 1 - 2 mm at Tyne Sands), "*Dichothrix*" community and *Ralfsia* (Tyne Sands only), were collected from rock substrate with forceps. The material was put into separate acid washed Petri dishes, with a few ml of stream or seawater. They were transported back to the laboratory on ice (1 - 4 h) and refrigerated (up to 24 h).

2.23 Taxonomy

On return to the laboratory, organisms were studied under a microscope and identified using Geitler's flora (1932).

On 19.10.1991 *Calothrix* was collected from Tyne Sands, identified and made axenic. After becoming axenic it appeared more similar to *Dichothrix* in Geitler's flora (1932). On 28.5.94 material from the same site appeared to be largely *Tolypothrix* (see 5.4). Morphological variability is known to exist in cyanobacteria, especially where the ecological features of environments may vary (Whitton, 1992). Without molecular studies the exact identification of this genera is impossible. For simplicity the field community from which the axenic culture originated is called the "*Dichothrix*" community.

Rivularia from Red Sike is referred to as *Rivularia biasolettiana* (see 1.42). *Rivularia* from Tyne Sands is *R. atra.*

2.24 Microscopy and photomicrography

Colonies were sectioned using a razor blade and mounted in a drop of the stream water or seawater and observed under a Nikon Fluophot microscope. Hairiness and the presence of hormogonia were recorded as percentage of trichomes with hairs or hormogonia. Colony diameter was also recorded.

Photomicrographs were taken with a 35-mm camera attached to the microscope on 100 ASA Tungsten film.

2.25 Alkaline phosphatase assay

2.251 Standard phosphatase assay

Phosphomonoesterase activity is referred to as phosphatase activity, as no other esters were used. Phosphatase activity was measured in organisms and filtered water. All methods used 100 mM buffers (Table 2.2) made up in assay medium.

Table 2.2 Buffers used in standard alkaline phosphatase assays

pH	Buffer	Buffering capacity	pKa at 20 °C
7.0 - 8.0	HEPES - NaOH	6.8 - 8.2	7.5
8.5	HEPPS - NaOH	7.3 - 8.7	8.0
9.0 -10.5	glycine - NaOH	8.6 - 10.6	9.6
11.0	CAPS - NaOH	9.8 - 11.1	10.4

Two types of assay medium were used. For freshwater assays Chu 10D assay medium (Table 2.3), based on Chu 10D medium (Chu, 1942) and modified by Gibson and Whitton (1987) but without N and Si, with 1.00 rather than 1.78 mg l^{-1} P and 0.124 rather than 1.44 mg l^{-1} B, was used. The concentration of these elements was that of Grainger *et al.* (1989) using *Calothrix parietina* D550. Assay medium was different from culture medium in that no phosphorus was present, K_2HPO_4 was replaced by KCl and the Fe-EDTA concentration was halved.

For saline assays the assay medium was modified by adding salts from the medium ASP-6 (Fries, 1963) at half concentration, to achieve 12 ‰ salinity and is referred to as 50 % ASP-6-Chu 10D (Table 2.4).

Table 2.3 Freshwater phosphatase assay medium based on Chu 10D

Chemical	mg l^{-1}	µM	Element	mg l^{-1}	µM
$CaCl_2 \cdot 2H_2O$	35.83	243.7	Cl	20.43	576.3
$MgSO_4 \cdot 7H_2O$	25.00	101.4	Ca	9.77	243.7
$NaHCO_3$	15.85	188.6	Na	4.74	198.1
KCl	4.28	57.4	S	3.26	101.7
Na_2 EDTA $\cdot 2H_2O$	1.67	4.20	Mg	2.46	101.4
$FeCl_3 \cdot 6H_2O$	1.21	4.50	K	2.24	57.3
AC micro elements			Fe	0.250	4.50
H_3BO_3	0.715	11.56	B	0.124	11.50
$ZnSO_4 \cdot 7H_2O$	0.056	0.190	Zn	0.013	0.190
$MnCl_2 \cdot 4H_2O$	0.045	2.280	Mn	0.012	0.218
$NiSO_4 \cdot 7H_2O$	0.038	0.135	Ni	0.008	0.030
$CuSO_4 \cdot 5H_2O$	0.020	0.078	Cu	0.005	0.078
$CoSO_4 \cdot 7H_2O$	0.010	0.035	Mo	0.003	0.013
$Na_2MoO_4 \cdot 2H_2O$	0.007	0.028	Co	0.002	0.037

Table 2.4 Saline phosphatase assay medium based on 50 % ASP-6-Chu 10D

Chemical	mg l^{-1}	µM	Element	mg l^{-1}	µM
Na Cl	12000	205340	Cl	7488	127147
$MgSO_4 \cdot 7H_2O$	4000	16240	Na	4720	80751
KCl	354.3	4750	S	520	2111
$CaCl_2 \cdot 2H_2O$	75.0	510	Mg	396	1620
$NaHCO_3$	15.8	189	K	186	2490
Na_2 EDTA $\cdot 2H_2O$	1.67	4.20	Ca	20.5	139.2
$FeCl_3 \cdot 6H_2O$	1.21	4.50	Fe	0.25	4.50
KI	0.10	0.60	I	0.08	0.460
KBr	0.08	0.70	Br	0.06	0.470
AC micro elements as for Chu 10D (see Table 2.3)					

There was no phosphorus present in either the buffers or the assay medium, so the organic P substrate was the only external source of phosphorus available to the organisms. As the substrates used were monoesters, the product formed was in direct proportion to the amount of organic P broken down.

Standard assays were carried out at 25 °C and 100 μmol photon m^{-1} s^{-1} from daylight fluorescent tubes. Gentle shaking was employed to ensure mixing during the assay.

The length of assay depended on a combination of the substrate concentration and enzyme activity and was calculated from time course experiments. This insured that the organisms were not substrate limited (< 15 % conversion to product) during the course of the assay. Colonies did not take up product (Appendix i).

Long-term field assays were 10 min duration for organisms and 1 h for water samples. Assays for the long-term field studies were carried out using 250 μM pNPP at pH 10.3, considered optimal for alkaline phosphatase activity (Grainger *et al.*, 1989), and those using 250 μM and 1 μM 4-MUP were carried out at pH 7.6, closer to the environmental pH. The lower concentration of 4-MUP was intended to be closer to that of organic P in the environment.

Kinetic assays and pH spectra using pNPP were run for 40 min while those using 4-MUP were run for 10 min, because of the higher sensitivity of 4-MUP. All kinetic assays and time courses (Chapter 6 and 7) were carried out at pH 9.0, unless otherwise stated (see 6.1 and 7.1).

In routine assays, two *Rivularia biasolettiana* and three *R. atra* colonies were used per sample. This number was chosen due to a combination of chlorophyll extraction and phosphatase activity. Colonies were teased with forceps and cultures were homogenised using needles. In both cases filaments were checked under the microscope and rejected if damaged.

In time course experiments 10 colonies of *R. biasolettiana* and 30 for *R. atra* were used. All time course experiments used a total of 30 ml assay volume. A quantity of assay was taken out per time interval and the decreasing assay volume accounted for in the calculation of activity (n=1).

2.252 pNPP assay

Using freshwater assay medium

The method for phosphatase activity using pNPP was based on Grainger *et al.* (1989). Cyanobacteria or algal material was added to 1.4 ml of Chu-10D assay medium mixed with 1.5 ml of buffer in a snap cap vial. The vials were transferred to a water bath at the correct temperature and allowed to equilibrate for 10 min. 0.1 ml of substrate, at 30 times the required concentration, was added to the assay. At the end of the assay, 2.5 ml of the assay solution was mixed with 0.25 ml of 5 M NaOH which enhances the colour, brings up the pH of the assay to above 11, terminates the activity of phosphatase enzymes and prevents further hydrolysis of the substrate (Boon, 1993). Control vials were set up in the same way as the experimental vials, but without organism present. Organism present controls (without substrate) were also set up when using homogenised material, and added to the substrate (without organism) controls. Readings were taken in 1 cm quartz cuvettes at 405 nm.

Using saline assay medium

An alteration was made to the above method to overcome the effect of the MgOH precipitation (caused by reaction between NaOH and $MgSO_4$ above pH 10.5). The volumes were doubled and 5 ml of assay was mixed with 0.5 ml of terminator in a narrow upright tube, to allow the precipitate to settle. The terminated solution was left for one hour, during which time the colour was stable, then readings could be taken at 405 nm as above. This method gave the same result as that used by Hernández *et al.* (1992). Buffer used in saline experiments were made up in saline assay medium.

Using filtered water (stream or seawater)

Filtered stream water or seawater was added to buffer instead of assay medium so that phosphatase activity of enzymes in the water sample could be tested. As above, assay medium (without filtered water) with substrate was used as a control.

2.253 4-MUP assay

Using both freshwater and saline assay medium

This method was based on a modification of that published by Fedde and Whyte (1990). As for pNPP, the material to be assayed was added to the assay medium and buffer totalling 2.9 ml in snap cap vials and transferred to a water bath to equilibrate for 10 min. 0.1 ml of substrate (dissolved in a small volume of warm 100 % ethanol then made up to volume with assay medium) at 30 times the final concentration required, was added at the beginning of the assay. The assay was terminated by taking 2.5 ml of assay solution and adding it to 0.25 ml of terminator (to give a final concentration of 2.5 mM EDTA, 50 mM K_2HPO_4, 50 mM NaOH) in 1 cm polystyrene cuvettes. The terminator maintained a constant reading. EDTA and K_2HPO_4 inhibit phosphatase activity and the concentration of NaOH ensured that the pH was above 10 which is the pH of maximum fluorescence (Chróst & Krambeck, 1986). Readings were taken on a BAIRD-ATOMIC spectrofluorimeter with excitation wavelength at 356 nm and emission wavelength at 444 nm. Controls (no organism present, unless homogenised material was used, see 2.252) were set up in the same way as for the experiment.

Using filtered water (stream and seawater)

As for the method using pNPP, assay medium was substituted for filtered water. Controls were assay medium rather than filtered water.

2.254 Calibration

pNPP and 4-MUP assay calibration curves of reading against product concentration, are given in Appendix ii for freshwater and saline assay media. Values for 1 μmol l^{-1} product, from these graphs were included in the calculation of activity (see 2.256).

Detection limits using pNPP were 3.52 x 10^{-4} μmol pNP μg chl a^{-1} h^{-1} for organisms and 0.252 μmol pNP l^{-1} h^{-1} for water. Using 4-MUP detection limits were 9.46 x 10^{-6} μmol 4-MU μg chl a^{-1} h^{-1} for organisms and 6.76 x 10^{-3} μmol 4-MU l^{-1} h^{-1} for water.

2.255 Biomass determination

Deposition of $CaCO_3$, external to the sheath of *Rivularia* trichomes (Pentecost, 1978), cause the use of dry weight to be invalid in determining colony biomass. Chlorophyll *a* is often used instead (Khoja *et al.*, 1984; Livingstone & Whitton, 1984).

This method follows the recommendations of Marker *et al.* (1980a) with reference to Marker *et al.* (1980b) and Marker and Jinks (1982). It was thought unnecessary to measure phaeopigments, due to a decrease in accuracy caused by this method using methanol as the solvent (A.F.H. Marker, pers. comm.), and because neither the field material or the cultured material appeared unhealthy.

Solid material

Assayed material was kept after the assay and frozen in the snap cap vials. After unfreezing, the material was washed in distilled water, chopped up to increase the surface area and transferred to universal bottles. 5 ml of 100 % boiling methanol was added to each universal bottle which was covered in aluminium foil to extrude light and stoppered to prevent loss of solvent. The bottles were left at least 30 min until material fragments went white, after the chlorophyll *a* had been extracted. Material fragments were then removed by filtration and the solution was diluted to 90 % methanol. The solution was read on a spectrophotometer at 665 nm and at 750 nm, previously blanked on 90 % methanol.

The following equation was adopted for the determination of chlorophyll *a* in each sample (Marker *et al.*, 1980a).

$$\text{Chlorophyll } a \ (\mu g \ l^{-1}) = K \ (A_{665} - A_{750}) \ v \ / \ L \qquad (2)$$

Where A is absorbance reading, K is 1000 x the reciprocal of the specific absorption coefficient (SAC) for chlorophyll *a* at 665 nm (the SAC for chlorophyll *a* in methanol was taken to be 13), v is the volume of solvent used to extract the sample in ml and L is the path length, in cm, of the cuvette.

Homogenised material

A proportion of homogenised material from the phosphatase assay was filtered. The filter paper, with the homogenised material on it, was treated with 100% methanol in the same way as above.

2.256 Calculation of phosphatase activity

For organisms using pNPP and 4-MUP phosphatase activity was expressed as μmol product μg chl a^{-1} h^{-1}, using the formula:

$$\text{Phosphatase activity} = A \times v \,/\, Ap \times c \times t \qquad (3)$$

Where A is absorbance / fluorescence reading after subtraction of control, v is the total assay volume in litres, A_p is the absorbance / fluorescence reading of 1 μmol l^{-1} standard product (see Appendix ii), c is chlorophyll a in μg and t is the assay time in hours.

For filtered water using pNPP and 4-MUP the equation is as above except v is the total assay volume / volume of filtered water. Phosphatase activity of filtered water is expressed as μmol product l^{-1} h^{-1}.

2.257 Staining

Material was stained for phosphatase activity using 1 mM BCIP. The procedure was exactly the same as that of the pNPP assay, except that BCIP was used as the organic P substrate and the reaction was not terminated (Grainger *et al.*, 1989). BCIP formed a blue dye where the phosphorus had been hydrolysed. After 30 min incubation, the material was mounted on a microscope slide and photographed.

2.3 General culturing techniques

2.31 Growth Medium

Two types of growth media were used; one freshwater Chu 10D medium (Chu, 1942), Table 2.5, incorporating the modifications of Gibson and Whitton (1987) and Grainger *et al.* (1989), and one artificial seawater 50 % ASP-6-Chu 10D, Table 2.6. As for assay medium, saline culture medium was based on Chu 10D with 50 % ASP-6 salts added; a modification of Fries (1963).

Table 2.5 Chu 10D growth medium (Chu, 1942)

Chemical	mg l^{-1}	µM	Element	mg l^{-1}	µM
$CaCl_2 \cdot 2H_2O$	35.87	243.7	Na	39.2	1061
$MgSO_4 \cdot 7H_2O$	25.00	101.4	Cl	19.3	544.4
$NaHCO_3$	15.85	188.6	Ca	9.77	243.7
KH_2PO_4	4.38	32.2	S	3.26	101.7
Na_2 EDTA $\cdot 2H_2O$	3.34	8.30	EDTA	2.66	6.60
$FeCl_3 \cdot 6H_2O$	2.42	8.90	Mg	2.46	101.4
NaOH	c 60	1500	K	1.26	27.5
HEPES	600	2517	P	1.00	7.30
			Fe	0.500	9.00
AC micro elements as for assay medium (see Table 2.3)					

HEPES (to give a final concentration of 2.5 mM) was dissolved in about 600 ml of MilliQ water, the pH was then adjusted to pH 7.6 by addition of approximately 1.5 ml 1 M NaOH; stocks were then added. KH_2PO_4 was added last to avoid precipitation. The volume was made up to 1.0 l with MilliQ water. 25 ml of liquid medium was added to each 100 ml conical flask, bungs (Sanko Plastics Co., Japan) were twisted down firmly and the flasks were autoclaved at 1 bar for 20 min. After autoclaving, the flasks were left for 12 h for the gasses to equilibrate before the material was subcultured.

Sterilised solid media (1 % w/v agar) was poured onto pre-sterilised disposable Petri dishes (Sterling) in a laminar flow cabinet, pre-sprayed with 70 % ethanol 15 min before use, and left in the laminar flow cabinet to set.

Table 2.6 50 % ASP-6-Chu 10D medium based on Chu (1942) and Fries (1963)

Chemical	mg l^{-1}	µM	Element	mg l^{-1}	µM
NaCl	12000	205340	Cl	7487	127115
$MgSO_4 \cdot 7H_2O$	4000	16240	Na	4755	81613
KCl	350	4695	S	520	2111
$CaCl_2 \cdot 2H_2O$	75	510	Mg	396	1620
$NaHCO_3$	15.8	189	K	185	2461
KH_2PO_4	4.38	32.2	Ca	20.5	139.2
Na_2 EDTA $\cdot 2H_2O$	3.34	8.30	EDTA	2.66	6.60
$FeCl_3 \cdot 6H_2O$	2.42	8.90	P	1.00	7.30
KI	0.10	0.60	Fe	0.500	9.00
KBr	0.080	0.70	I	0.080	0.460
NaOH	c 60	1500	Br	0.060	0.470
HEPES	600	2517			
AC micro elements as for assay medium					

2.32 Subculturing

Cultures were subcultured once a month in a laminar flow cabinet. Aseptic techniques were adopted. Approximately 200 µl of the culture was transferred in a wire loop, from the old to the new medium.

2.33 Isolation of cyanobacteria

Immediately on return to the laboratory, samples were inoculated on to Petri dishes with several types of appropriate media and incubated at 25 °C in 100 µmol photon m^{-2} s^{-1} continuous light. For *Dichothrix* D861, 862, 863 & 866 (Durham

Culture Collection); ASP-6 Chu 10D (Table 2.6) was used with different concentrations of agar and salts. Cycloheximide was added at 50 mg l^{-1} (Zehnder & Hughes, 1958) to remove eukaryotes. Once the material had started growing (2 - 4 days), it was possible to see hormogonia spreading out from the initial inoculum. Hormogonia farthest from the other species of cyanobacteria or bacteria were picked off the agar with a fine needle under a dissecting microscope and removed to fresh media to minimise transferring contaminants.

Once the cyanobacterium was axenic, it was cultured in liquid medium. Bacterial test media (nutrient broth, SST and peptone - glucose) were used to check for the absence of bacteria (Hoshaw & Rosowski, 1979). After inoculation, the plates were incubated in the same conditions as the culture and also at 37 °C in darkness.

2.4 Computing and Statistics

Most of the computing was done on a Macintosh Quadra 610 though a Viglen 486 PC was used for SPSS. MS Excel (version 4.0) was used as a spreadsheet for storing data, calculating phosphatase activity and for basic statistics (mean, standard deviation and t-test). SPSS for Windows was used for calculating Spearman's rank correlations, and MS Word (version 5.1) was used for word processing. Mac Draw Pro was used for drawing maps and Cricket Graph (version 1.5) was used for constructing graphs.

All the data (nutrient concentrations and phosphatase activity) for the long-term studies at both sites are largely low values which peak sharply on several occasions. The data are not normal neither can it be readily normalised using natural logarithms or transformations worked out using Taylor's Power Law and the F_{max} test (Kelly & Onyeka, 1992). Non-parametric statistics were therefore adopted to analyse these data. Sanden and Danielsson (1995) used non-parametric statistics when analysing nutrients "to reduce problems with the distributional properties of the data". Since the sequence of the maxima is important in comparing the changes in variables, Spearman's rank correlation was used. The interpretation of the data is not compromised by losing the actual concentrations or phosphatase activity, however

highly variable data may decrease the significance between compared samples because of the ranking procedure. For this reason the significance of correlations were always compared to graphs.

Graphs, single factor Analysis of Variance and Student's t-tests were used to analyse the intensive study data. r^2 was used as a measure of the proportion of variability explained by the regression. All regression lines were significant ($p \leq 0.050$) unless otherwise stated. The equation of the line is positioned on the graph according to the order on the graph. An equality of slopes test (Zar, 1984), based on regression coefficients, was used to compare the slopes of time courses or Lineweaver - Burk kinetic plots in the experimental studies.

Where < 20 variables are compared, *** $p \leq 0.001$, ** $p \leq 0.010$, * $p \leq 0.050$. Where > 20 variables are compared * ≤ 0.050 is dropped to avoid Type 1 error (Kelly & Onyeka, 1992). All error bars indicate s.d.

V_{max} was calculated from Lineweaver - Burk kinetic plots (1/v vs. 1/S) (Hernández *et al.*, 1995) and K_m values were calculated from Eadie - Hofstee plots (v vs. v/S) as the distribution of errors in this plot is more even (Price & Stevens, 1982). V_{max}/K_m was used to compare the competitive ability of phosphatase enzymes (Healey, 1982). Where Lineweaver - Burk plots appeared to be non-linear, Hill plots (log [v / V_{max}-v] vs. log [S]) were used to obtain n_H. If $n_H < 1.0$ non Michaelis - Menten kinetics is implied, indicating apparent negative cooperativity (Hernández *et al.*, 1995).

CHAPTER 3

STUDY AREA AND SAMPLING PROGRAM

3.1 Red Sike

Red Sike is one of a series of similar streams in Upper Teesdale, England, where the water chemistry has been studied (Livingstone and Whitton, 1984). The phosphatase and nitrogenase activity of *Rivularia biasolettiana* colonies have also been studied (Livingstone & Whitton, 1984; Livingstone *et al.*, 1984, respectively). The presence of *Rivularia* throughout the year and the results of the previous studies, made Red Sike an ideal site to choose to compare with a marine site.

The colonies range in size from 2-15 mm in diameter (Fig. 3.1). The smaller colonies are not apparent throughout the whole year. It was observed that colonies vary in calcification, with colonies closest to the sides of the stream being more calcified than those in the centre.

Fig. 3.1 *Rivularia biasolettiana* colonies dominating the bottom of Red Sike (large dark patches - amalgamation of colonies, and dots - individual colonies, 2 - 15 mm), during a period of extremely low flow (D. Livingstone, May 1981)

Red Sike is a freshwater stream running N-E to S-W, in the Upper Teesdale National Nature Reserve, County Durham, grid reference NY 816 295 (Fig. 3.2). It is one of a series of streams running into Cow Green Reservoir, at an altitude of approximately 500 m. Pigott (1978) found ice cover to be present throughout many of the winter months but during the three years of this study, the winters were milder than a decade or so ago. The stream is shallow, < 180 mm deep, with a flat bottom of bedrock and a maximum width < 10 m. Flow levels fluctuate depending on precipitation, but the stream has an approximate mean discharge of 0.04 m^{-2} s^{-1} (Crisp & Howson, 1982). Red Sike is dominated to the N-E by Widdybank Fell, approximately 30 m higher than the stream, with Cow Green Reservoir to the S-W.

Fig. 3.2 Red Sike study area looking up-stream to the N-E (24.4.1993)

The stream cuts through glacial drift, exposing a bedrock of limestone with occasional bands of shale and sandstone (Johnson, 1978). Coarsely crystalline marble ("sugar limestone") has been formed in this area by the metamorphosis of a quartz-

dolerite intrusion within some of the limestone. Minerals have been mined from the area which has led to spoil heaps.

The vegetation in the catchment of the stream is a complex mosaic which comprises of three types of flushed ecosystems described by Bellamy and Tickle (1964):

1. Drainage axes of the blanket mire
2. Spring-water from acid rocks, mainly the quartz-dolerite and glacial drift
3. Spring-water from limestone

The most abundant cyanobacteria are *Rivularia biasolettiana* Menegh. (see 1.42) and *Schizothrix lardacea*, (Ces.) Gom (Livingstone & Whitton, 1984). The red colour of the sheath of *Schizothrix*, as well as the iron oxide present, is probably responsible for the name Red Sike. In the spring there is typically a growth of diatoms, mainly *Cymbella* spp. this is followed by filamentous Conjugales: *Mougeotia*, *Spirogyra* and *Zygnema* (Livingstone & Whitton, 1984).

3.2 Tyne Sands

Tyne Sands, the marine study site, is very different to the freshwater site. Like the freshwater site it has been studied previously (Khoja *et al.*, 1984). These authors found some phosphorus features similar to those of the freshwater site; high levels of organic P. However, this site had higher concentrations of total P and less abundant *Rivularia* than the freshwater site. The site was chosen because *Rivularia* were present, it was apparently contrasting to the freshwater site and previous data were available for comparison.

Tyne Sands is a sheltered bay, surrounded by agricultural farmland on the estuary of the Scottish River Tyne. The site is located at grid reference NT 636 812 and is part of the John Muir Country Park, Dunbar. The mean annual rainfall is 560 mm. The bay is protected by a spit.

The study site is classified as upper eulittoral intertidal region (Dring, 1992). The upper eulittoral consists of rock pools (Fig. 3.3). All the pools chosen for study

contained *Rivularia atra* and *Ralfsia verrucosa* and were in the zone covered by > 4.5, < 4.8 m tides, except *Rivularia* pool 9 (R9) which was chosen for sampling the "*Dichothrix*" community from mats surrounding it (see 5.4). R9 was covered by tides ≥ 5.2 m. It was not until July 1992 that *R. atra* were discovered in this pool. The circumference of the *Rivularia* pools varied between 1 - 25 m, and the pools were < 200 mm deep (Fig. 3.4). R5 was much smaller than the other pools.

Fig. 3.3 Tyne Sands from R9, looking west over the rock pool zone (10.6.1993)

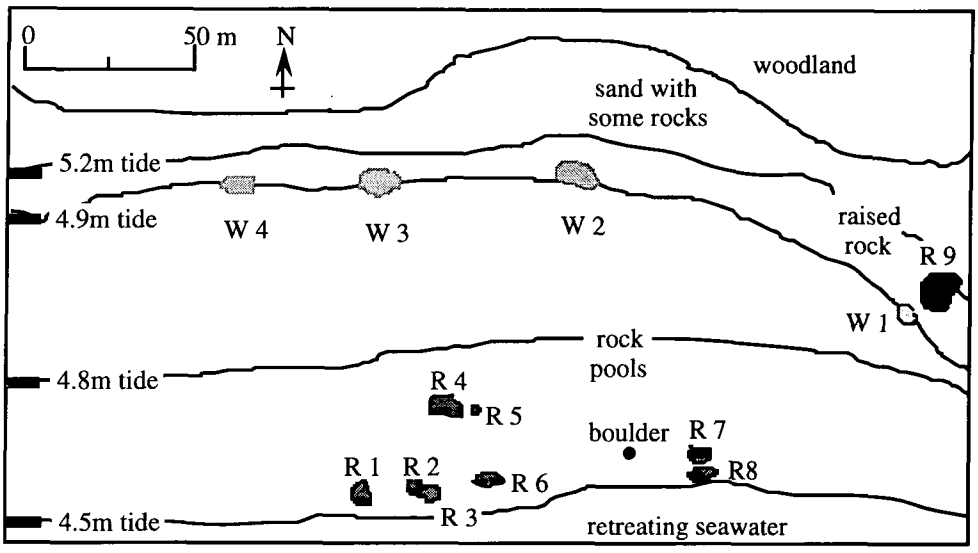


Fig. 3.4 Map of Tyne Sands study area

The pools of the upper eulittoral cover a zone of about 75 m width on calciferous sandstone or loose rocks overlying sand and silt. Within this zone of rock pools, is a zone of about 25 m width, at the lower end of the rock pool zone (Fig. 3.4), where *Rivularia atra* (Roth) is seasonally abundant (Fig. 3.5). *Ralfsia verrucosa* (Areschoug) is abundant in almost all the rock pools (Fig. 3.5). *Chondrus crispus, Cladophora laetevirens* and *Enteromorpha intestinalis* were observed growing in some of the *Rivularia* pools, at times. *Fucus spiralis* L. sometimes grew in the pools, but was much more common on the exposed rock above R1-8. After neap tides, especially in summer, *Fucus* became very desiccated on the outer layers. *Gammarus* spp. were present in the pools as were periwinkles (*Littorina* spp.) which were extremely abundant, at times. Periwinkles were observed feeding on *Ralfsia* but not on *Rivularia* (Fig. 3.5).

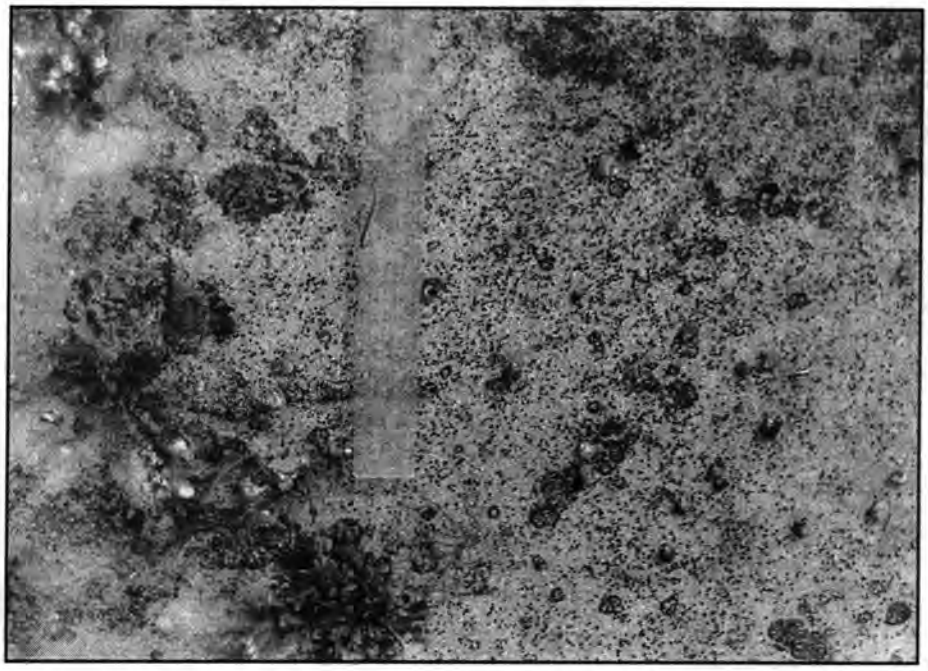


Fig. 3.5 *Rivularia atra* (dark spots < 2 mm) and *Ralfsia verrucosa* (dark patches > 5 mm) with periwinkles and *Fucus spiralis* , 300 mm ruler also shown (10.8.93)

Throughout most of the year there were piles of seaweed, mostly *Laminaria* species, deposited by the high tides (Fig. 3.6). At some times of year these piles were larger and / or in a greater state of decay (Khoja *et al.*, 1984).

R9 and weed pool 1 (W1) were situated on rock (Fig. 3.4), at the most easterly end of the bay. Because of the height of the rock at R9, only tides above 5.2 m, covered this pool. Tide heights were based on Admiralty Tide Tables (1992 - 4) and are approximate as wind conditions are important in determining the tide height on particular days.

W1 was based on rock but the other weed pools were less permanent because they were based on sand. This meant that W2 - 4 were not always the same pools on each sampling occasion although they were always located in the same area (within 5 m).

Fig. 3.6 Decomposing seaweed deposited on the strand line, at the top of the eulittoral, at Tyne Sands (D. Livingstone, 13.11.1981)

3.3 Sampling programme

Long-term study

The long-term study period began, at both sites, in April 1992 and ended in May 1993 at Red Sike, and October 1993 at Tyne Sands. Both sites were sampled monthly. Red Sike was sampled usually on the first Monday of the month and Tyne Sands on the second. Samples from Tyne Sands were collected 1 - 3 h after high tide.

At Red Sike water temperature, conductivity, pH and alkalinity were measured. Current speed and estimation of flow were taken. Water was sampled for absorbance (420 nm), phosphorus, nitrate, nitrite, ammonium, cation analysis and phosphatase assays. *Rivularia biasolettiana* and other algal species were collected for identification and phosphatase assays. Percentage *R. biasolettiana* on the stream floor

and proportion of colonies in each size class, (small (‹ 3 mm), medium (3 - 9 mm) and large (› 9 mm)) were estimated.

At Tyne Sands water temperature, conductivity and salinity were measured in each pool and seawater. Water pH was measured after May 1993. Water samples were taken for phosphorus, nitrate, nitrite, ammonium analysis and phosphatase assays. *R. atra* abundance was estimated in each *Rivularia* pool. *R. atra*, "*Dichothrix*" community (throughout the study period) and *Ralfsia verrucosa* (after December 1992) were collected for phosphatase assays.

At both sites morphological studies of *Rivularia* were taken, whereby the percentage of hair / hormogonia and the length of the hair were measured.

Intensive studies

Water chemistry was studied at Red Sike during the spring of 1993 and 1994, and at Tyne Sands during summer of 1993 and 1994. These were the times just before and during maximal phosphorus concentrations at both sites in 1992. Intensive studies were initiated to obtain more information about the phosphorus maxima.

Experimental studies

During the intensive study period, maxima in phosphatase activity were different for the two substrates at Tyne Sands and the organisms appeared to show maxima at different times (see 5.5). With the isolation of "*Dichothrix*" from Tyne Sands (*Dichothrix* 861) and the axenic freshwater *Calothrix parietina* D550, studies could be undertaken with field material and axenic cultures to investigate the kinetics of the different species using the two substrates. Field material was collected in June 1994 at Red Sike, and July 1994 at Tyne Sands.

CHAPTER 4
RED SIKE FIELD STUDY

4.1 Introduction

Previous studies found *Rivularia biasolettiana* colonies to be dominant on the bottom of this small oligotrophic stream, Red Sike (Livingstone & Whitton, 1984; Livingstone *et al.*, 1984). Livingstone and Whitton (1984) found extremely high levels of organic P in spring 1981 (see 1.21) and speculated that this was one important reason why the stream was dominated by an organism that was able to utilise organic P.

This chapter contains data collected during the long-term study, April 1992 - May 1993 (and meteorological data January 1992 - May 1994), together with intensive studies of water chemistry, spring 1994 (see 3.3). The purpose of the long-term study was to find out if there was a relationship between phosphorus fractions in the stream compared to the phosphatase activity and morphology of *R. biasolettiana* colonies (see 1.5). The intensive studies were undertaken in order to focus on the spring peak in phosphorus found by Livingstone and Whitton (1984) and also found in the long-term study (see 4.21).

Pools, approximately 50 m from Red Sike on slightly higher ground, appeared on top of the blanket mire in spring. Four of these bog pools and a spring, 300 m upstream from the sampling point in Red Sike, were sampled in the intensive studies in spring 1994.

4.2 Environment

4.21 Long-term study

Physical variables

Mean monthly meteorological data (I. Findlay, pers. comm.), from a station 0.5 km from Red Sike, showed clear seasonal maxima and minima temperatures, with minima rarely falling much below freezing. Sunshine showed similar seasonal variation, with the exception of November 1993, an exceptionally sunny month (Fig. 4.1).

Snowfall showed a similar seasonal pattern with highest levels of snow falling in the coldest periods, with least sunshine. However rainfall was more erratic, with no clear seasonal pattern (Fig. 4.2).

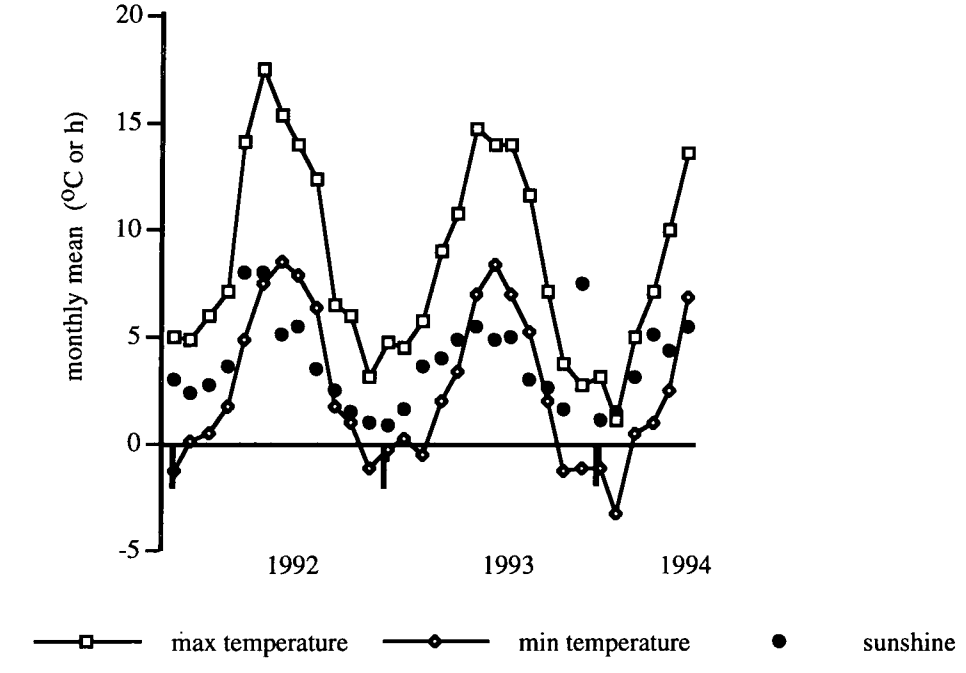


Fig. 4.1 Mean temperature (maximum and minimum) and sunshine at meteorological station, 0.5 km from Red Sike, January 1992 - June 1994

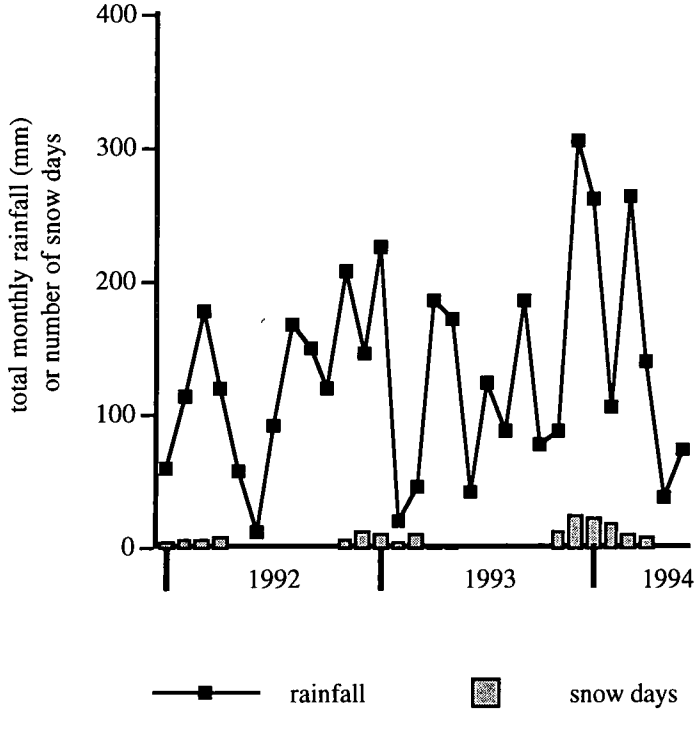


Fig. 4.2 Total monthly rainfall and total number of snow days per month from meteorological station, 0.5 km from Red Sike, January 1992 - June 1994

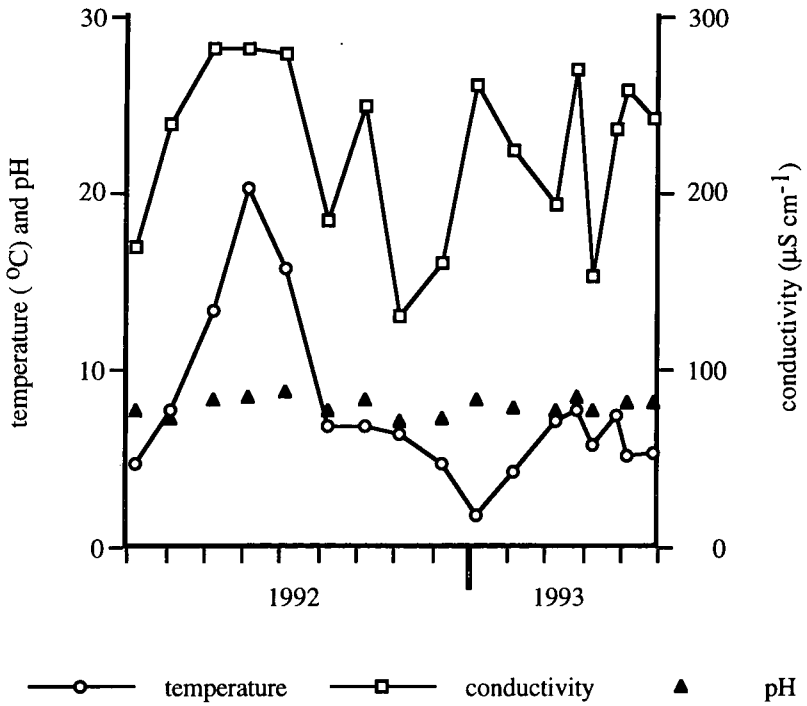


Fig. 4.3 Temperature, conductivity and pH of water from the stream, Red Sike, during monthly sampling, April 1992 - May 1993 (two samples March - May 1993)

The pattern and values of water temperature spot samples (Fig. 4.3), were similar to maximum air temperature monthly means (Fig. 4.1). Physical data obtained during the long-term study period showed the seasonality of temperature, though conductivity was less clearly seasonal. pH appeared stable around a mean of 7.99, ranging from a minima of 7.2 in November 1992 and maxima of 8.8 in August 1992 (Fig. 4.3).

Current speed showed a similar pattern to width (Fig. 4.4), with the stream tending to flow faster when it was wider. Total alkalinity was positively correlated (Appendix iii) with conductivity and negatively correlated to current speed and width, suggesting greater buffering capacity when the stream was slower flowing. Total alkalinity showed an inverse relationship to absorbance: absorbance may be higher when drainage was mainly from the bog since bog pool water had far lower conductivity.

In the spring and summer, absorbance of the water at 420 nm increased and decreased at the same time as stream width; indicating clearer water when the width was less, perhaps because there was less runoff (Fig. 4.5). However in the winter months absorbance of the water at 420 nm showed a reciprocal relationship to width.

Although these were spot samples, they may be indicative of the type of drainage the stream was subjected to; i.e. from blanket bog when the width and the absorbance (420 nm) were high, and from the spring when absorbance was low.

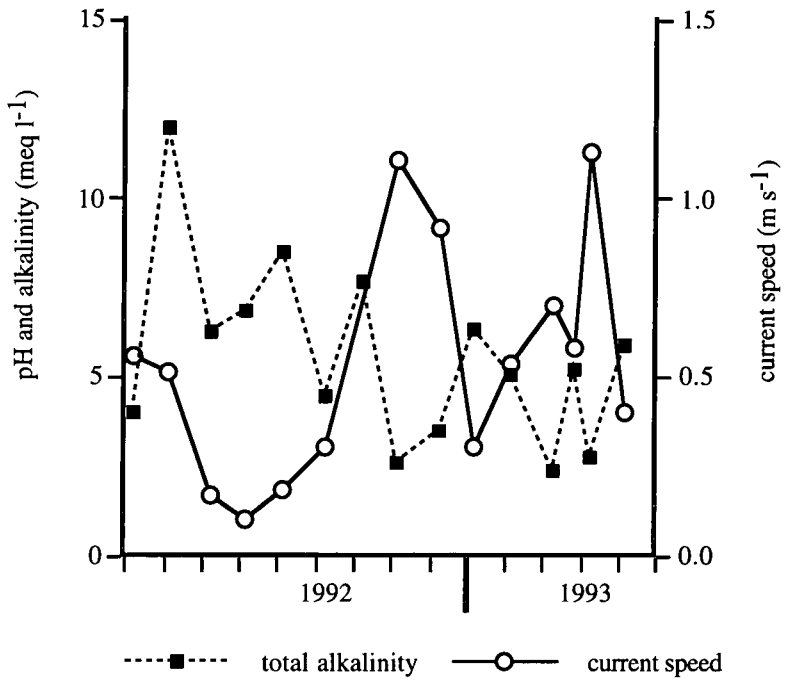


Fig. 4.4 Total alkalinity and current speed of stream water during monthly sampling at Red Sike, April 1992 - May 1993 (two samples March 1993)

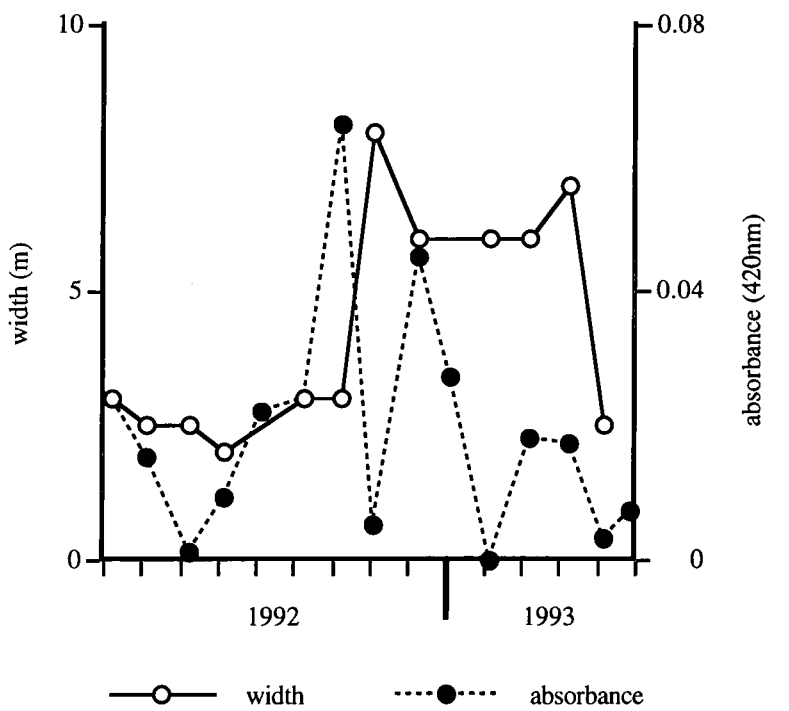


Fig. 4.5 Width of the Red Sike at sampling point and absorbance (420 nm) of water samples taken monthly, April 1992 - May 1993

Chemical variables

Phosphorus

Phosphorus was slightly higher in spring (Fig. 4.6). This appeared to be largely inorganic. Inorganic P peaks were 35.1 μg l^{-1} in 1992; 9.6 μg l^{-1} in 1993 and 6.7 μg l^{-1} in 1994 with a mean of 0.8 (± 1.15 μg l^{-1}) μg l^{-1} at other times. In 1992 organic P peaked in June and August. For most of the year the low levels of total P were predominantly organic but these levels were often very close to detection limits. Organic P was positively correlated to total P, but inorganic P was not correlated to either total or organic P (Appendix iii).

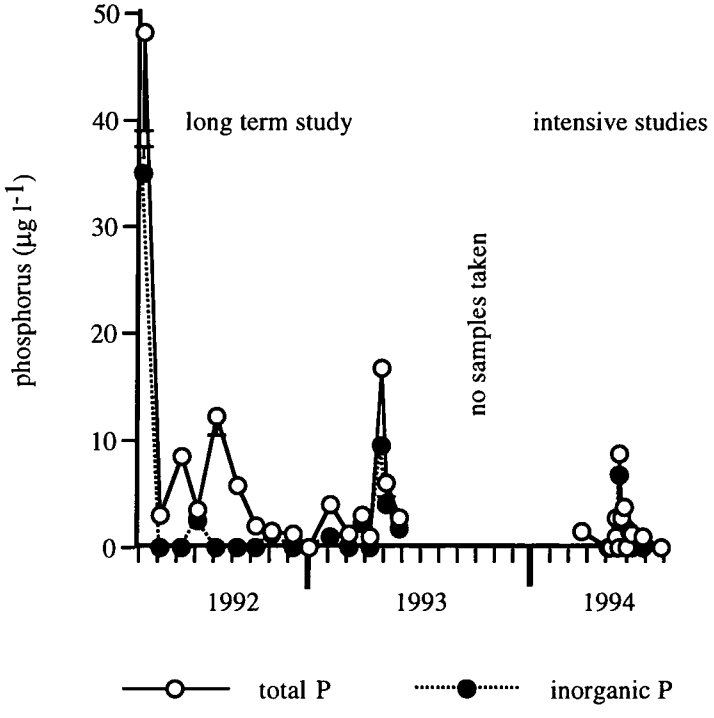


Fig. 4.6 Total and inorganic P concentrations in water sampled monthly from Red Sike, April 1992 - May 1993 (two samples March - May 1993) and sampled intensively (see Fig. 4.10) March - July 1994 (n = 4)

Nitrate and Ammonium

Nitrate and ammonium showed a similar seasonal pattern to phosphorus with higher concentrations in spring and lower concentrations in the autumn / winter (Fig. 4.7). Maximum values of both fractions occurred in April 1992 and March 1993.

Concentrations of ammonium make up the smaller proportion of the total N. Both N fractions were positively correlated (Appendix iii).

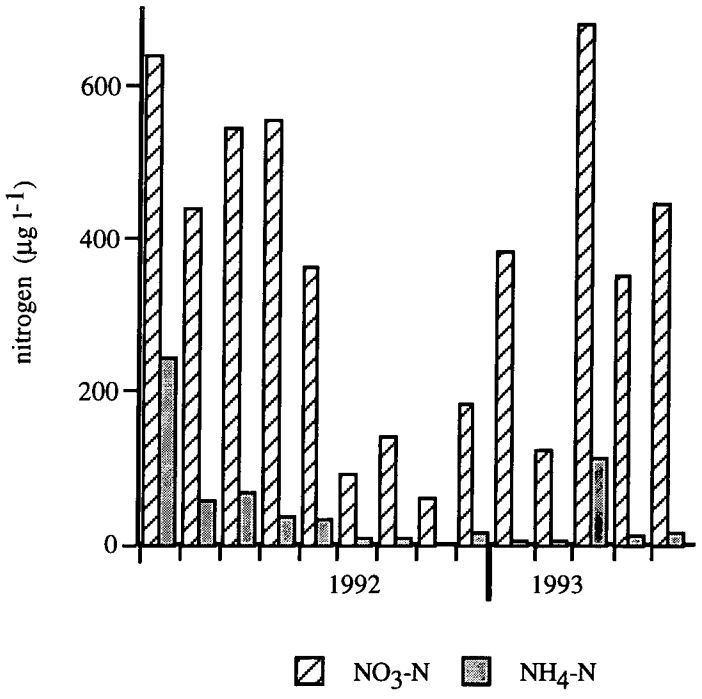


Fig. 4.7 Nitrogen (NH_4-N and NO_3-N) fractions in water from Red Sike sampled monthly April 1992 - May 1993

Cations

Fig. 4.8 gives an indication of the changes in the concentrations of Fe, Mn, Ca, Mg and K in Red Sike water during the study period. The relationship of these to one another and the other variables is given in Appendix iii.

Total and filtrable Fe were highly correlated, as were total and filtrable Mn. Total Fe was also correlated to the absorbance of water at 420 nm, though filtrable Fe was not. Ca and Mg were positively correlated. Both were also correlated to conductivity, but were negatively correlated with current speed and stream width (Appendix iii).

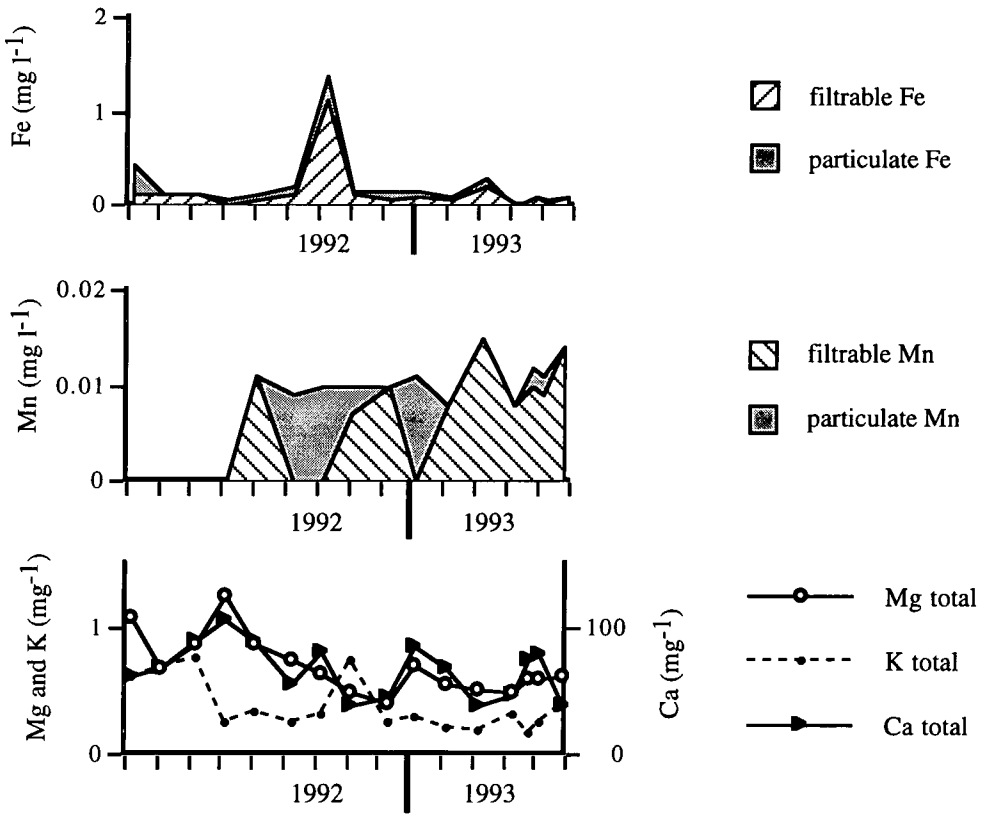


Fig. 4.8 Particulate and filtrable concentrations of Fe and Mn, and total concentrations of Mg, K and Ca in water from Red Sike taken at monthly intervals between April 1992 - May 1993

4.22 Intensive studies

Intensive studies were carried out in the spring of 1994 with the aim of finding possible sources for the increased phosphorus concentrations in Red Sike during the spring of the long-term study. Therefore in addition to sampling water from Red Sike, a small spring feeding into Red Sike and four bog pools, on a slight elevation above Red Sike (< 50 m), were selected for sampling. Intensive studies in 1994 involved:

1. Studies on P fractions of water from Red Sike, a spring and bog pools using different porosity filters
2. Routine sampling of phosphorus, temperature, conductivity, pH and absorbance (420 nm) carried out every few days during the spring 1994
3. Hourly samples taken on 27.4.94 for phosphorus, temperature, conductivity and pH
4. An overnight study of phosphorus fractions was also carried out on 1-2.5.94

Filter Studies

The difference in concentration of total and inorganic P was tested on unfiltered water (total) and water filtered through different porosities (filtered): 1.2 µm (GF/C), 0.7 µm (GF/F) and 0.2 µm (Nuclepore). Four samples were taken from each site. The results showed that there was no significant difference in phosphorus concentration between any of the filtrates and total phosphorus in the stream or spring (Fig. 4.9). Analysis of Variance however, showed that total unfiltered P in bog pool water was significantly different from the other fractions ($p \leq 0.001$). There was no significant difference between any of the inorganic P samples from any of the sites. Inorganic P was very low at all three sites. No samples were taken for unfiltered inorganic P at any of the sites. These results suggest that a large proportion of the phosphorus in the bog pools was probably particulate organic P.

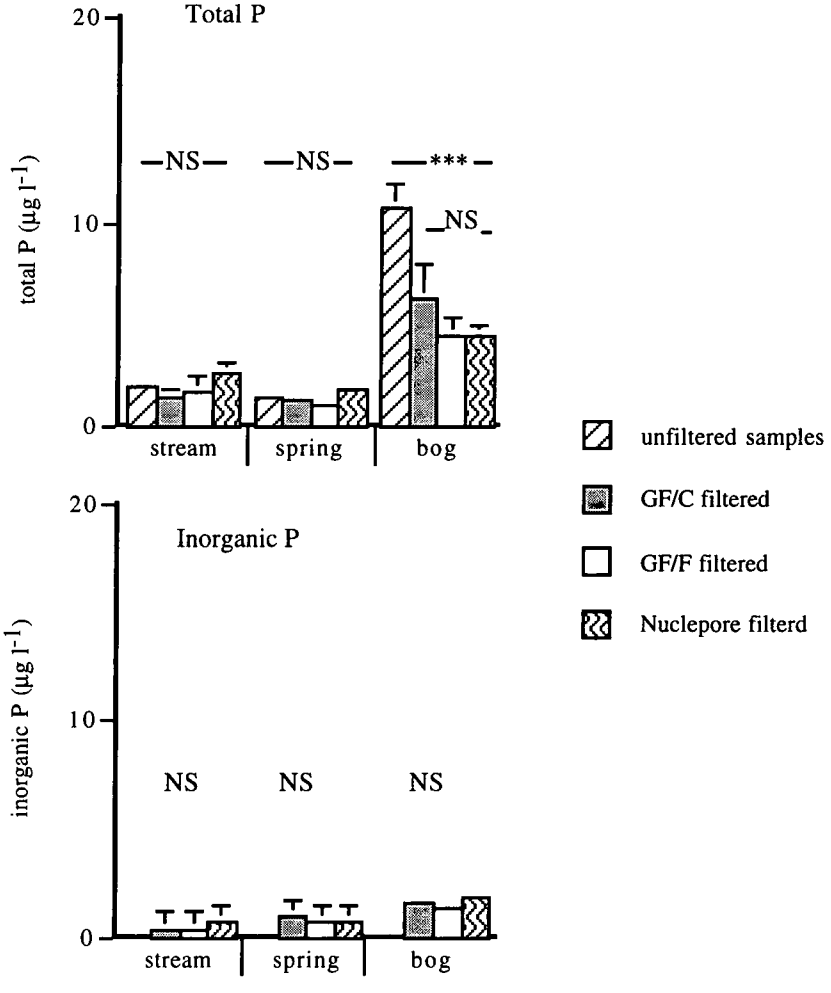


Fig. 4.9 Comparison of total and inorganic P concentrations in unfiltered water and water filtered through 1.2 µm, 0.7 µm and 0.2 µm porosity filters from Red Sike, spring and bog pools, on 23.5.1994 (n = 4) No samples taken for unfiltered inorganic P

Routine spring sampling

The studies of phosphorus fractions taken between March and May 1994 in Red Sike (Fig. 4.10), show that the proportion of organic P in the spring was near detection (3 $\mu g\ l^{-1}$). When the total P was greatest, on 12.5.94 inorganic P was 6.7 $\mu g\ l^{-1}$ and total P was 8.8 $\mu g\ l^{-1}$. Most other sampling occasions the proportion of inorganic P was also the larger proportion of total P, though phosphorus levels were generally close to detection limits. The degree of variation between the samples was small, when measured on 5.5.94.

Water from the spring also contained a larger proportion of total P in the inorganic form. On 27.4.94 there was a peak of 31.2 $\mu g\ l^{-1}$ total P of which 97 % was inorganic.

The bog pool samples, unlike samples from the spring or stream, were taken from four separate pools on the bog, rather than from the same location. The error bars indicate large variation between the four pools. Inorganic P appears less variable than organic P. The highest levels of phosphorus were found on 9.5.94, with mean total P of 9.6 $\mu g\ l^{-1}$ and mean inorganic P of 4.3 $\mu g\ l^{-1}$ and on 12.5.95 with mean total P of 11.5 $\mu g\ l^{-1}$ and mean inorganic P of 3.6 $\mu g\ l^{-1}$ (Fig. 4.10). The pattern of change of phosphorus fractions in the spring and bog pools is similar to that of Red Sike. However, a more detailed study is needed to determine the influence of each on Red Sike.

There was quite a large degree of variability between the stream samples even though they were collected from the same location. It may be that drainage predominantly from the bog or stream or a combination of the two, alters the phosphorus fractions in the stream. It was thought that absorbance may indicate the source of water, being higher when originating from the peat bog. However, the absorbance of the spring water, suspected to be clear, did change slightly during the sampling period (Fig. 4.11). It seems possible that a pulse of inorganic P in the stream may originate from the spring, whereas a pulse of organic P may originate from the bog. Spot samples may miss pulses in phosphorus as it was likely that they are

flushed quickly from the stream. Increased intensity of spot sampling was undertaken to try to increase the likelihood of monitoring such pulses.

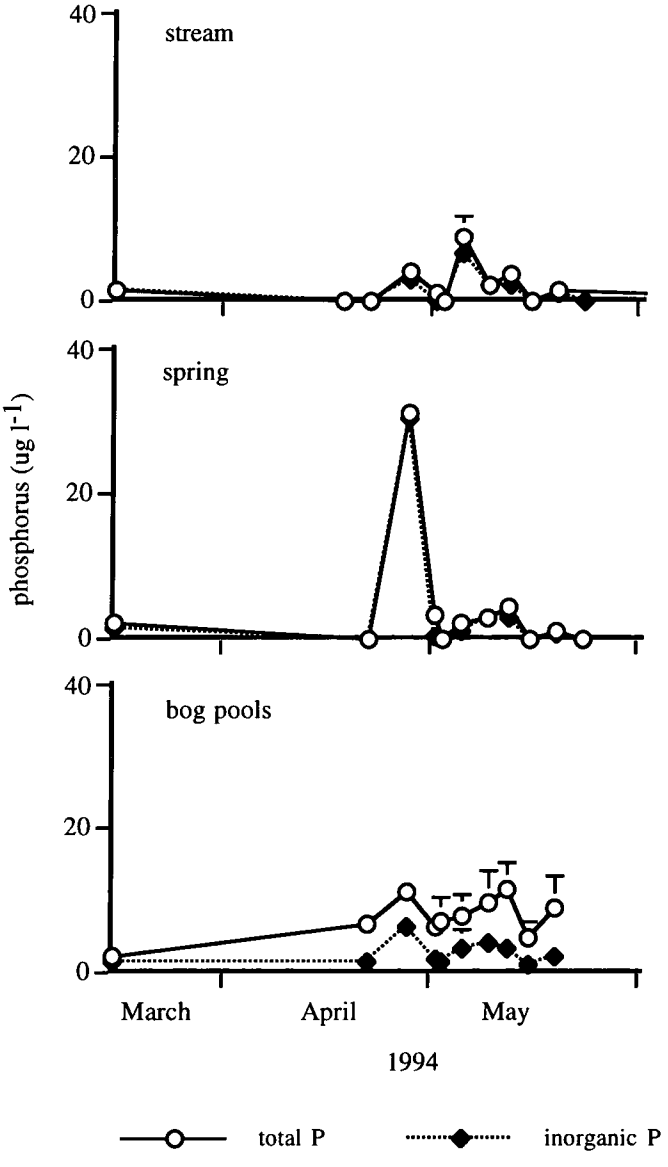


Fig. 4.10 Mean concentrations of total and inorganic P in water from Red Sike, spring and bog pools, March - May 1994 (n = 4)

During this period of study, conductivity and pH remained fairly stable in the water at each site: 8.2 (± 0.10) in Red Sike, 7.8 (± 0.20) in the spring and 4.6 (± 0.2) in one bog pool. However all sites showed changes in temperature, especially the stream and the bog pools (Fig. 4.11). The spring temperature remained colder than the coldest samples in the stream and bog (Fig. 4.11), never rising above 6 °C. The absorbance of water was highest in the bog pools, though these were highly variable

(Fig. 4.11). Water from Red Sike and the spring had lower absorbance than the bog pools.

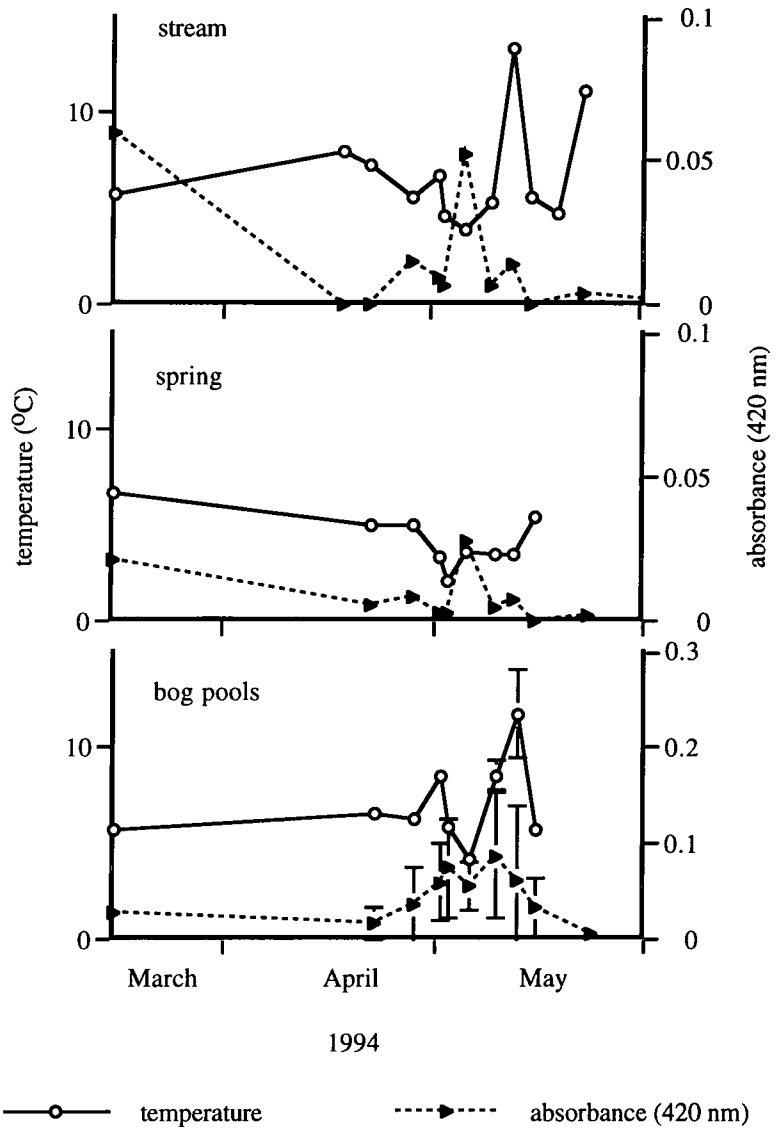


Fig. 4.11 Temperature and absorbance of water from Red Sike, spring and 4 bog pools sampled intensively (every few days), March - May 1994 (n = 4, bog pools only)

Day study

The day study of the Red Sike, spring and one bog pool water showed the same result to the routine sampling: water from the spring was largely inorganic and from the bog pools had a greater proportion of organic P (Fig 4.12). However, Red Sike was more similar to the spring than during the long-term study with most of the phosphorus in the water being inorganic. At 1000 the sample containing the largest

concentration of phosphorus was collected from the spring, more than 90 % was inorganic.

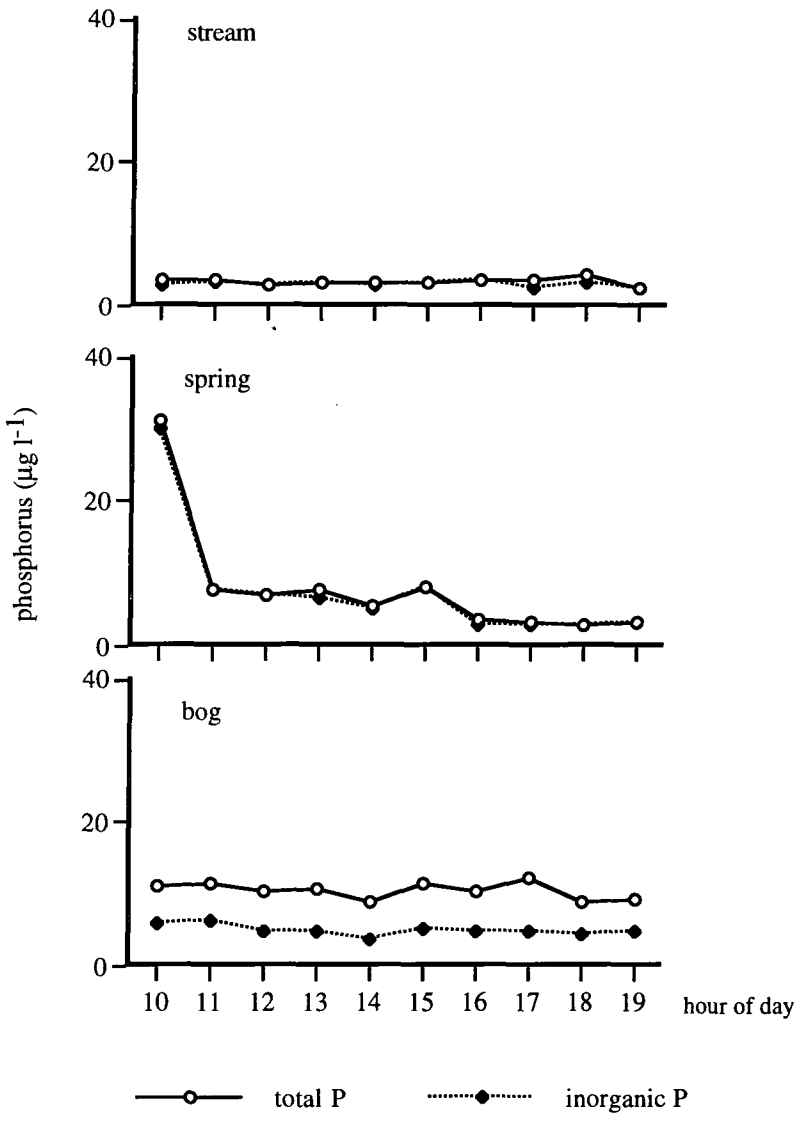


Fig. 4.12 Total and inorganic P concentrations of water samples taken hourly (1000 - 1900 BST) from Red Sike, a spring and a bog pool during one day, 27.4.94 (n = 4, taken on first stream sample only)

Conductivity increased during the day in the bog pool and the stream, but in the spring the conductivity remained stable, except at 1100 when it rose 4 fold (Fig. 4.13).. Conductivity in the stream was similar to that of the spring but at least four times higher than in the bog. pH remained stable, at 8.2 (± 0.08) in Red Sike and 8.1 (± 0.34) in the spring and 5.0 (± 0.08) in the bog pool. The temperature in the Red Sike

peaked to 8.6 °C at 1300 and peaked to 9.3 °C at 1600 in the bog, after a brighter period. The temperature of the bog was slightly higher than Red Sike, 7.6 °C (± 0.81) and 6.5 °C (± 0.90) respectively, and both were higher than the spring, with a mean of 4.6 °C (± 0.51).

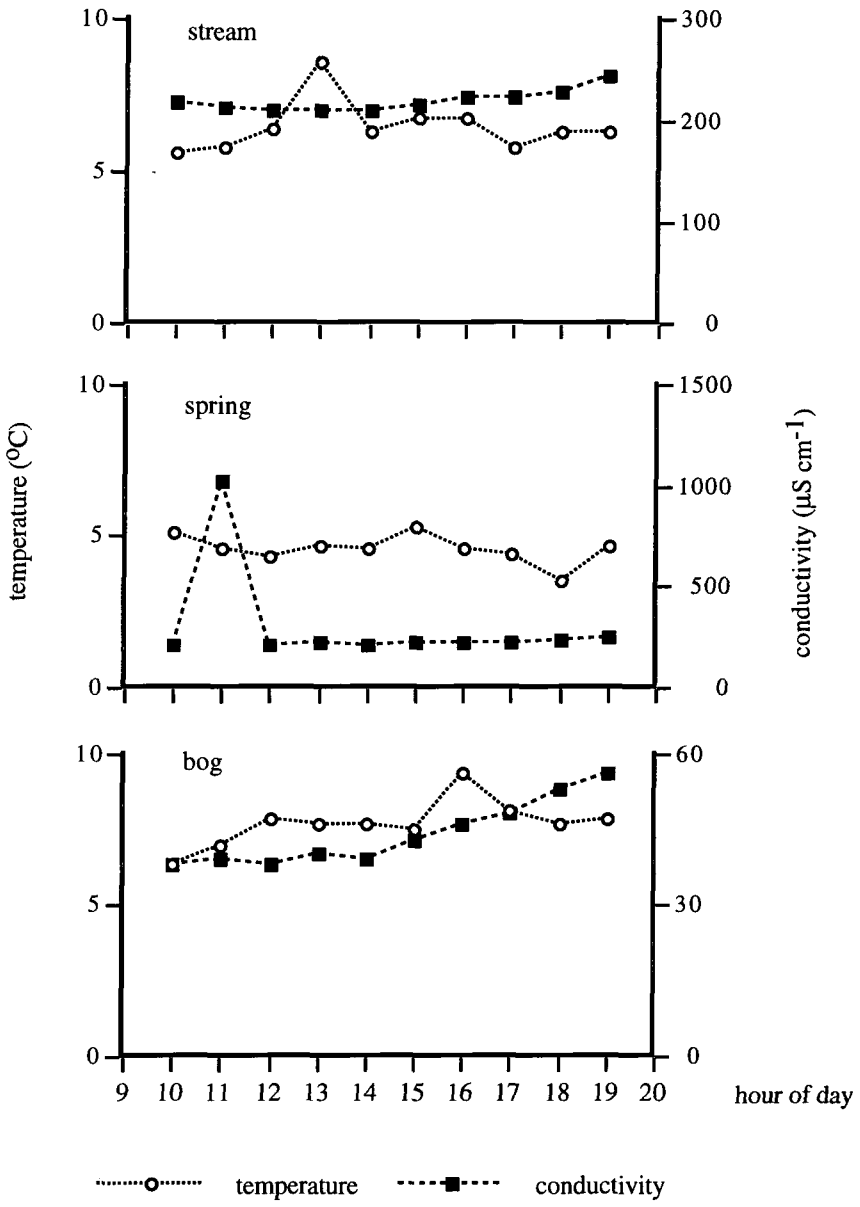


Fig. 4.13 Temperature, conductivity and pH of water from Red Sike, a spring and a bog pool, sampled hourly during one day from 1000 - 1900 BST, 27.4.94

Overnight study

During the overnight study there was little difference in the phosphorus concentration in the four bog pools between samples taken at 1900 (1.5.94) and those

taken the next morning, at 0700 (Fig. 4.14). Total P was largely organic on both occasions, though variability in the organic fraction was slightly greater in the morning. All total P in Red Sike was organic, though at 1900 the concentration of phosphorus was barely above detection (1.5.94). There was no detectable phosphorus in the morning. In the spring, the majority of phosphorus was in the organic fraction at 1900 (1.5.94) and there was no detectable phosphorus in the morning. Previously all phosphorus in the spring had been inorganic. At all three sites the temperature was higher in the evening than the morning: lowest in the spring and highest in the bog pools.

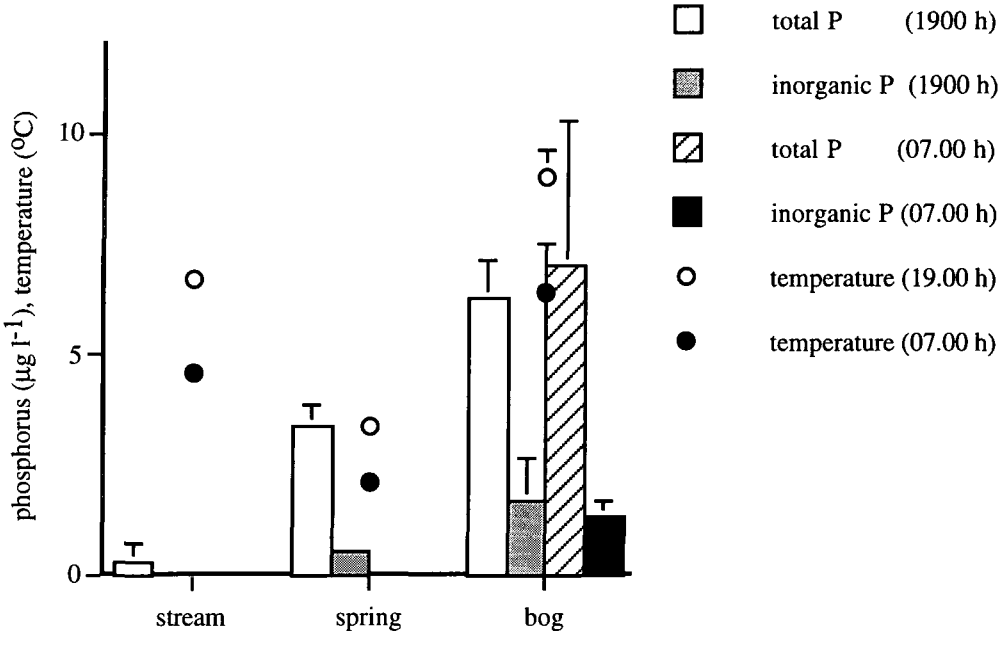


Fig. 4.14 Temperature, total P and inorganic P of water from Red Sike, a spring and 4 bog pools sampled at 1900 h BST on 1.5.94 and again at 0700 h BST on 2.5.94 (n = 4)

pH was slightly higher in the evening at all sites (Fig. 4.15). Absorbance was low in the stream and the spring but high in the bog.

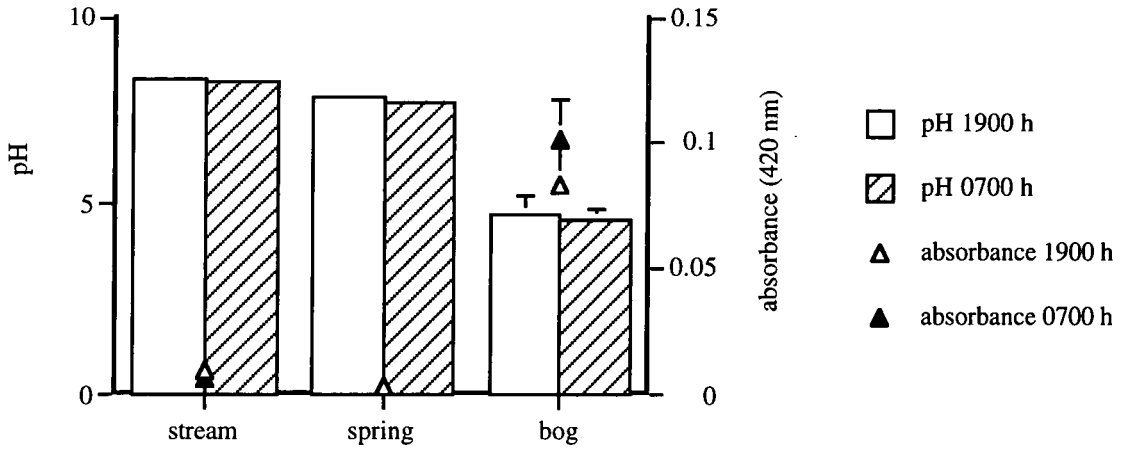


Fig. 4.15 Absorbance (420 nm) and pH of water from Red Sike, a spring and 4 bog pools taken at 1900 h BST (1.5.94) and again at 0700 h BST (2.5.94) (n = 4, bog pools only)

Phosphorus fractions appear changeable especially overnight. The spot sampling undertaken in this study may not be frequent enough to pick up phosphorus pulses which may be present in this stream.

4.3 Occurrence and morphology of *Rivularia biasolettiana*

During most of the sampling period, *Rivularia* appeared to be the dominant organism (> 50 % cover of stream floor). However the percentage cover of *Rivularia* was less during late winter and early spring, building up to nearly 100 % in May or June (Fig. 4.16). Hormogonia production appears to be restricted to the late winter / early spring when *Rivularia* abundance was lowest, suggesting that the hormogonia were responsible for the increased abundance in late spring. Small colonies may not be detectable, by eye, until they have grown beyond the microscopic stage, which may take several weeks. The colonies possessed a large percentage of hairs throughout the sampling period, dropping slightly when hormogonia were observed. It was often the case that the colonies would remain hairy even when hormogonia were visible, these appeared on the outer edge of the colony and did not appear to be associated with a particular filament. These observations suggest that the peak time of hormogonia production was never sampled: it may be comparatively transitory.

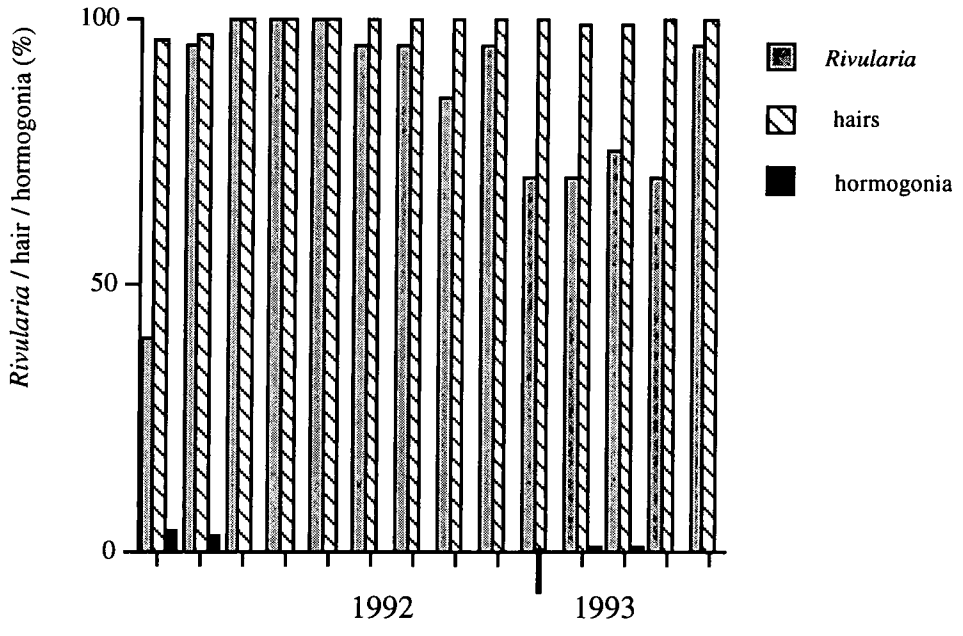


Fig. 4.16 Percentage cover of *Rivularia biasolettiana* colonies, % filaments containing hairs and % filaments containing hormogonia, collected from Red Sike once a month between April 1992 - May 1993

The percentage cover of *Rivularia* was positively correlated to temperature (Appendix iii). Percentage of hairs in colonies were negatively correlated to percentage of hormogonia in colonies. Hormogonia were seasonally restricted in occurrence (Fig. 4.16) and, due to the growth of microscopic colonies (Fig. 1.5), hormogonia were not present in colonies by the time new small colonies became visible. Percentage cover of *Rivularia* increased a few weeks after hormogonia were found in colonies, in summer 1992.

4.4 Phosphatase activity

Although 250 μM pNPP and 250 μM 4-MUP were assayed at different pH, the two are graphed together in order to compare the timing of peaks in activity rather than to compare the values of activity (see 2.251).

4.41 Filtered water

Phosphatase activity of filtered stream water was not assayed until May 1992. At the higher substrate concentration, phosphatase activity of filtered water was highest

in May 1992 (10 μmol pNP l^{-1} h^{-1} and 0.6 μmol 4-MU l^{-1} h^{-1}). Using 250 μM pNPP, activity peaked again in September (7.2 μmol pNP l^{-1} h^{-1}) and November 1992 (8.8 μmol pNP l^{-1} h^{-1}), Fig. 4.17. Activity using 250 μM 4-MUP showed a general decline after the May 1992 peak, with a slight peak in March 1993 (0.1 μmol 4-MU l^{-1} h^{-1}). Using 1 μM 4-MUP activity peaked in July (0.16 μmol 4-MU l^{-1} h^{-1}) and March 1993 (0.03 μmol 4-MU l^{-1} h^{-1}) and was nearly as great as activity using 250 μM 4-MUP; being a maximum of half the activity at 250 μM 4-MUP in July 1992 and May 1993 and a minimum of 40 times less than the activity using 250 μM 4-MUP in May 1992, perhaps indicating enzyme affinity for 4-MUP.

Phosphatase activity of filtered water using 4-MUP showed a positive correlation to temperature (Appendix iii). 250 μM pNPP and 250 μM 4-MUP were positively correlated to one another. Activity using all three substrates showed a negative influence of inorganic P.

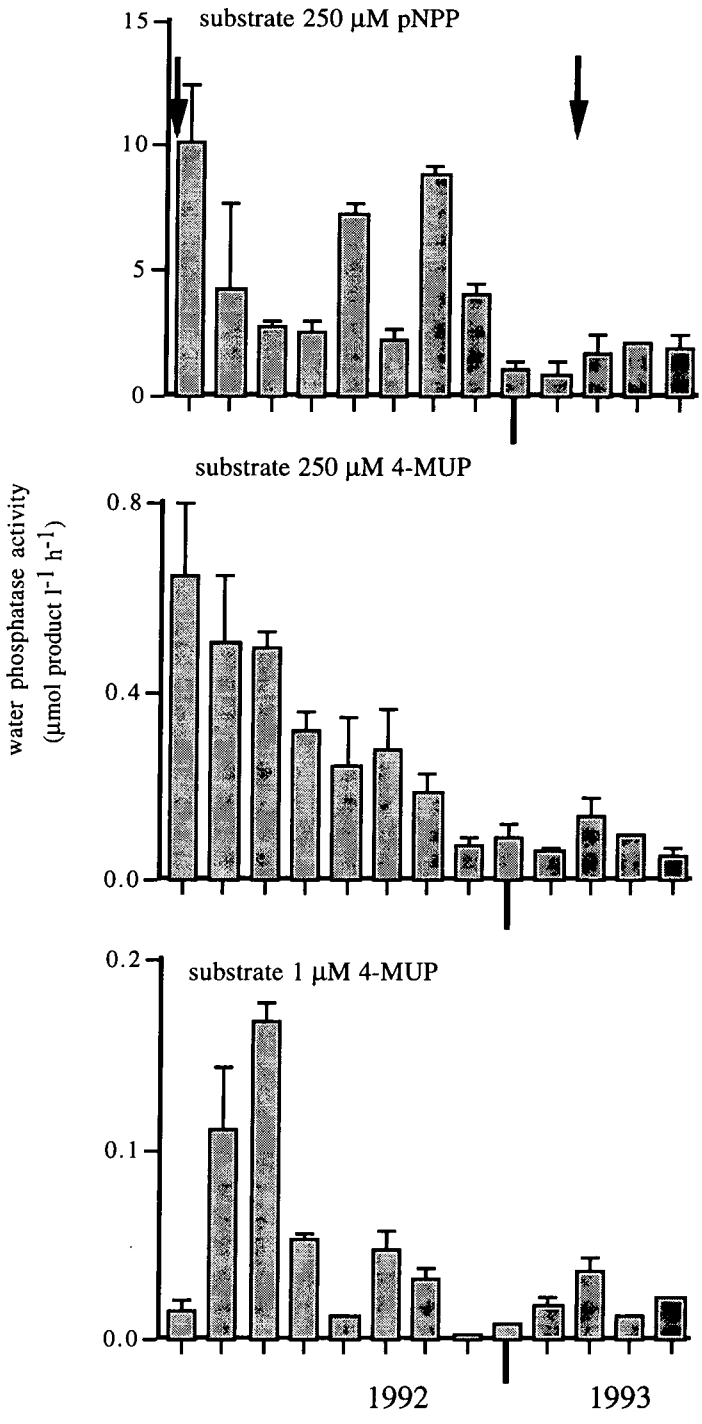


Fig. 4.17 Phosphatase activity of GF/F filtered water from Red Sike, using 250 µM pNPP at pH 10.3 and using 250 µM and 1 µM 4-MUP at pH 7.6, sampled monthly May 1992 - May 1993 arrows indicate when hormogonia were present in *Rivularia* colonies (n = 4)

4.42 *Rivularia biasolettiana*

Rivularia phosphatase activity was generally high and very variable, as can be seen in the error bars, with no clear distinctive seasonal peaks in any of the substrates. There were slight troughs in activity, indicated by the arrows in Fig. 4.18, when hormogonia were found in colonies. Peaks in activity using 250 μM pNPP and 250 μM 4-MUP occurred in November 1992 (0.076 μmol pNP μg chl a^{-1} h^{-1} and 0.013 μmol 4-MU μg chl a^{-1} h^{-1}) and April 1992 (0.12 μmol pNP μg chl a^{-1} h^{-1} and 0.014 μmol 4-MU μg chl a^{-1} h^{-1}). Both at 250 μM and at 1 μM 4-MUP, activity peaked in January 1993 (0.014 and 0.0014 μmol 4-MU μg chl a^{-1} h^{-1}). Activity was high using 1 μM 4-MUP throughout autumn 1992 (above 0.0009 μmol 4-MU μg chl a^{-1} h^{-1}). In April 1993 the peak was 0.002 μmol 4-MU chl a^{-1} h^{-1}.

As with the filtered water, activity using 1 μM 4-MUP as a substrate was a large proportion of that at 250 μM: maximum difference was 23 times less than that at 250 μM in November 1992, and a minimum of 2.7 times less in August 1992. This suggests high affinity of the enzyme(s) for 4-MUP (see enzyme characteristics in 6.3). Activity may vary more markedly than monthly spot samples would indicate.

Activity using 250 μM 4-MUP was positively correlated to activity using 1 μM 4-MUP (Appendix iii). Phosphatase activity of colonies using pNPP was positively correlated to percentage of hairs in colonies and negatively correlated to hormogonia. Phosphatase activity of *Rivularia* colonies was negatively related to inorganic P (250 μM pNPP and 1 μM 4-MUP). Phosphatase activity of *Rivularia* using 250 μM pNPP and 1 μM 4-MUP showed the positive influence of temperature and hair length.

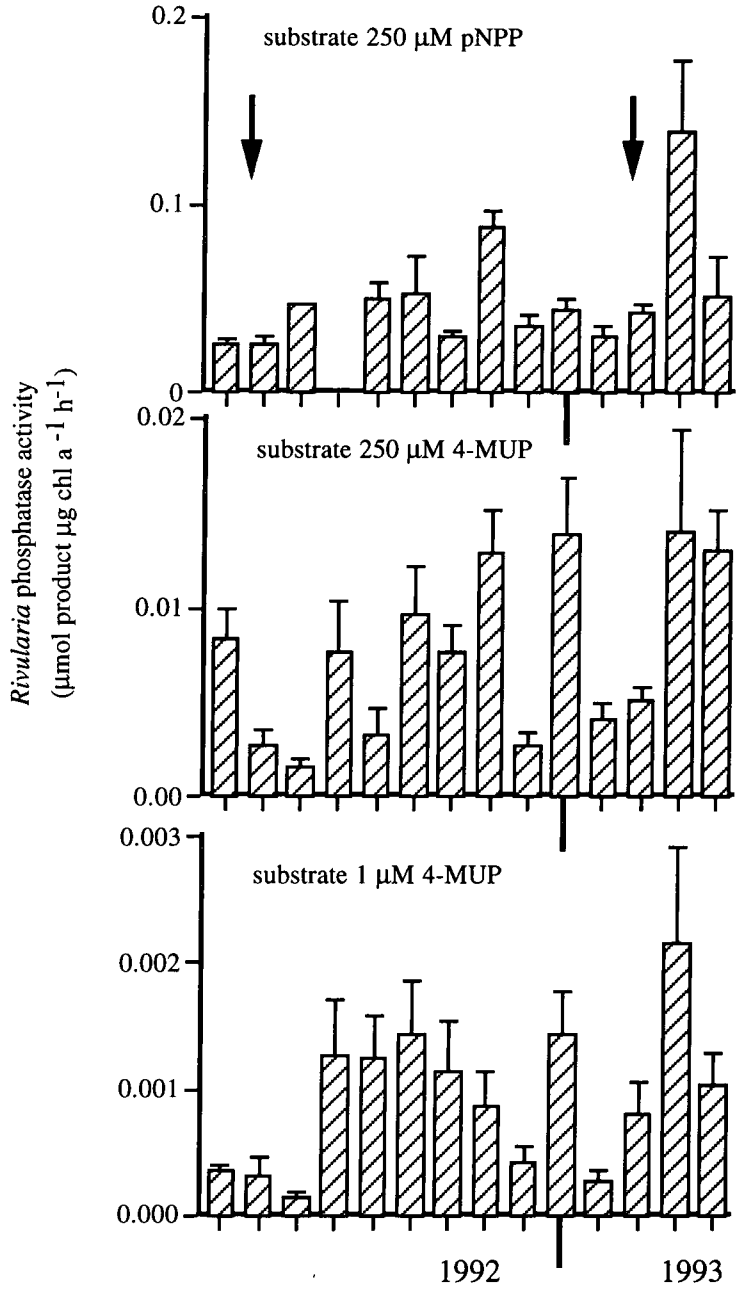


Fig. 4.18 Phosphatase activity of *Rivularia biasolettiana* colonies from Red Sike, using 250 µM pNPP at pH 10.3 and using 250 µM and 1 µM 4-MUP at pH 7.6, sampled monthly April 1992 - May 1993.

Arrows indicate when hormogonia were present in colonies (Samples were lost in July 1992 using 250 µM pNPP) (n = 4)

4.5 Summary

Inorganic P concentrations in the stream peaked in spring 1992, 1993 and 1994. Organic P peaked at other times of year. N fractions showed a similar pattern to phosphorus concentrations, with highest concentrations in spring. Total iron concentrations showed a positive correlation to absorbance of water at 420 nm.

The intensive studies showed that, during the spring, the total filtrable phosphorus in the spring and stream were nearly all inorganic P. However, water from the bog pools had a larger proportion of organic P. There was one occasion during the intensive study, when both total and inorganic P in the spring were extremely high, though there were no replicates to qualify this (Fig. 4.12).

Phosphatase activity in the filtered water was much higher using pNPP than 4-MUP at 250 μM. Activity using 1 μM 4-MUP was always a large proportion of activity at 250 μM 4-MUP. *Rivularia biasolettiana* showed higher activity using 250 μM pNPP than 250 μM 4-MUP, and also showed a high proportion of activity at 1 μM 4-MUP compared to activity at 250 μM 4-MUP, suggesting that at 250 μM the enzyme(s) may be substrate saturated (see 6.32). Activity in all three substrates troughed when hormogonia were present in colonies.

CHAPTER 5

TYNE SANDS FIELD STUDY

5.1 Introduction

Previous studies at Tyne Sands found *Rivularia atra* colonies were abundant in the upper eulittoral (Khoja *et al.*, 1984) and, similar to Red Sike (Livingstone & Whitton, 1984), a large proportion of the phosphorus in the pool water where *Rivularia* colonies were growing, was organic. Higher concentrations of total P, of which organic P was a still larger proportion, were found near detached seaweed at the high tide mark. The *Rivularia* colonies had high phosphatase activity. Khoja *et al.* (1984) suggested that the high phosphatase activity of the colonies and the high levels of organic P were probably related.

The present study was undertaken to test the hypothesis that the proportion of organic P may relate to the phosphatase activity, morphology and abundance of the *Rivularia* colonies. From December 1992 phosphatase assays of the brown alga *Ralfsia verrucosa*, a non-N_2 fixing probable competitor of *Rivularia* in the pools, were included in the long-term study. Phosphatase activity of the "*Dichothrix*" community (see 5.4), situated on rock above R9, and filtered water were also assayed.

More detailed studies were carried out mainly after the initial 19 month period to focus on the possible origin and dynamics of the phosphorus fractions. As in Chapter 4, the intensive studies form the second half of the section 5.2 (environmental variables), giving more detail to the first half of 5.2, the long-term studies.

In this chapter R refers to *Rivularia* pools and W to the weed pools.

5.2 Environment

5.21 Long-term study

Physical variables

As expected, temperature was highest during the summer; W1-4 reached slightly higher temperatures than the other pools, especially during the summer (Fig. 5.1). Seawater was generally colder than water in any of the pools.

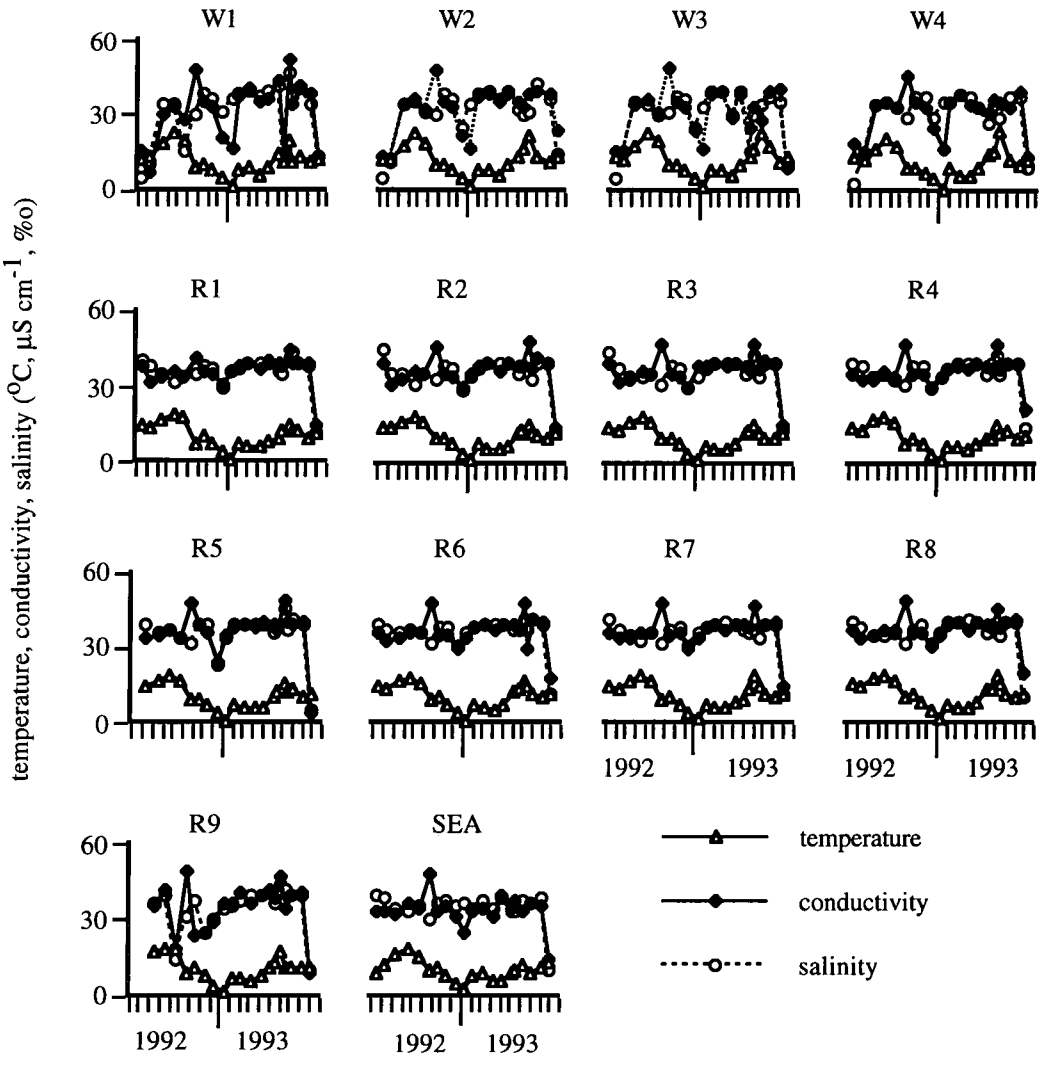


Fig. 5.1 Temperature, conductivity and salinity in water from W1-4, R1-9 and seawater sampled monthly, April 1992 - October 1993

Conductivity and salinity showed a similar pattern in R1-8. R9 and W1-4 showed greater fluctuations. In 5 out of the 20 sampling days, the tide was less than 4.9 m and did not reach W1-4 (see Fig. 5.2). R9 was reached on 9 occasions, when tides were above 5.2 m.

Conductivity and salinity were lower when it snowed (January 1993) in W1-4 and rained heavily (October 1993) in all the pools and seawater. This may indicate that the "seawater" was influenced by terrestrial drainage.

Salinity was negatively correlated to temperature during the study, probably because the colder water was likely to be a slightly higher proportion of seawater when

tides were high (see Appendix iv). However, the changes in salinity in the pools were very small during the whole period (except October 1993) so Spearman's rank correlation associated changes in salinity of only a few ‰. Temperature was negatively correlated to tide height; and salinity was positively correlated to tide height. Higher tides produced colder slightly more saline water in the *Rivularia* pools probably because they caused greater mixing of the pools by seawater. It is unlikely that there was evaporation in the *Rivularia* pools during the tidal cycle as the salinity in the pools did not change more than 3 ‰, even on hot days (see 5.22).

Chemical variables

Total P

From Table 5.1, it can be seen that there were significant correlations between R1-8 during the study period (see also Fig. 5.2). W1-4 were also highly correlated. The overlap between the two sets of pools is shown by the highly significant correlations between W1-4 and R1-9, especially 1, 4 and 9. Seawater was correlated with all the pools.

Table 5.1 Intercorrelation matrix of total P concentrations in water from W1-4, R1-9 and seawater, sampled monthly, April 1992 - October 1993

	W1	W2	W3	W4	R1	R2	R3	R4	R5	R6	R7	R8	R9
W1													
W2	***												
W3	***	***											
W4	**	***	***										
R1	***	***	***	***									
R2	*	**	*	**	***								
R3		*	*	*	***	***							
R4	***	***	***	***	***	***	***						
R5	**	***	**	***	***	***	***	***					
R6	*	**	**	***	***	***	***	***	***				
R7	*	**	*	**	***	***	***	**	***	***			
R8		*	*		**	***	***	*	**	***	***		
R9	***	***	***	***	***	*		**	***	*	**		
SW	*	*	*	*	***	***	***	**	*	***	***	*	*

The graphs of phosphorus fractions (Fig. 5.2) shows that R1, 4 and 9 were most similar, in terms of total P, to the weed pools. R5 appears similar in the correlation matrix, although it appears different to the other pools in Fig. 5.2 probably because of high concentrations of phosphorus in October 1993.

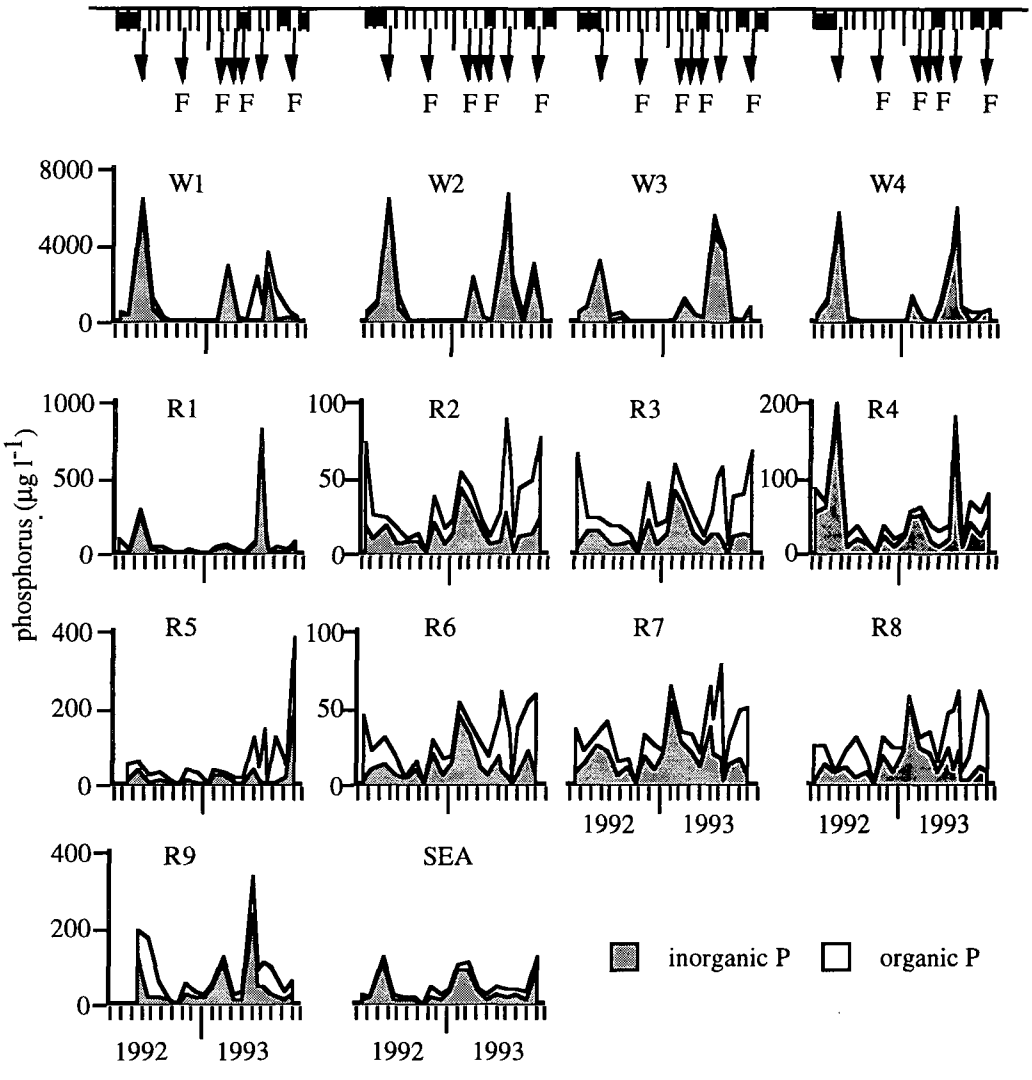


Fig. 5.2 Phosphorus fractions in W1-4, R1-9 and seawater, sampled monthly, April 1992 - October 1993. Arrows indicate when seaweed deposits were present; F indicates when these were fresh deposits; the black square indicates tides that did not reach W1-4 (< 4.9m). Graph shows total P concentration (inorganic P + organic P), on each occasion

R1 and R9 and weed pools 2, 3 and 4, had total P highly correlated with inorganic and organic P; and also organic with inorganic P. These were pools where inorganic P formed a large proportion of the total P, with peaks in June 1992 (ranging from 6500 µg PO_4-P l^{-1} in W2 - 197 µg PO_4-P l^{-1} in R9), March 1993 (2317 µg PO_4-

P l^{-1} in W2 - 67 µg PO_4-P l^{-1} in R1) and June 1993 (6757 µg PO_4-P l^{-1} in W2 - 336 µg PO_4-P l^{-1} in R9), Fig. 5.2.

R3, 4, 5, 6, 7, 8, W1 and seawater all showed high correlations of both inorganic P and organic P to total P, but the inorganic and organic P were not significantly correlated. These were pools where organic P was a larger proportion of the total P or where the organic fraction was not showing synchronised changes with the inorganic fraction and where the February / March peak in phosphorus (2964 µg PO_4-P l^{-1} in W1 - 36 µg PO_4-P l^{-1} in R5) was larger than at least one of the June peaks.

W1 would have been expected to fit into the same category as the other weed pools, when comparing all weed pools in Fig. 5.2. However, this pool was different because it was more contained on rock than sand, and was far from the other weed pools (Fig. 3.4). The high levels of organic P in W1 in the summer of 1993 was probably responsible for the difference between the organic and inorganic fractions throughout the study period.

Inorganic P

Inorganic P showed less correlation between the pools than total P. From the graph (Fig. 5.2) and correlation (Table 5.2), its clear that R4 and R5 were different to R7 and R8. R4 and R5 were higher up the eulittoral than the other pools and R5 was much smaller in volume (Fig. 3.4).

Table 5.2 Intercorrelation matrix of inorganic P concentrations in water from W1-4, R1-9 and seawater, sampled monthly, April 1992 - October 1993

	W1	W2	W3	W4	R1	R2	R3	R4	R5	R6	R7	R8	R9
W1													
W2	**												
W3	**	***											
W4		***	***										
R1	*	***	***	***									
R2				*	**								
R3				*	**	***							
R4	**	*	**	**	***	***	***						
R5		*	*	*	**	**	***	**					
R6					*	***	***	*	**				
R7		*		*	**	**	***		*	***			
R8					*	*	***			***	***		
R9		*	**	**	***	*	*	***	**		**		
sw			*	*	**	***	***	***	*	**	**	*	**

Of the *Rivularia* pools that show a similar correlation to the weed pools, R1 and R4 showed highly significant correlations with W1-4. W3 and W4 were directly above R1 and R4. R9 also showed highly significant correlations to W2, 3 and 4, but not to W1 which was the nearest weed pool to it. R1, 4, 5 and 9 were positively correlation to W2-4.

Fig. 5.2 shows that the pattern of inorganic P in R1, 4 and 9 were very similar during the study period. These pools all had higher correlations with the weed pools. R1, 4 and 9, had high concentrations of inorganic P, above 100 $\mu g\ l^{-1}$ in June 1992 and June / July 1993, which was similar, though at a lower concentration, to the weed pools. A peak in inorganic P also occurred in March 1993 in these pools, but the concentration of this peak was approximately 50 $\mu g\ l^{-1}$, as in all the other *Rivularia* pools. In all the other *Rivularia* pools the March peak was similar or higher to the June peaks (Fig. 5.2). R9 was not covered by all the tides during the study period, but R1 and R4 were. It may be that these pools are similar to the weed pools above them because there is some interchange of water between the weed pools and these *Rivularia* pools i.e. drainage after high tide.

Table 5.2 shows that W1 and W4 were not correlated. These pools were the furthest apart and W1, like R9, was at the very far end of the study area, on slightly higher ground; rock based rather than sand based (Fig. 3.4). Seawater was similar to all pools except W1 and W2.

All *Rivularia* pools, including R1, 4 and 9, showed a peak in inorganic P concentration in February / March 1993 in the order of 50 $\mu g\ l^{-1}$ (Fig. 5.2). This was higher than either of the June peaks in *Rivularia* pools 2, 3, 6, 7 and 8. The tide height in February and March 1993 covered all the weed pools. Fresh weed was deposited in February and weed was also present in March (Fig. 5.2) The seawater and weed pools also showed this February / March peak in inorganic P.

Because these February and March tides were so large (5.8 and 5.9 m), most of the water in the *Rivularia* pools was likely to be similar to seawater. The concentration of inorganic P in the seawater averaged $93 \pm 1.8\ \mu g\ l^{-1}$ in February and $94 \pm 1.2\ \mu g\ l^{-1}$ in March (n=4) and in R1-8 mean $46 \pm 7.5\ \mu g\ l^{-1}$ in February and 35 ±

11.0 μg l^{-1} in March (n=8). In R9 the inorganic P concentration was 52 μg l^{-1} in February and 112 μg l^{-1} in March. The inorganic P concentration in W2-4 was 85 ± 17.1 μg l^{-1} in February and 2087 ± 914.6 μg l^{-1} in March (n=3) but in W1 the inorganic P concentration was 1487 μg l^{-1} in February and 266 μg l^{-1} in March. During these very high tides the inorganic P concentration were higher than the mean for the whole study period (R1-8 mean 27 μg l^{-1}, W2-4 mean 1104 μg l^{-1} and seawater 37 μg l^{-1}, Table 5.4), however, the mean inorganic P in the *Rivularia* pools was still lower than the seawater mean. This suggests that the seawater sample may have been slightly influenced by the weed pools (Fig. 5.2) and that the biota of the *Rivularia* pools were probably utilising inorganic P very rapidly, within 1-2 h.

Organic P

Changes in organic P between the pools were different to those of inorganic P (Table 5.3). The *Rivularia* pools were all highly correlated. The weed pools were also highly correlated.

Table 5.3 Intercorrelation matrix of organic P concentrations, in water from W1-4, R1-9 and seawater, sampled monthly, April 1992 - October 1993

	W1	W2	W3	W4	R1	R2	R3	R4	R5	R6	R7	R8	R9
W1													
W2	***												
W3	***	***											
W4	***	***	***										
R1	***	***	*	*									
R2	*	*			**								
R3	*	*			***	***							
R4	***	**		*	***	***	***						
R5	**				*	***	***	***					
R6	***	**		*	***	***	***	***	***				
R7	**	**			***	***	***	***	***	***			
R8	*	*	*		***	***	***	***	**	***	***		
R9	***	***	***	**	***		*	**	***	**	**	*	
SW	*	**			**	***	***	**	*	**	**	*	**

The seawater was similar to all the *Rivularia* pools and to W1 and W2. The *Rivularia* pools that were similar to the weed pools were not those directly above them. W1 and W2 (the furthest east weed pools), contrary to the inorganic P patterns, were similar to all *Rivularia* pools. W3 and W4, directly above the

Rivularia pools were not similar to R2, 3, 5 and 7. This may be coincidental or it may be due to inorganic P being transferred down the eulittoral, either by the tide or by drainage, whereas organic P may not be allochthonous.

The *Rivularia* pools were all highly similar to one another, R5 showed slightly less similarity to the other pools probably because it is so small. R9 was also more different to the other *Rivularia* pools probably because of its location, tidal influence (covered by tides ≥ 5.2 m) and was the only *Rivularia* pool not surrounded by *Fucus spiralis*. R5 had higher concentrations of organic P when tides were below 4.8 m (summer 1993).

Looking at the whole data set, both organic and total P were positively correlated to temperature and negatively correlated to tide height (see 5.22, tide studies); when tides were low, and pools were exposed for longer giving higher temperatures, total P, of which organic P was a large proportion, was also higher (see Appendix iv). All phosphorus fractions were positively correlated to one another. Inorganic P was positively correlated to NO_3-N.

Table 5.4 Mean and s.d. of P and N fractions ($\mu g\ l^{-1}$) sampled monthly in water from W1-4, R1-9 and seawater, April 1992 - October 1993. NO_2-N was negligible during the study period

Pool	Inorganic P	Organic P	NH_4-N	NO_3-N
R1-8	26.6 ± 65.7	22.2 ± 24.6	1.3 ± 6.7	1.3 ± 2.4
R9	48.4 ± 58.8	39.9 ± 38.4	5.5 ± 15.5	0.8 ± 1.8
W2-4	1103.7 ± 1678.8	178.1 ± 254.5	87.9 ± 315.7	24.5 ± 59.0
W1	716.2 ± 1417.3	407.9 ± 648.6	57.7 ± 190.6	51.8 ± 154.7
seawater	36.6 ± 33.0	14.0 ± 17.2	6.8 ± 7.6	33.2 ± 50.9

Nitrate and Ammonium

Concentrations of both nitrate and ammonium were often very low (Fig. 5.3). Maxima, largely ammonium, occurred in June 1992 when the smell of ammonia was noticed, in all the weed pools and in R9. These maxima were not observed in the other *Rivularia* pools and were only just apparent in W2, 3 and 4 in June 1993. There was a small amount of ammonium present in February / March 1993 in W1, 2 and 3. In the winter 1992/3, there were small peaks in nitrate in all the *Rivularia* pools, except R5 and R9, which corresponded to times when seaweed was deposited on the strand line. In R1 - 8, in contrast to the weed pools and the seawater, the N present, especially during the peaks, was largely nitrate rather than ammonium. N levels in the seawater were low, especially during the summer (1992 and 1993) but there was a maxima in November / December 1992 of 200 $\mu g\ l^{-1}$ of which most was ammonium. This peak corresponded to one of the peaks of nitrate in the *Rivularia* pools, except R5 and R9. As with phosphorus, the nitrogen concentration of the seawater was often greater than that of the *Rivularia* pools (Fig. 5.3 and Table 5.4).

From the graphs (Fig. 5.2 and Fig. 5.3) the similarity of inorganic P and nitrogen fractions can be seen, especially in the weed pools and seawater. Because the NH_4-N concentrations are so low in the *Rivularia* pools and these make up the majority of the data, only the correlation between NO_3-N and inorganic P (and the correlations which are associated with it) were significant (see Appendix iv). TIN : inorganic P was positively correlated with both N fractions since they contribute to it. N fractions were positively correlated to tide height, since high tides were responsible for the mixing of weed deposits (which is likely to be the source of N, Fig. 5.3 shows the dilution of N from the weed pools down the eulittoral) on the upper shore with the pools lower down.

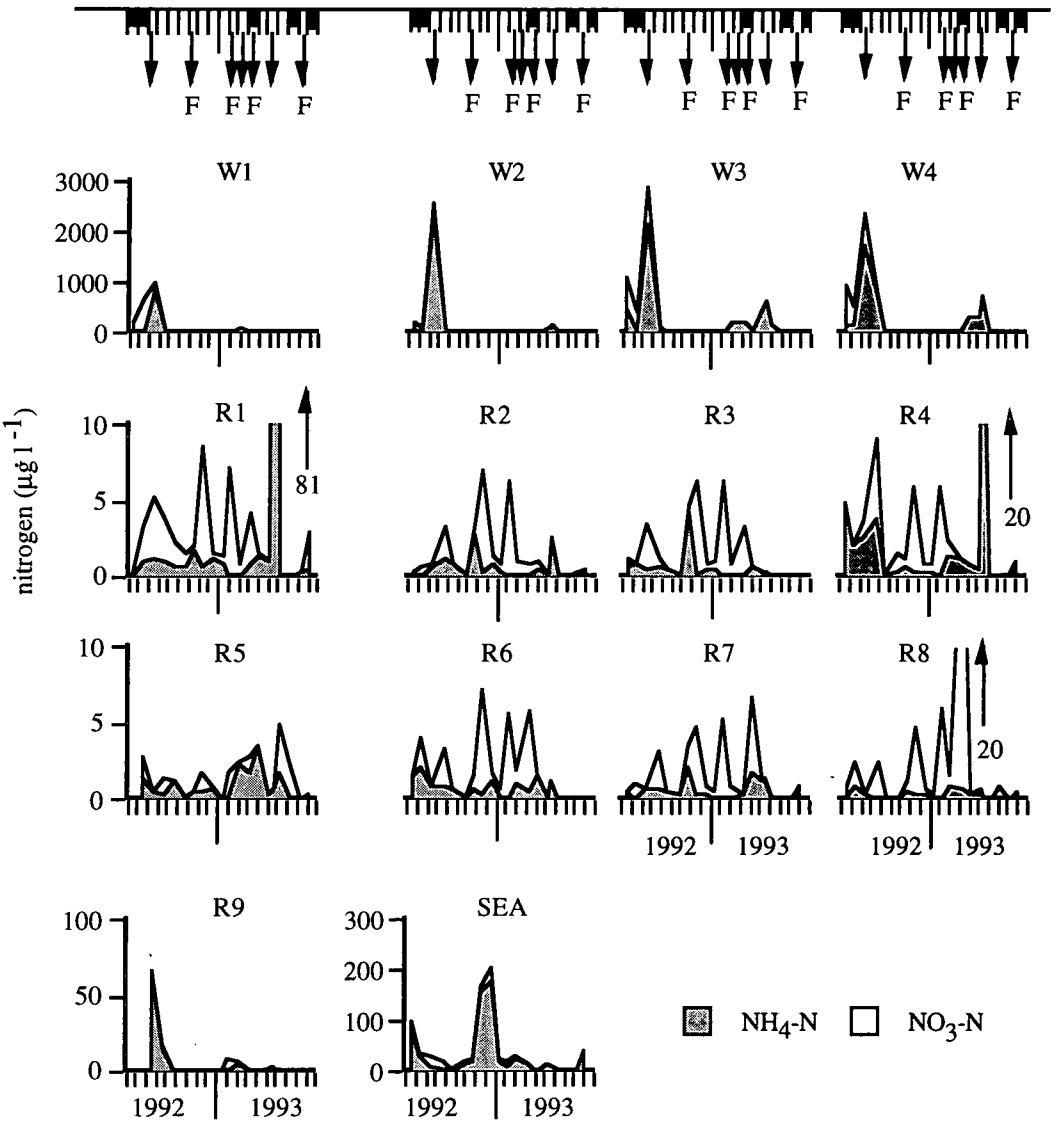


Fig. 5.3 Nitrogen fractions in water from W1-4, R1-9 and seawater, sampled monthly, April 1992 - October 1993. Arrows indicate when seaweed deposits were present; F indicates when these were fresh deposits; the black square indicates tides that did not reach the weed pools (< 4.9m). Graph shows total nitrogen concentration (NH_4-N + NO_3-N (NO_2-N negligible)), on each occasion

5.22 Intensive studies

Tide studies

Inorganic P in R1-8 showed some similarity to the weed pools above them, especially when tides were very high. Organic P showed less similarity between the weed pools directly above R1-8 and R1-8 were more similarly correlated to one another than was the case with inorganic P.

Because it was thought that the source of the inorganic P was likely to be the seaweed deposits, it seemed likely that the tidal cycle and tidal height would have an

influence on the phosphorus fractions within the *Rivularia* pools. Samples were taken 1 - 3 h after the high tide, when the influence of the weed pools was likely to be greatest.

During two summer days, when phosphorus concentrations were expected to be high, based on results from the long-term study, samples were taken from the R1-9 and W3, directly above R1-6 and therefore most likely to drain into them after the tide retreated. One spring and one neap tidal cycle were sampled. Samples were taken at 2 hourly intervals between the two high tides on each of the two days.

During the time after the spring tide (Fig. 5.4, top), which covered all the pools, the total P rose although the inorganic fraction remained constant in R1-8, indicating a rise of organic P as the tidal cycle progressed. R 1, 4 and 9 had slightly higher levels of inorganic P than the other pools.

Seawater at the end of the first high tide appeared to be different in each of the pools, indicating mixing with the pool water. However, seawater coming into the pools during the beginning of the second high tide diluted the water in the pools R1, 6 and W3 (Fig. 5.4). Seawater at the end of the high tide appeared to be influenced by the eulittoral whereas that at the beginning of the tidal cycle may be closer to "true" seawater.

During the neap tidal cycle (Fig. 5.4, bottom), where the tide did not reach any of the *Rivularia* pools, the total P concentrations in all the pools were higher than the previous study. In most of the pools, both the fractions appear fairly stable except in the weed pool where the total P and the inorganic P decreased during the cycle. Because the scale on the y axis is larger, the increase in organic P is not as noticeable as in the previous study. In both tidal cycles organic P was the major fraction in all the *Rivularia* pools.

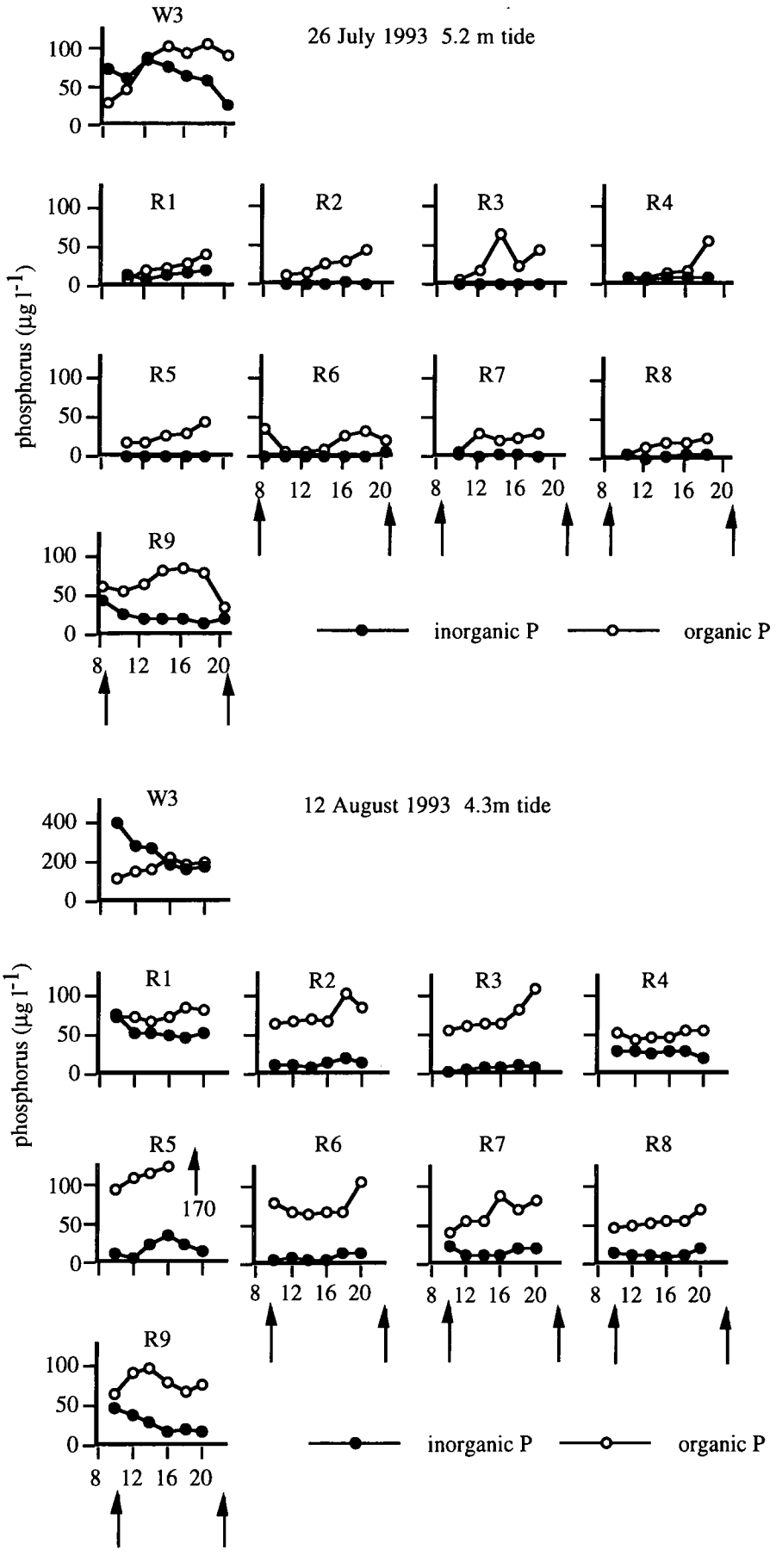


Fig. 5.4 Inorganic P and organic P from R1-9 and W3 during 2 tidal cycles. Time of high tide indicated by arrow

N levels were generally below detection limits, though NH_4-N was detectable especially 4 hours after high tide (Table 5.5) during the spring tidal cycle.

Table 5.5 NH_4-N concentrations ($\mu g\ l^{-1}$) in R1-9 and W3 during one tidal cycle (high tide 5.2m at 08.30 BST), on 26.7.1993 (nd = below detection)

Time	R 1	R2	R3	R4	R5	R6	R7	R8	R9	W
8.30	nd	nd	nd	nd	nd	2.7	nd	nd	4.4	13.0
10.30	nd	nd	nd	nd	4.2	nd	nd	nd	4.0	12.8
12.30	nd	nd	nd	nd	5.3	nd	2.6	nd	4.1	16.6
14.30	nd	nd	nd	nd	8.8	nd	nd	nd	7.7	18.9
16.30	nd	2.8	2.2	nd	5.2	5.5	nd	6.5	8.0	11.4
18.30	nd	nd	nd	nd	5.7	nd	nd	nd	7.2	8.0
20.30	nd	nd	nd	nd	nd	nd	nd	nd	10.1	nd

During the neap tidal cycle NO_3-N concentrations were only detectable in W3 which averaged 2.4 $\mu g\ l^{-1} \pm 0.96$ (n = 6). NH_4-N was not detectable.

In order to test whether the higher inorganic P concentrations were the result of drainage in R1 and R4 during inorganic P peaks observed in June, samples were taken directly following tide cover from the pools (Table 5.6). R1 and R9 were the only pools to show inorganic P concentrations higher than seawater, but R1, 4, 5, 6 and 9 all showed organic P concentrations higher than seawater. R9 was not reached by this tide of 5.0 m. Inorganic P concentrations in the weed pools were above 1000 $\mu g\ l^{-1}$ during sampling in June 1992, June 1993 and June 1994.

Table 5.6 Phosphorus fractions ($\mu g\ l^{-1}$) immediately after R1-8 were uncovered by the tide (16.6.1994). High tide height 5.0 m, inorganic P = Pi, organic P = Po

	w1	w2	w3	w4	R1	R2	R3	R4	R5	R6	R7	R8	R9	SW
Pi	1001.3	1858.6	1267.4	822.3	29.7	0	0	1.5	0	1.6	0	0	539.4	2.2
Po	284.2	244.1	277.1	163.9	24.6	4.7	3.6	17.2	39.7	39.2	3.6	6.3	72.1	15.9

Microhabitat studies

The effect of sample location within the pools was tested by taking 4 replicates from the middle and 4 from the edge of selected *Rivularia* pools. Samples were collected on a comparatively cold and a warm day (Table 5.7).

Table 5.7 Temperature (°C) of R1, 2, 6 and 7 on a comparatively cold day, 16.5.94 and a warm day, 26.7.94; high tide height given in brackets

Pool	16.5.94 (4.9 m)		26.7.94 (5.4 m)	
	middle	edge	middle	edge
1	15.1	16.4	18.8	19.4
2	17.6	15.0	20.0	19.5
6	15.5	15.0	19.3	18.1
7	14.4	-	20.1	20.1

The cold samples, taken in May, were taken just after a storm when a lot of fresh *Laminaria* was deposited in R7. There was no significant difference using a t-test, between edge or middle samples from any of the pools on the cold day. There was a great deal of variability between samples in R2 (Fig. 5.5).

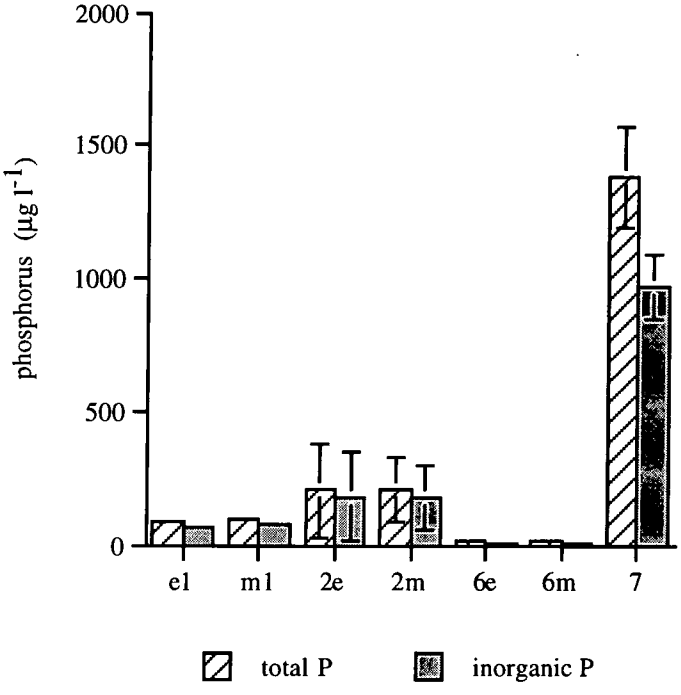


Fig. 5.5 Total and inorganic P concentrations in water taken from the middle (m) or edge (e) of R1, 2 and 6, on a relatively cool weather (16.5.94). Pool 7 samples taken from middle only (see text) (n = 4)

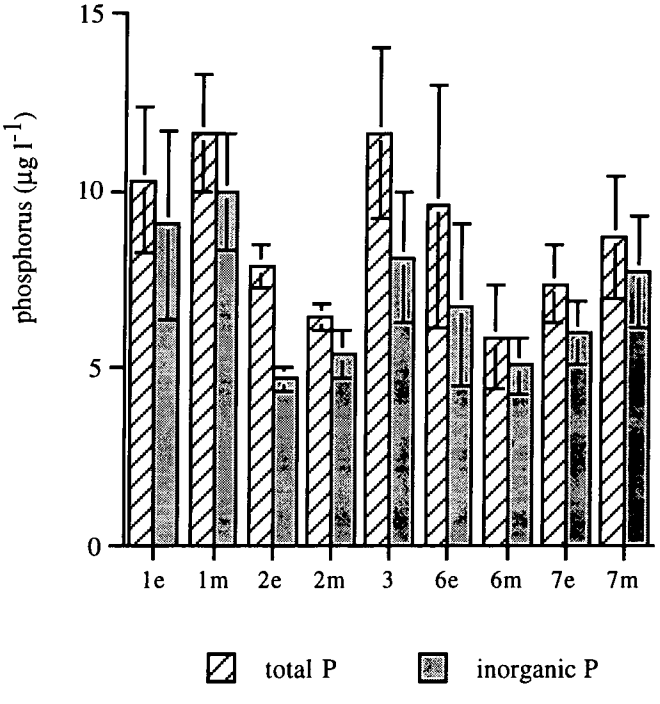


Fig. 5.6 Total and inorganic P concentrations in water taken from the middle (m) or edge (e) of R1, 2 and 3 (middle only) and R6 and 7 on a relatively warm day 26.7.94. (n = 4)

On the warm day the phosphorus concentrations were much lower in all the pools (Fig. 5.6). There was no significant differences using a t-test, between the middle and the edge of the pools.

Filter studies

The effect of different porosity filters on phosphorus concentration were compared to those of unfiltered water from R1, W3 (above the *Rivularia* pools) and seawater. There was no significant difference using Analysis of Variance, in the concentration of the phosphorus fractions between the filters or unfiltered sea or *Rivularia* pool water, but there were significant differences in the concentrations of weed pool water depending on filter porosity (Fig. 5.7). The phosphorus concentration was much higher in the W3 than in R1 or seawater. Unfiltered samples were not used for inorganic P.

In weed pool water t-tests were used in conjunction with Analysis of Variance to identify which porosities were significantly different (Fig. 5.7). There were significant differences in total P concentration between all the treatments. The only non-significant difference in inorganic P concentration was between water filtered using GF/F (0.7 μm) and GF/C (1.2 μm) filters.

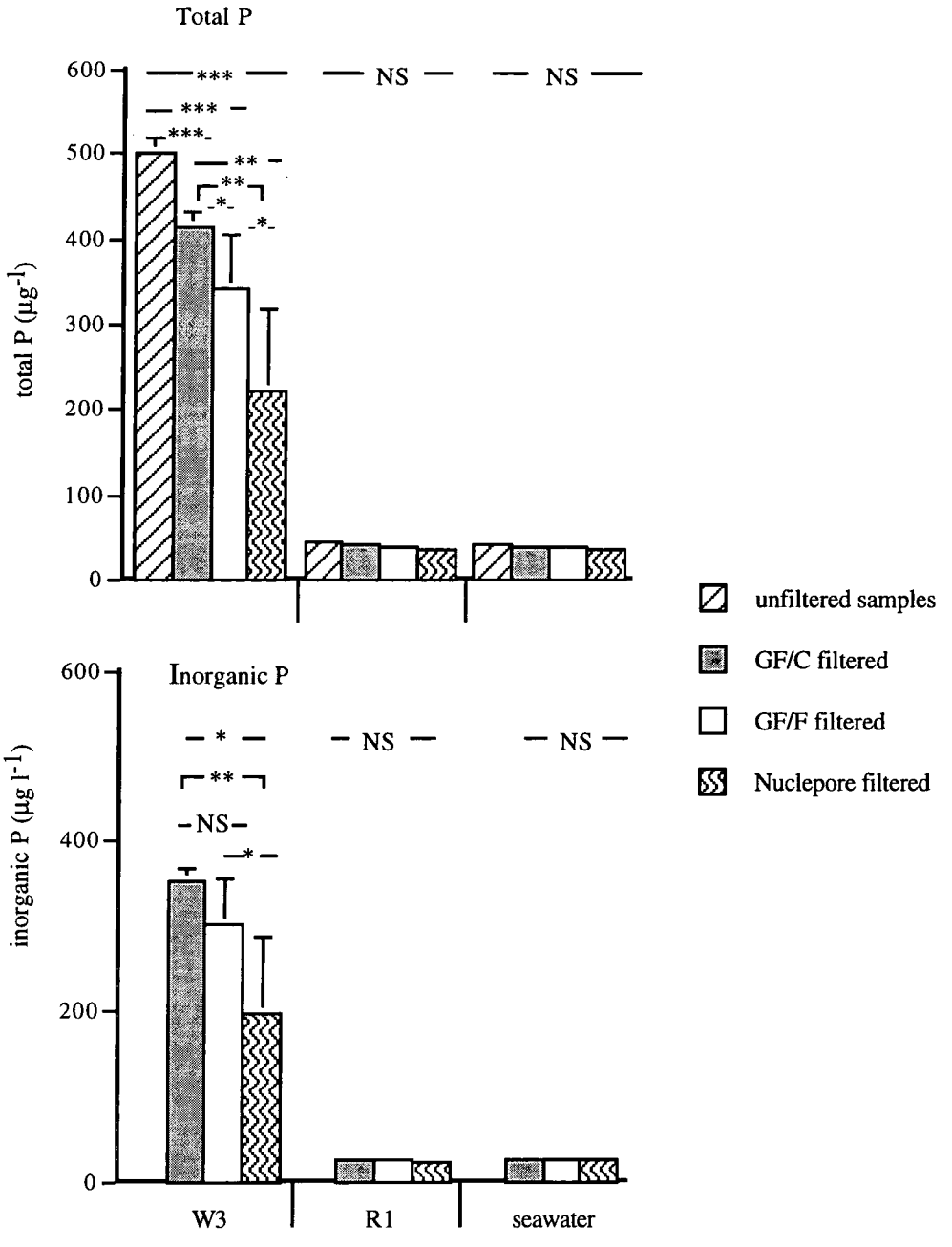


Fig. 5.7 Comparison of total and inorganic P concentrations in unfiltered water and water filtered using GF/C (1.2 µm), GF/F (0.7 µm) and Nuclepore (0.2 µm) filters, from W3, R1 and seawater (n = 4)

5.3 Occurrence and morphology of *Rivularia atra*

From studies carried out on *Calothrix parietina* (Livingstone & Whitton, 1983), it was know that increased inorganic P in a previously phosphorus limited culture caused the formation of hormogonia (see 1.43). Fig. 5.8 shows that *Rivularia atra* were rare when inorganic P concentrations in the pools were above 100 μg l^{-1}. Hormogonia may have formed from rare colonies when inorganic P was higher, resulting in more abundant *Rivularia* once the peak in inorganic P had dropped. This is shown by the correlations of *Rivularia* abundance to other variables (Appendix iv).

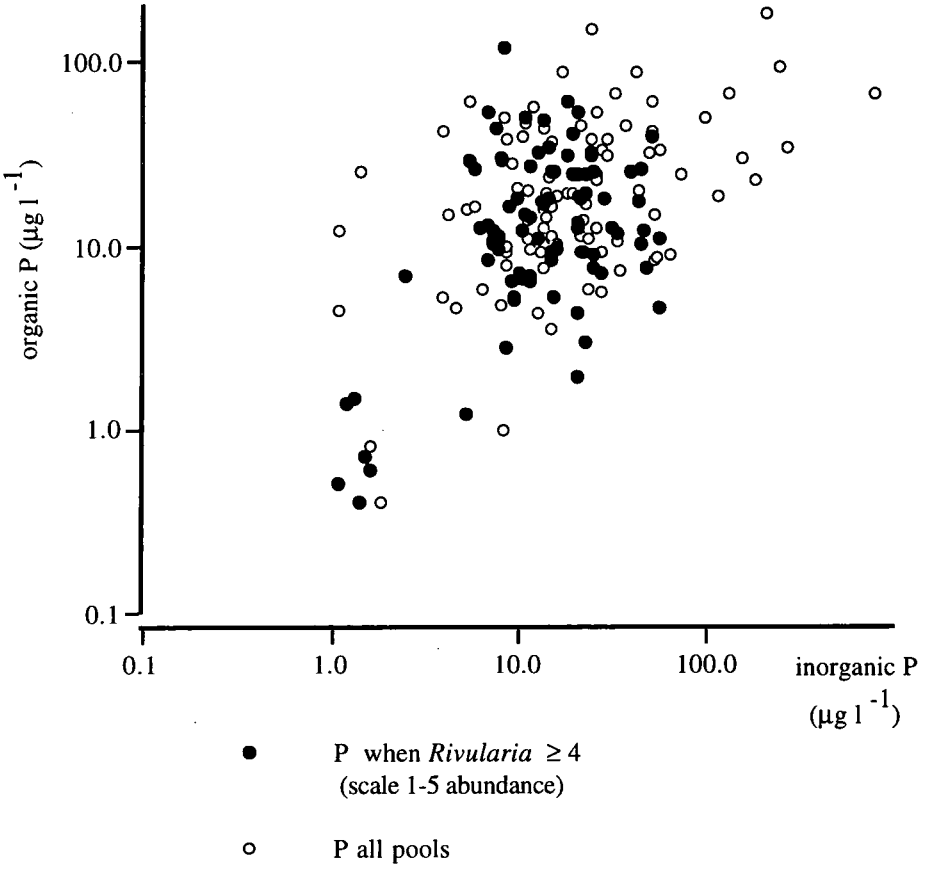


Fig. 5.8 Comparison of *Rivularia atra* abundance with inorganic P and organic P concentrations, on a log scale, from R1-9 April 1992 - October 1993. Dark circles superimposed when *Rivularia atra* abundance in the pools ≥ 4

Rivularia abundance was negatively correlated to all phosphorus fractions while *Rivularia* chlorophyll *a* content was positively correlated to all phosphorus fractions. So when *Rivularia* were rare, the chlorophyll *a* content of the colonies was probably high. Colonies were rare at the time phosphorus concentrations were high. By the

time phosphorus concentrations had dropped, *Rivularia* colonies had become abundant (July / August), with less chlorophyll *a* per colony. The percentage of filaments with hairs was positively correlated with the hair length and temperature of pool water; and negatively correlated with colony diameter, NO_3-N, salinity and tide height. *Rivularia* colony diameter was negatively correlated with % hair: small, new colonies tended to be those with hairs, since the phosphorus levels had dropped soon after their formation, when phosphatase activity was highest, July/August - October. Colony diameter was positively correlated with the concentration of chlorophyll *a* in the colony.

5.4 Identification of "*Dichothrix*" community

Samples of crust material were collected from three different locations near R9 on 28.5.94, to identify the genera of material in samples of the "*Dichothrix*" community (Table 5.8). The three locations were:

1. Rock surface just above the water level where material was collected for assays during the long-term study and the experimental studies
(Initial material taken from this site was isolated and became *Dichothrix* D861. Material for isolation was taken on 19.10.1991)
2. On rock 15 cm higher than the above
3. Small freshwater pool at the top of the rock (+ 30 cm from 1); dried rapidly but nevertheless with water 8 h after previous high tide

Schizothrix and *Lyngbya* may have been the same, but there were probably two different organisms present. *Schizothrix* cells were approximately 1.8 μm with cells up to 6 times longer than wide, often with one prominent granule at one end. *Calothrix* trichomes had colourless regions and in some cases short hairs. The *Tolypothrix* trichome reached 12-13 μm (see 2.23).

Table 5.8 Genera of material collected from 3 locations just above R9 (see text); +15 cm and + 30 cm on 28.5.94

	Sites		
Organism	1	2	3
Gloeocapsa	2		
Entophysalis			5
Schizothrix calcicola (bundles)		4	2
? *Lyngbya / Schizothrix*	4	4	
Calothrix parietina			4
Tolypothrix byssoidea	5	5	? 2
1-cellular green	3		
young *Enteromorpha*	3		

5.5 Phosphatase activity

Although 250 µM pNPP and 250 µM 4-MUP were assayed at different pH, the two are graphed together in order to compare the timing of peaks in activity rather than to compare the values of activity (see 2.251).

5.51 Filtered water

Fig. 5.9 shows that all pools showed a peak in activity using pNPP in July or August 1992. In R7 this peak was less pronounced than in the other pools and R3 also peaked in activity in January 1993 (32 µmol pNP l^{-1} h^{-1}). Phosphatase activity was highest in R1 (74 µmol pNP l^{-1} h^{-1}). The phosphatase activity of filtered water from the weed pools was similar to that of the *Rivularia* pools. Seawater showed much lower activity with peaks in April and October 1992 and May and August 1993 (16 µmol pNP l^{-1} h^{-1}).

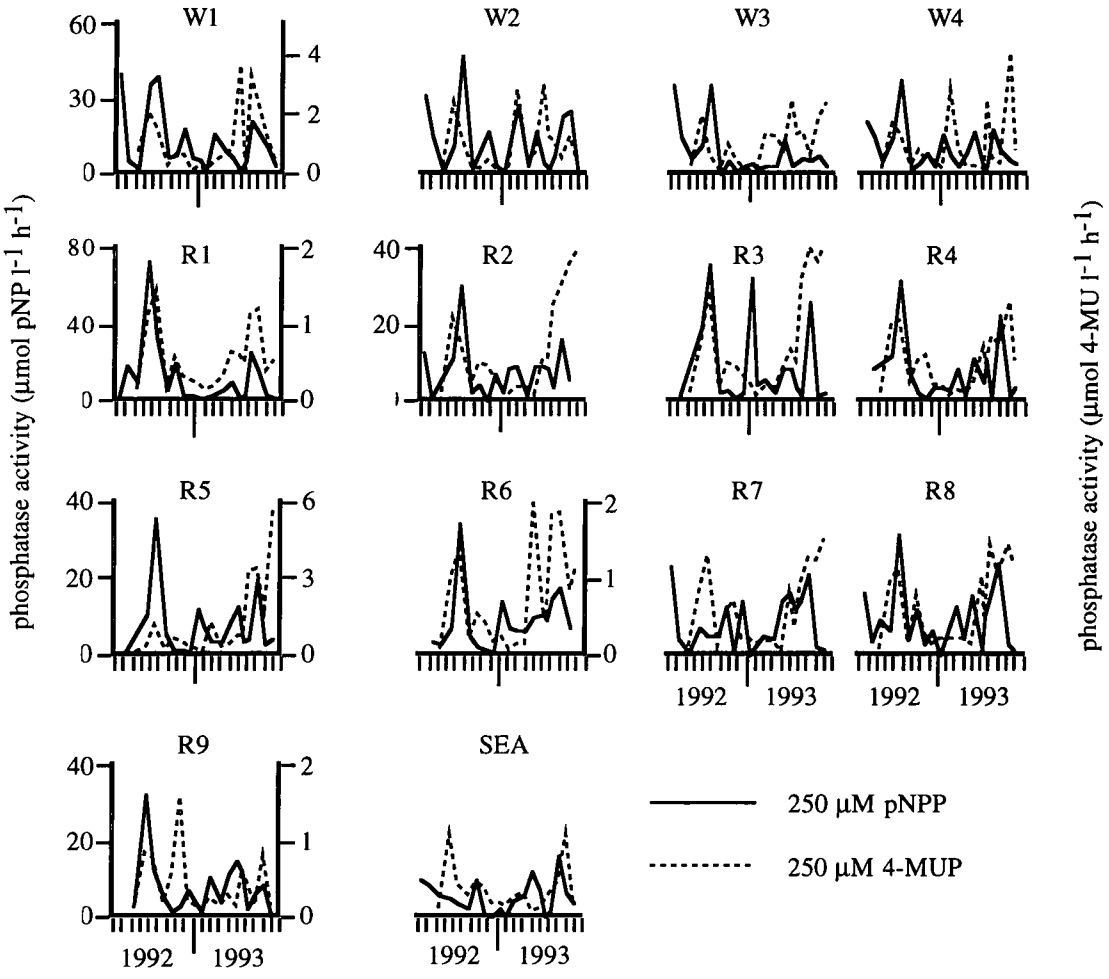


Fig. 5.9 Phosphatase activity of GF/F filtered water using 250 µM pNPP (y axis) at pH 10.3 and 250 µM 4-MUP (yy axis) at pH 7.6, from W1-4, R1-9 and seawater, sampled monthly April 1992 - October 1993. Samples were not taken from R5 or R9 or for 4-MUP until June 1992

Using 250 µM 4-MUP, activity peaked (0.7 µmol 4-MU l^{-1} h^{-1} in R9 - 1.4 µmol 4-MU l^{-1} h^{-1} in W2) at a similar time to that of pNPP, July or August 1992, in all the pools and seawater (Fig. 5.9). R9 showed a larger peak in activity in November 1992 (1.6 µmol 4-MU l^{-1} h^{-1}). R6 showed a peak in activity in May 1993, but most pools showed a peak in activity for 1993 between August and October (1 µmol 4-MU l^{-1} h^{-1} in R9 - 6 µmol 4-MU l^{-1} h^{-1} in R5). Filtered water phosphatase activity from the weed pools was slightly higher than that in the *Rivularia* pools. Weed pools showed peak activity at a similar time to the *Rivularia* pools; W2 and W4 also showed a peak in activity in March 1993 (2.8 µmol 4-MU l^{-1} h^{-1}). Activity using 4-MUP was much lower than that using pNPP, which may be related to the lower pH used in the 4-MUP assay.

Using 1 μM 4-MUP activity peaked in July / August 1992 (0.14 μmol 4-MU l^{-1} h^{-1} in W1 - 0.35 μmol 4-MU l^{-1} h^{-1} in R4) in all pools and in July and September / October 1993 (0.18 μmol 4-MU l^{-1} h^{-1} in R1 - 0.4 μmol 4-MU l^{-1} h^{-1} in R8) though in W4 there was also a peak in February 1993 (0.43 μmol 4-MU l^{-1} h^{-1}), Fig. 5.10. Activity was approximately 10-20 % that of 250 μM 4-MUP. TIN : inorganic P appears to show a different pattern of peaks. It was plotted to give a comparison to filtered water phosphatase activity at 1 μM 4-MUP.

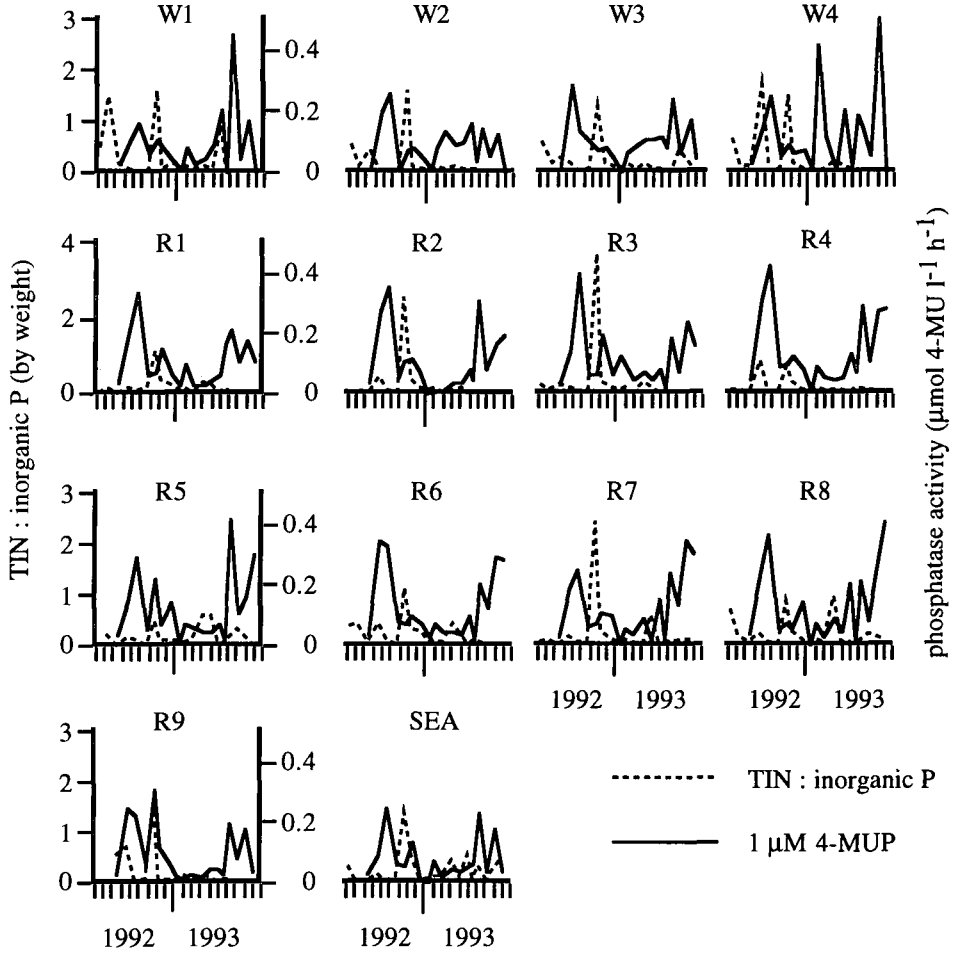


Fig. 5.10 TIN : inorganic P ratio (y axis) compared to phosphatase activity using 1 μM 4-MUP at pH 7.6 (yy axis) of GF/F filtered water from W1-4, R1-9 and seawater, sampled monthly April 1992 - October 1993 (samples for 4-MUP not taken until June 1992)

Although Fig 5.9 shows the similarity of the major peaks in filtered water phosphatase activity, Table 5.9 shows that the correlations were not high. This may

be related to the many small peaks which may affect the ranking order. R1 was different to most pools. R 2, 3, 4, 5 and 6 were similar to one another. The weed pools were not highly correlated to one another, or the *Rivularia* pools. The seawater was only correlated to W3.

Table 5.9 Intercorrelation matrix of phosphatase activity of filtered water using 250 µmol pNPP between W1-4, R1-9 and seawater, April 1992 - October 1993

	W1	W2	W3	W4	R1	R2	R3	R4	R5	R6	R7	R8	R9
W1													
W2	***												
W3													
W4	*		**										
R1													
R2		*											
R3						***							
R4						***	***						
R5						**	***	**					
R6			*			*	**		***				
R7						**							
R8	*	*			*	**					**		
R9						**	*	***					
sw			*										

Using 250 µM 4-MUP, all phosphatase activity from R1-8 was highly correlated, in contrast to pNPP, perhaps because there are fewer small peaks between maxima (Table 5.10). Activity from W3 was highly correlated to R2 - 8.

Table 5.10 Intercorrelation matrix of phosphatase activity of filtered water using 250 µmol 4-MUP between W1-4, R1-9 and seawater, April 1992 - October 1993

	W1	W2	W3	W4	R1	R2	R3	R4	R5	R6	R7	R8	R9
W1													
W2	*												
W3	*	***											
W4													
R1	**	*											
R2			**		**								
R3			*		***	***							
R4	*		*		***	***	***						
R5		*	**		**	***	***	**					
R6	*				***	**	***	***	***				
R7			*		**	***	***	***	***	**			
R8	*		**	*	**	***	***	***	***	**	***		
R9								**					
sw				*		*		**			*	*	**

Using 1 μM 4-MUP, all the *Rivularia* pools were similar. W4 was only correlated with R1 and R9 (Table 5.11). With the lowest substrate concentration the correlation of activity between the *Rivularia* pools, weed pools and seawater, was greatest.

Table 5.11 Intercorrelation matrix of phosphatase activity of filtered water using 1 μmol 4-MUP between W1-4, R1-9 and seawater, April 1992 - October 1993

	W1	W2	W3	W4	R1	R2	R3	R4	R5	R6	R7	R8	R9
W1													
W2	***												
W3	***	***											
W4		*	*										
R1	*		*	*									
R2	**		*		***								
R3	*				*	***							
R4	**	*	*		***	***	**						
R5	*				**	***	*	***					
R6	**	*	**		***	***	***	***	***				
R7	*	*	*		**	***	***	***	***	***			
R8	*	*	*		**	***	***	***	***	***	***		
R9	***	*	*	*	***	***		***	***	***	*	*	
sw	***	**	***	**	***	***	*	***		**	*		**

Correlations of both filtered water phosphatase activity using 4-MUP were positive to temperature and organic P and negative to salinity, tide height, either NH_4-N or NO_3-N and TIN : inorganic P, and weed deposits; reinforcing that organic P increased when the pools were exposed longer and temperatures of the pools, therefore were higher and salinity was lower (Appendix iv). Filtered water phosphatase activity using 250 μM pNPP was also positively correlated to temperature. Filtered water phosphatase activity using 4-MUP was positively correlated to *Rivularia* and *Ralfsia* phosphatase activity using 250 μM 4-MUP and *Ralfsia* phosphatase activity using 1 μM 4-MUP. Filtered water phosphatase activity using 250 μM 4-MUP was positively correlated to both other filtered water phosphatase activity and was negatively correlated to inorganic P. Filtered water phosphatase activity using 1 μM 4-MUP was positively correlated to filtered water phosphatase activity using 250 μM 4-MUP and all phosphatase activity of *Rivularia*.

5.52 *Rivularia atra*, "*Dichothrix*" community and *Ralfsia verrucosa* phosphatase activity

Rivularia atra

Rivularia phosphatase activity was maximal in July / August 1992 in colonies from all pools using pNPP (0.02 µmol pNP chl a^{-1} h^{-1} in R8 - 0.26 µmol pNP µg chl a^{-1} h^{-1} in R4), Fig. 5.11. Colonies from R8 showed continuously higher activity from July 1992 to October 1992 (0.02 µmol pNP µg chl a^{-1} h^{-1}) and did not show an overall peak in 1993. Using 250 µM 4-MUP, maximal activity was found one to two months after the peak using pNPP; in August - October 1992 (0.07 µmol 4-MU µg chl a^{-1} h^{-1} in R6 - 0.003 µmol 4-MU µg chl a^{-1} h^{-1} in R2) and also in July 1993 in colonies from R1, 2, 3, 4, 5, 7 and 8 though approximately 20 % that of the 1992 peak (Fig. 5.11). Colonies from R1, 2, 3 and 8 showed high activity, though not maximal, using 4-MUP at the time of the peak using pNPP.

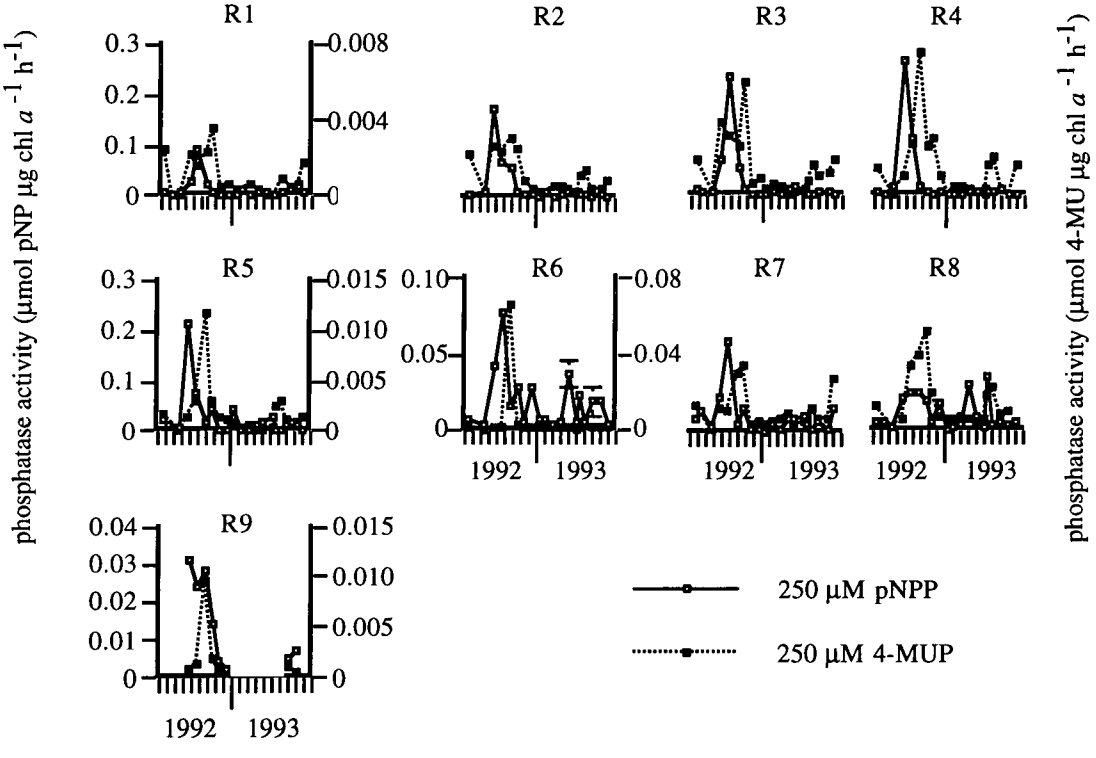


Fig. 5.11 Phosphatase activity of *Rivularia* colonies from R1-9 using 250 µM pNPP (y axis) at pH 10.3, and 250 µM 4-MUP (yy axis) at pH 7.6, sampled monthly April 1992 - October 1993. (All samples for 4-MUP lost in May 1992; R9 no *Rivularia* present during April - July 1992 or January - August 1993 or October 1993; error bars n = 4, R6 only, April - October 1993)

Using 1 µM 4-MUP activity peaked in colonies from all pools in September 1992 (0.0016 µmol 4-MU µg chl a^{-1} h^{-1} in R5 - 0.0002 µmol 4-MU µg chl a^{-1} h^{-1} in R7) though this peak was not maximal in R6, 7 and 8 (Fig. 5.12). The colonies from these pools showed maximal activity in April or July 1992 (0.0002 µmol 4-MU µg chl a^{-1} h^{-1}). Colonies from all pools peaked at either the end of June (2 samples were taken for phosphorus and phosphatase activity in June 1993, two weeks apart) or July 1993, though this peak was much smaller than in 1992 (0.0003 µmol 4-MU µg chl a^{-1} h^{-1} in R4 - 0.00006 µmol 4-MU µg chl a^{-1} h^{-1} in R2). Colonies also showed a peak in activity in September / October 1993. Generally, inorganic P appeared to show a negative influence on phosphatase activity in *Rivularia* colonies at 1 µM 4-MUP, except in June 1993.

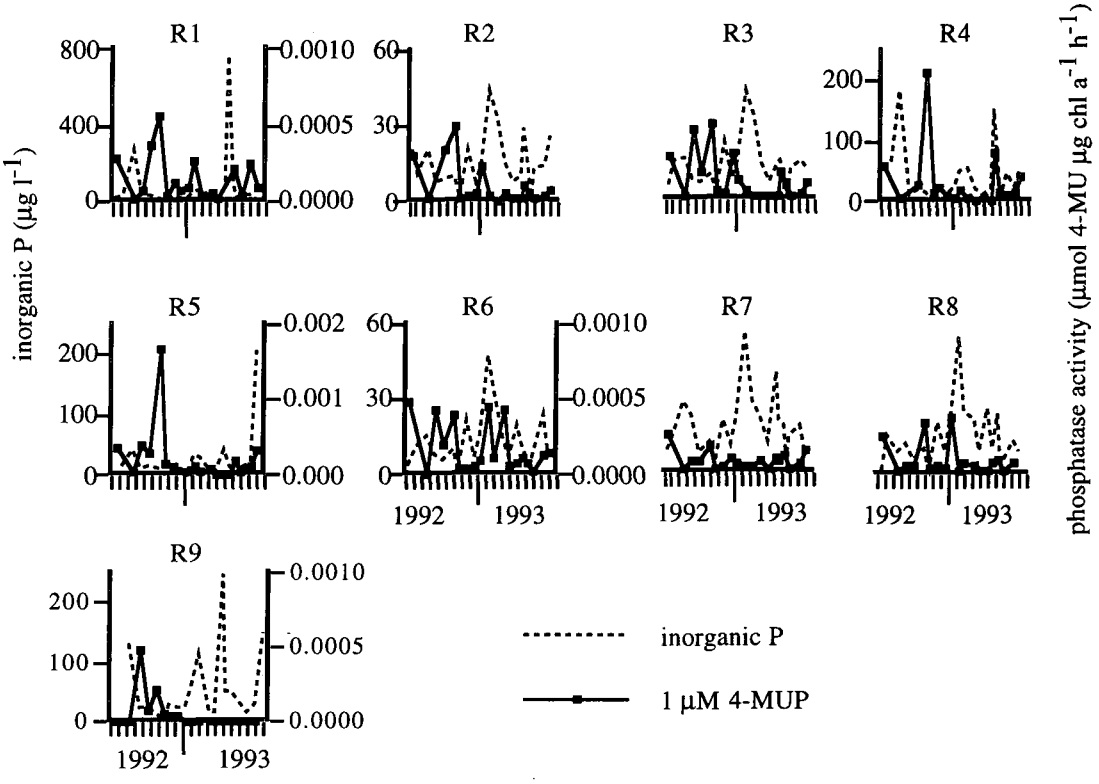


Fig. 5.12 Inorganic P concentration (y axis) of water from R1-9 compared to phosphatase activity of *Rivularia atra* colonies using 1 µM 4-MUP (yy axis) at pH 7.6, sampled monthly April 1992 - October 1993 (Samples for 4-MUP in May 1992 lost; no *Rivularia* present in R9 during April - July 1992 or January - October 1993; error bars n = 4 R6 only, April - October 1993, bars may be smaller than symbol)

Generally, *Rivularia* phosphatase activity between the *Rivularia* pools was similar using pNPP (Table 5.12). But as with the phosphatase activity of filtered water, *Rivularia* phosphatase activity using pNPP was less highly correlated than using 4-MUP. The high correlation of colony activity from R1, 3 and 5 to R9 is probably due to similar trends in activity when *Rivularia* were present in R9.

Table 5.12 Intercorrelation matrix of phosphatase activity of *Rivularia atra* colonies using 250 µmol pNPP between R1-9, April 1992 - October 1993

	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9
R 1									
R 2	**								
R 3	**	***							
R 4	*	**	**						
R 5			***	*					
R 6	*	*	*	*	*				
R 7		*	**	*	*	*			
R 8		*		**		**	*		
R 9	**		***		***				

Using 4-MUP all pools were similar except R9 as *Rivularia* was absent from R9 for half the sample period (Tables 5.13 and 5.14). 250 µM 4-MUP showed the highest correlation of *Rivularia* phosphatase between the pools.

Table 5.13 Intercorrelation matrix of phosphatase activity of *Rivularia atra* colonies using 250 µmol 4-MUP between R1-9, April 1992 - October 1993

	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9
R 1									
R 2	***								
R 3	***	***							
R 4	**	***	**						
R 5	***	***	***	***					
R 6	**	***	**	**	***				
R 7	***	***	***	**	***	***			
R 8	***	***	***	***	***	***	***		
R 9				*	**			*	

Table 5.14 Intercorrelation matrix of phosphatase activity of *Rivularia atra* colonies using 1 µmol 4-MUP between R1-9, April 1992 - October 1993

	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9
R 1									
R 2	***								
R 3	**	***							
R 4	***	**	***						
R 5	**	*	**	**					
R 6	*	*	*		*				
R 7	**	***	***	*	*	*			
R 8	**	**	**	*		*	**		
R 9			*		**	***			

All *Rivularia* phosphatase activity was positively correlated to % trichomes with hairs in a colony (% hair) and filtered water phosphatase activity at 1 µM 4-MUP, and negatively correlated to chlorophyll *a* in the colony and salinity (see Appendix iv). In conjunction, activity using 250 µM and 1 µM 4-MUP was negatively correlated to % hormogonia in the colonies. Activity using 250 µM pNPP and 250 µM 4-MUP was negatively correlated to inorganic P and positively correlated to hair length. Activity using 250 µM 4-MUP was also positively correlated to *Rivularia* abundance and activity using 1 µM 4-MUP was also negatively correlated to colony diameter. These correlations associated phosphatase activity with hairs in colonies, hair length, with small colonies (with little chlorophyll *a*), the absence of hormogonia, when colonies were abundant and when inorganic P was low. However, activity at 250 µM pNPP was positively correlated to TIN : inorganic P (and NH_4-N) while using 250 µM 4-MUP it was negatively correlated. All *Rivularia* phosphatase activity was positively correlated.

"*Dichothrix*" community

The "*Dichothrix*" community was always present on rock just above the water in R9. Water from this pool was not collected for analysis until July 1993, two months after the start of the long-term field study. All substrates show a similar trend, with phosphatase activity peaking in October 1992 and October 1993 (0.04 and 0.08 µmol pNP µg chl a^{-1} h^{-1}; 0.04 and 0.03 µmol 4-MU µg chl a^{-1} h^{-1}; 0.0004 and 0.004 µmol 4-

MU μg chl a^{-1} h^{-1}, respectively), Fig. 5.13. The October 1992 peak coincided with a peak in the TIN : inorganic P ratio of 1.5.

The phosphatase activity of the "*Dichothrix*" community at 250 μM 4-MUP, like that of *Rivularia,* was negatively correlated to salinity and positively correlated to activity at 1 μM (Appendix iv). Using 1 μM 4-MUP activity was correlated to activity at 250 μM. Given that the peaks in activity using all the substrates occur at the same time, and so distinctively, it is surprising that activity using 250 μM pNPP is not related to activity using 4-MUP.

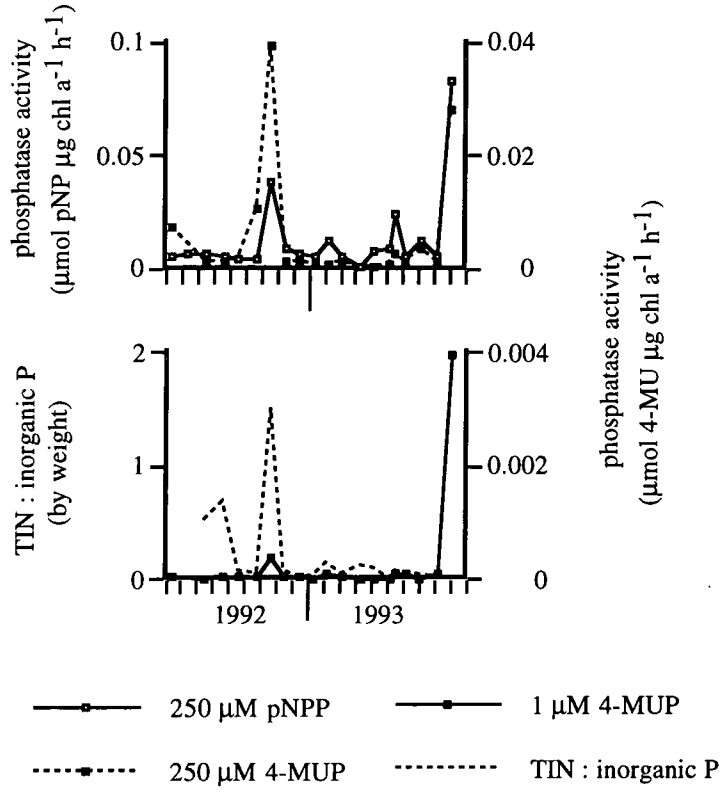


Fig. 5.13a Phosphatase activity of the "*Dichothrix* " community, sampled monthly from above R9, April 1992 - October 1993) using 250 μM pNPP (y axis) at pH 10.3, and 250 μM 4-MUP (yy axis) at pH 7.6 (sample for 250 μM 4-MUP May 1992 lost)

Fig 5.13b TIN : inorganic P ratio in R9 (y axis); sampled from June 1992 - October 1993, compared to phosphatase activity of the "*Dichothrix*" community using 1 μM 4-MUP (yy axis) at pH 7.6 (sample for 1 μM 4-MUP May 1992 lost)

Ralfsia verrucosa

Ralfsia was always present in all the *Rivularia* pools. Samples of *Ralfsia* were taken from each pool to compare its activity with the phosphatase activity of *Rivularia atra*. Activity of *Ralfsia* using 250 µM pNPP peaked in May 1993 in R1, 3, 6, 7 and 8 (0.003 in R8 - 0.08 in R7 µmol pNP µg chl a^{-1} h^{-1}), Fig. 5.14. Activity peaked in June 1993 (0.005 µmol pNP µg chl a^{-1} h^{-1} in R5 - 0.04 µmol pNP µg chl a^{-1} h^{-1} in R9) in the other *Rivularia* pools (R2, 4, 5 and 9) Using this substrate, a larger peak of activity occurred in September or October in R3, 5, 8 and 9 (0.010 µmol pNP µg chl a^{-1} h^{-1} in R3 - 0.051 µmol pNP µg chl a^{-1} h^{-1} in R9). Activity was generally higher in *Ralfsia* from R7 and R9.

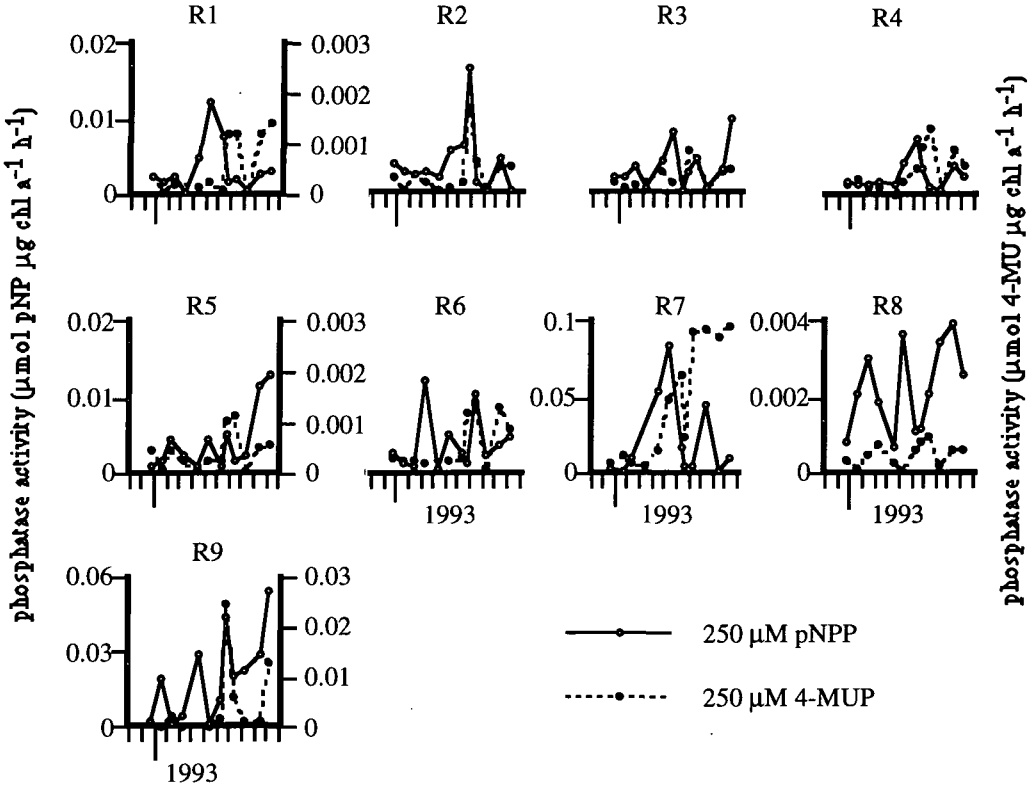


Fig. 5.14 Phosphatase activity of *Ralfsia verrucosa* from R1-9 using 250 µM pNPP (y axis) at pH 10.3 and 250 µM 4-MUP (yy axis) at pH 7.6 sampled monthly from December 1992 - October 1993

Using 250 µM 4-MUP (Fig. 5.14), *Ralfsia* phosphatase activity peaked in June / July (0.0007 µmol 4-MU µg chl a^{-1} h^{-1} in R8 - 0.025 µmol 4-MU µg chl a^{-1} h^{-1} in R9) and in September / October (0.0005 µmol 4-MU µg chl a^{-1} h^{-1} in R3 - 0.013 µmol 4-MU µg chl a^{-1} h^{-1} in R9) in all the pools (Fig. 5.14). As with pNPP, *Ralfsia* from R9

had generally higher activity. Unlike the phosphatase activity of the *Rivularia* colonies, there was no clear indication that *Ralfsia* phosphatase activity using pNPP reached a peak before that of 4-MUP (Fig. 5.14).

Using 1 μM 4-MUP there was a small peak in the phosphatase activity of *Ralfsia* in February / March in all pools (1.2 x 10^{-5} μmol 4-MU μg chl a^{-1} h^{-1} in R4 - 1.2 x 10^{-4} μmol 4-MU μg chl a^{-1} h^{-1} in R9), Fig. 5.15. A peak in activity occurred in September / October in all the pools which was the highest in R1, 3, 5, 6, 7, 8 and 9 (8.8 x 10^{-5} μmol 4-MU μg chl a^{-1} h^{-1} in R6 - 1.8 x 10^{-3} μmol 4-MU μg chl a^{-1} h^{-1} in R9). Activity using 1 μM 4-MUP showed very similar peaks to that using 250 μM 4-MUP. At the lower substrate concentration activity was approximately 10 % that of the higher substrate concentration. Generally, activity showed an apparently inverse relationship to TIN : inorganic P which peaked in October 1992 (0.7 in R4 - 3.6 in R3).

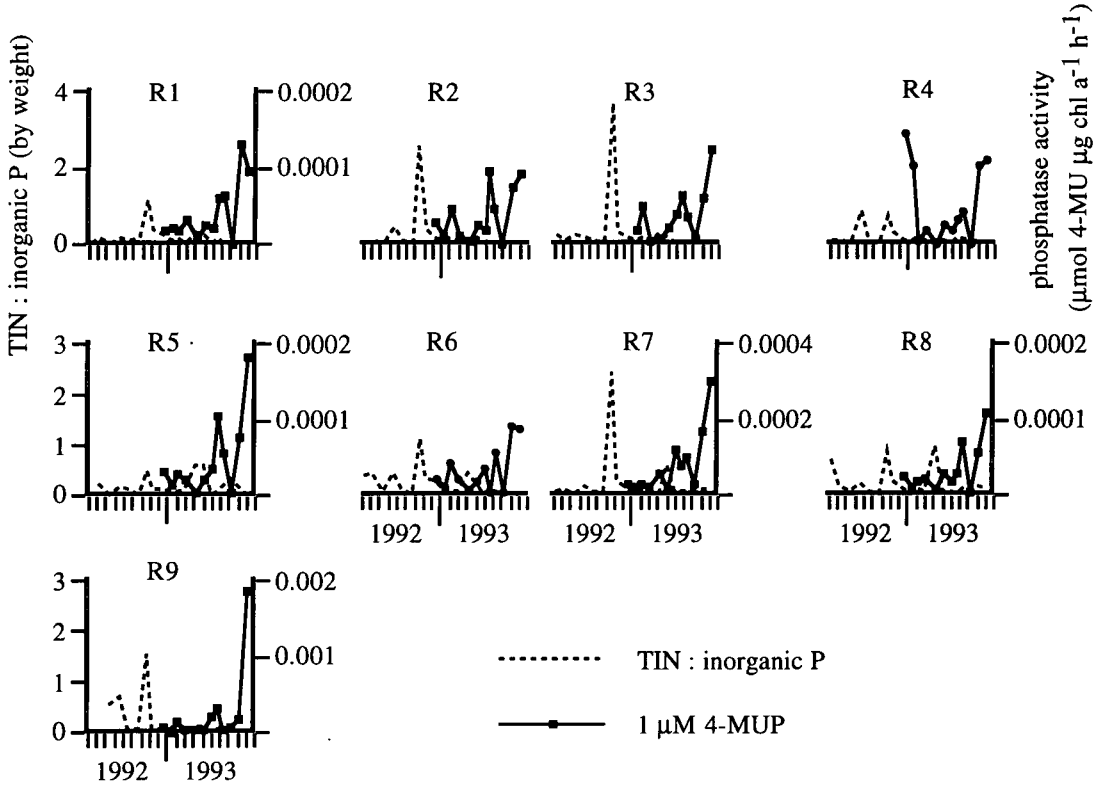


Fig. 5.15 TIN : inorganic P ratio (y axis), sampled April 1992 - October 1993 compared to phosphatase activity of *Ralfsia verrucosa* from R1-9 using 1 μM 4-MUP (yy axis) at pH 7.6, sampled from December 1992 - October 1993

Using 250 µM pNPP phosphatase activity of *Ralfsia* between all pools was not highly correlated except between R2 and R4; and R5 and R8. Using 250 µM 4-MUP, activity between all pools was correlated except R7 (Table 5.15a). Using 1 µM 4-MUP activity of all pools was less highly correlated (Table 5.15b).

Table 5.15a Intercorrelation matrix of phosphatase activity of *Ralfsia verrucosa* using 250 µM 4-MUP between R1-9, December 1992 - October 1993

Table 5.15b Intercorrelation matrix of phosphatase activity of *Ralfsia verrucosa* using 1 µM 4-MUP between R1-9, December 1992 - October 1993

250 µM	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9
R 1									
R 2	**								
R 3	**	*							
R 4	*	**	*						
R 5	***	***	*	***					
R 6	**	**	**	***	***				
R 7									
R 8	*	***	*	*	*	*			
R 9	*	**	*	*	**	*		*	

1 µM	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9
R 1									
R 2	**								
R 3		***							
R 4	*		*						
R 5	**	***	***	*					
R 6									
R 7			*		*				
R 8	***	***	*	*	***	*			
R 9		**	***		***		*		

Phosphatase activity of *Ralfsia* was positively correlated using each substrate. Activity using 4-MUP was similarly correlated at both substrate concentrations: positively to organic and total P, temperature and both *Rivularia* and filtered water activity using 4-MUP and negatively to salinity and TIN : inorganic P (Appendix iv).

5.6 Summary

Total phosphorus concentrations, predominantly inorganic P, were high in June (all 3 years) in the weed pools. There was also a slight peak in February / March. R1, 4 and 9 showed similar peaks to those of the weed pools, though of lower concentration, but in the other *Rivularia* pools phosphorus concentrations were higher

after very high spring tides (February - March 1993). Organic P was a higher proportion of total P in the *Rivularia* pools and showed a high degree of correlation within the weed pools and within the *Rivularia* pools, with some overlap between the two. Inorganic P was less highly correlated. Nitrogen showed a similar pattern to that of phosphorus. Nitrogen was often very low in the *Rivularia* pools and, like inorganic P, concentrations were sometimes lower in the *Rivularia* pools than in either the weed pools or the seawater.

Intensive studies showed that tidal cycle may play an important role in nutrient dynamics. Organic P was found to increase during the course of a tidal cycle, which was especially obvious when the previous high tide had covered the *Rivularia* pools. There was a significant difference between unfiltered and filtered (1.2, 0.7, 0.2 μm porosity) water from W3 although no significant differences were found between these fractions in water from R1 or seawater. There was very little difference in phosphorus concentration between water collected from the middle and the edge of *Rivularia* pools. Following recent *Laminaria* deposition in R7, phosphorus concentration, largely inorganic P, was considerably higher than in the other pools.

Phosphatase activity of filtered water showed similar peaks with all three substrates. Phosphatase activity of *Rivularia* colonies showed an apparent difference in activity in 1992, between the two substrates, with pNPP appearing to peak at least one month before 4-MUP in all the pools. There was a higher degree of correlation between the activity of *Rivularia* colonies from different pools, measured using 4-MUP than using pNPP. This may have been because pNPP showed a higher degree of variability between peaks. Phosphatase activity of *Ralfsia* showed no apparent difference in peaks of activity between pNPP and 4-MUP, though, as with *Rivularia*, there was less correlation of *Ralfsia* activity from different pools using pNPP than using 4-MUP. Activity at 1 μM 4-MUP peaked at similar times to that using 250 μM 4-MUP. The "*Dichothrix*" community showed the clearest peaks in phosphatase activity, in October 1992 and October 1993 with all three substrates.

Throughout the study, phosphatase activity was mainly low with occasional peaks. There appeared to be periods when activity was especially high, though these

were different for different organisms and, apparently, when assayed with different substrates in the case of *Rivularia*. Phosphatase activity in *Rivularia* was negatively correlated to inorganic P (except using 1 μM 4-MUP), and salinity, and positively correlated to *Rivularia* abundance (using 250 μM 4-MUP), % hairs in colonies and the temperature of pool water (except using 1 μM 4-MUP). Temperature showed a positive correlation and salinity a negative correlation to phosphatase activity in *Ralfsia* and filtered water using both concentrations of 4-MUP, also (see Appendix iv).

Organic P was negatively correlated with tide height and *Rivularia* abundance, and positively correlated with total and inorganic P, temperature of the pool water and chlorophyll *a* content of the *Rivularia* colonies. Inorganic P was negatively correlated with *Rivularia* abundance. Inorganic P was positively correlated to total and organic P and chlorophyll *a* of the *Rivularia* colonies (see Appendix iv). Colonies were most abundant when inorganic P was < 100 $\mu g\ l^{-1}$. *Rivularia* colonies were most active when they were most abundant, small and with little chlorophyll *a*. This tended to be in the summer when water temperature was higher, tide heights were lower, salinity was lower and inorganic P was lower but organic P was generally higher.

CHAPTER 6

EXPERIMENTAL STUDIES ON UPPER TEESDALE ORGANISMS

6.1 Introduction

The long-term field studies showed that there may be differences in phosphatase activity between pNPP and 4-MUP. Although this was not apparent with *Rivularia biasolettiana* colonies (see 4.42), *R. atra* showed differences between the two substrates clearly (see 5.52). The aim of this chapter was to find out more about the differences in phosphatase activity of freshwater organisms using the two substrates.

Assays for the long-term field studies were carried out at different pH so phosphatase activity throughout a pH spectrum was obtained for each organism. Other experiments were carried out at pH 9, a pH between those used in the field studies (pH 7.6 and 10.3), and the mean environmental pH of the stream (pH 8). pH 9 is also the lowest pH approaching the optimal pH using both substrates (see 6.23 and 6.33).

An axenic culture of *Calothrix parietina* D550 was used as a comparison to the field population of *Rivularia biasolettiana*. Also, variables in growth conditions could be manipulated in the axenic culture.

6.2 Axenic culture: *Calothrix parietina* D550

Calothrix parietina was isolated from Sand Sike in Upper Teesdale. This stream was similar in water chemistry to Red Sike, with pulses of phosphorus in spring (Livingstone & Whitton, 1984). *Calothrix parietina* has been kept as an axenic culture since 1979. Cultures for these experiments were grown in Chu 10D medium (Chu, 1942) as modified by Gibson and Whitton (1987) and Grainger *et al.* (1989), see 2.31. Nitrogen was not added to the medium and the phosphorus concentration was 1 mg l^{-1} at the time of subculture.

6.21 Time course

Time course experiments were used on the axenic culture to find out if the older cultures (assumed to be more P deficient) had a higher rate of activity and if there was a difference in rate of substrate cleavage at different substrate concentrations. Time course experiments were also used to check that activity was linear, especially at higher concentrations of substrate where enzyme limitation might occur. The rates of activity were linear with both substrates (Fig 6.1).

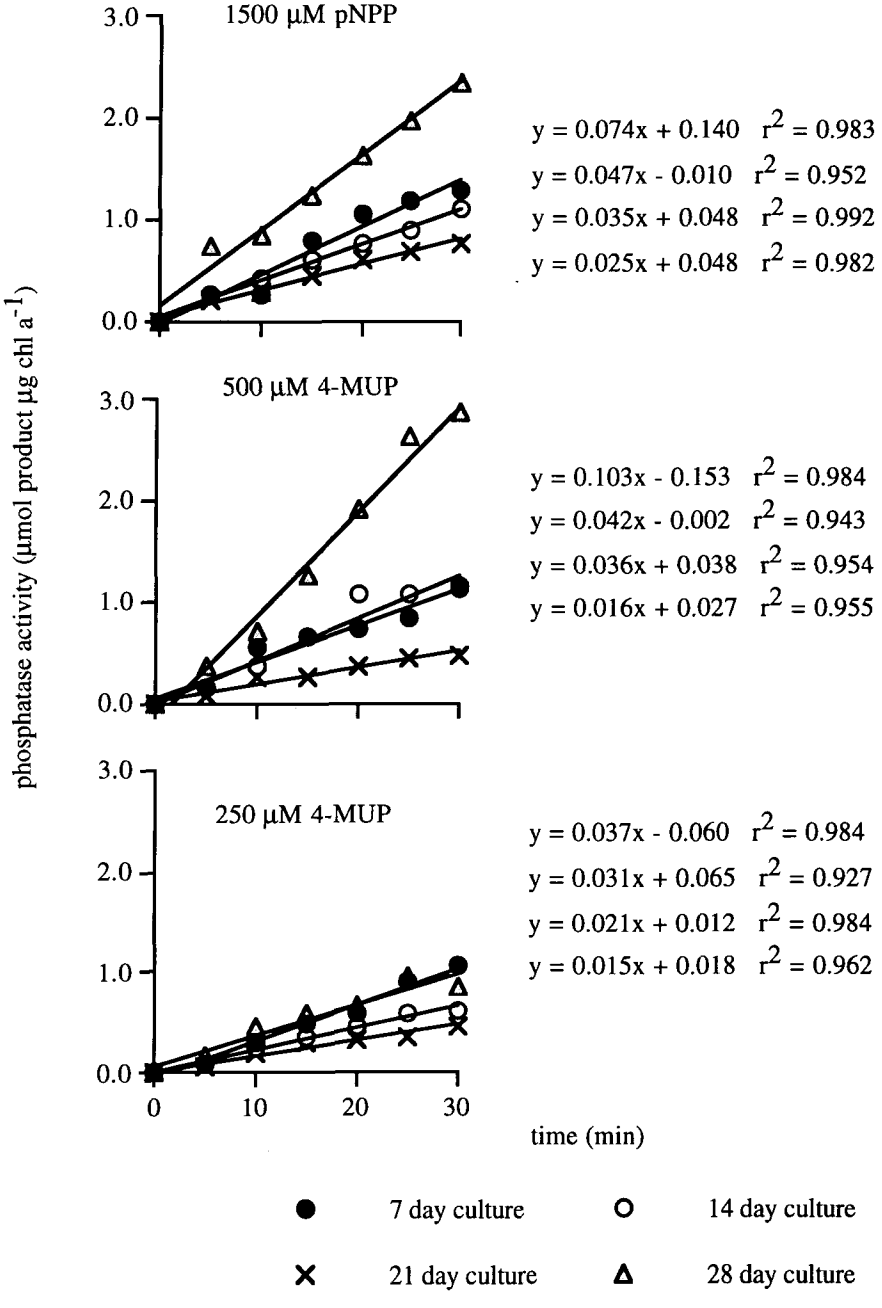


Fig. 6.1 Time course of phosphatase activity of *Calothrix parietina* D550 at 7, 14, 21 and 28 d, using 1500 µM pNPP, 500 and 250 µM 4-MUP ; all at pH 9.0

With 1500 μM pNPP, the 21 day old culture showed the lowest rate of activity (0.7 μmol pNP μg chl a^{-1} after 30 min), Fig. 6.1. This was 32 % that of the 28 day old culture which showed the highest rate of activity (2.2 μmol pNP μg chl a^{-1} after 30 min). Although the 14 day old culture showed a lower rate of activity than the 7 day old culture, the difference in rate of activity between these two was not significantly different (Table 6.1), using a test for equality of slopes (Zar, 1984).

Table 6.1 Comparison of phosphatase activity using 1500 μM pNPP, 500 and 250 μM 4-MUP between 7, 14, 21 and 28 d cultures of *Calothrix parietina* D550, (df = 10)

Cultures	1500 μM pNPP	500 μM 4-MUP	250 μM 4-MUP
7 : 14	NS	NS	***
7 : 21	**	***	***
7 : 28	**	***	NS
14 : 21	***	***	**
14 : 28	***	***	NS
21 : 28	***	***	*

Using 500 μM 4-MUP the rate of activity was again greatest in the oldest culture (2.9 μmol 4-MU μg chl a^{-1} after 30 min), Fig. 6.1. The 7 and 14 day cultures had a higher rate of activity than the 21 day culture (0.4 μmol 4-MU μg chl a^{-1} after 30 min) which was less than 14 % that of the 28 day culture. The rates of activity of all the cultures were significantly different ($p \leq 0.001$) except the 7 and 14 day old cultures. These results were similar to those using 1500 μM pNPP (Table 6.1).

Using half the substrate concentration, 250 μM 4-MUP, the rate of activity of the 28 day old culture was much less distinctive. The 21 day old culture still showed the lowest rate of activity (Fig. 6.1), which was the same as the rate of activity at twice the substrate concentration, as was the 7 day old culture. The rate of activity in the 28 day old culture was much less, one third of that at the higher substrate concentration, and was not significantly different to the 7 day or 14 day old cultures (Table 6.1).

6.22 Substrate concentration

The results of the rates of activity at 250 µM 4-MUP and at 500 µM 4-MUP may be related to enzyme characteristics. Kinetic experiments on the different aged cultures were used to try to determine characteristics of phosphatase activity using both substrates.

The 7 day old culture had the lowest V_{max} for both pNPP (1.1 µmol pNP µg chl a^{-1} h^{-1}) and 4-MUP (0.5 µmol 4-MU µg chl a^{-1} h^{-1}) and the 14 day old culture had the highest V_{max} for both substrates (4.4 and 4.7 µmol product µg chl a^{-1} h^{-1}, respectively), Table 6.2. The 7 day culture assayed with 4-MUP is not clearly linear so high and low substrate concentrations were regressed separately. The regression line for the total spectrum of substrate concentrations is also shown (Fig. 6.2).

The possible non-linearity of the Lineweaver - Burk plot (and Eadie - Hofstee plot) may indicate non-Michaelis - Menten type kinetics in the 7 day old culture assayed with 4-MUP (Fig. 6.2); with high substrate concentrations giving a higher V_{max} and K_m than the lower substrate concentrations (Table 6.2). n_H (see 2.4) was calculated and found to be < 1 (Table 6.2), indicating negative cooperativity. Negative cooperativity is defined as a protein molecule having two or more sites where the event of binding at one site interferes with the same event of binding at other sites in the molecule (Bohinski, 1987). As the substrate concentration increases enzyme-substrate binding becomes less efficient. K_m increases as the enzyme - substrate affinity is lessened.

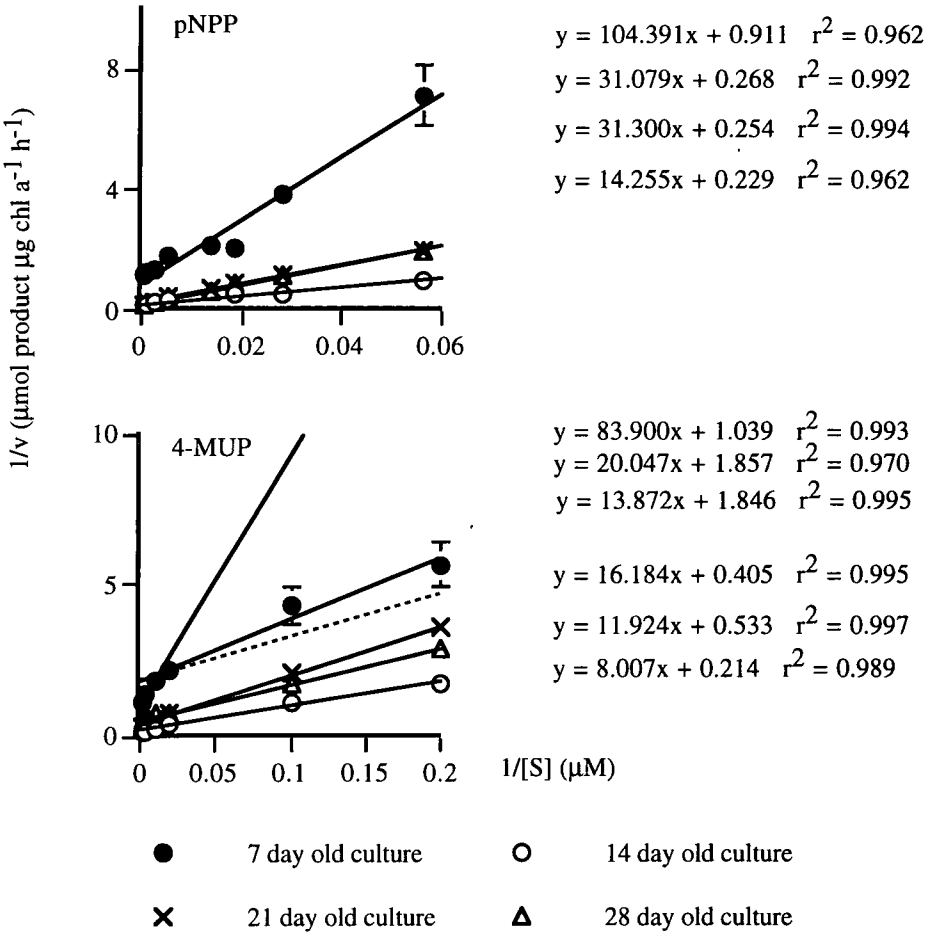


Fig. 6.2 Lineweaver - Burk plot of *Calothrix parietina* D550 using pNPP and 4-MUP, at 7, 14, 21 and 28 d, at pH 9.0 (n = 4)

The 7 day old culture showed the lowest K_m, and thus indicates the greatest affinity of all cultures to both substrates (Table 6.2). However V_{max}/K_m was highest (0.060 using pNPP and 0.086 using 4-MUP) in the 14 day old culture using both substrates (Table 6.2), indicating the highest competitive ability (see 2.4), except at low concentrations of 4-MUP when 7 day old culture gave a V_{max}/K_m ratio of 0.123. The data for 21 and 28 days were similar using pNPP but using 4-MUP, 21 day cultures had a higher V_{max} and K_m. The K_m value was always lower and the ratio V_{max}/K_m was always higher using 4-MUP.

Table 6.2 Kinetic values for cultures of *Calothrix parietina* D550 7, 14, 21 and 28 d using pNPP and 4-MUP

Culture (d)	V_{max}		K_m		V_{max}/K_m		n_H
	pNPP	4-MUP	pNPP	4-MUP	pNPP	4-MUP	4-MUP
7 low [S]		0.5		4		0.123	
high [S]		1.0		52		0.018	
all [S]	1.1	0.5	64	12	0.017	0.045	0.730 ***
14	4.3	4.7	72	54	0.060	0.086	
21	3.7	2.5	150	49	0.025	0.050	
28	3.9	1.8	134	23	0.029	0.082	

Since Lineweaver - Burk plots are expected to be linear throughout substrate concentration in classical Michaelis - Menten kinetics, an equality of slopes test was performed on plots to compare the activity using pNPP to that using 4-MUP on same age cultures. The test for equality of slopes from the Lineweaver - Burk plots showed that the same age cultures gave a significantly different response when using pNPP than when using 4-MUP (Table 6.3). Seven day cultures using 4-MUP may be non-linear through the total substrate range, so high and low concentrations were compared separately. There was a significant difference between the slope of 7 day old cultures assayed with low concentrations (5-50 µM 4-MUP) to those assayed at high concentrations (100-1000 µM 4-MUP).

Table 6.3 Comparison of Lineweaver - Burk plots of *Calothrix parietina* D550 7, 14, 21 and 28 d using pNPP to those using 4-MUP (high and low concentrations of 7 day cultures using 4-MUP compared separately)

Culture and substrate	df	p
7 pNPP : 7 4-MUP	11	***
14 pNPP : 14 4-MUP	10	**
21 pNPP : 21 4-MUP	11	***
28 pNPP : 28 4-MUP	11	***
7 4-MUP low : 7 4-MUP high	4	***

Using pNPP, there were significant differences between all the different age cultures except 21 and 28 day old cultures (Table 6.4). Using 4-MUP, 7 day old cultures showed no significant difference to any of the other cultures; all the other cultures were significantly different from one another ($p \leq 0.001$), Table 6.4. However, all concentrations were used to compare 7 day old cultures to the other cultures. Because the r^2 value of the 7 day old cultures was lower, the variance was increased which may result in the slope being not significantly different to any of the others cultures.

Table 6.4 Comparison of Lineweaver - Burk plots using pNPP and 4-MUP between *Calothrix parietina* D550 (7, 14, 21, 28 d cultures)

Cultures	pNPP		4-MUP	
	df	p	df	p
7 : 14	11	***	10	NS
7 : 21	11	***	11	NS
7 : 28	11	***	11	NS
14 : 21	12	***	9	***
14 : 28	12	***	9	***
21 : 28	12	NS	10	***

6.23 pH spectrum

At all substrate concentrations and with both substrates the optimum pH of phosphatase activity in *Calothrix parietina* D550 was between 9.5 - 11.0 (Fig. 6.3). Activity using pNPP showed a more gradual response to pH.

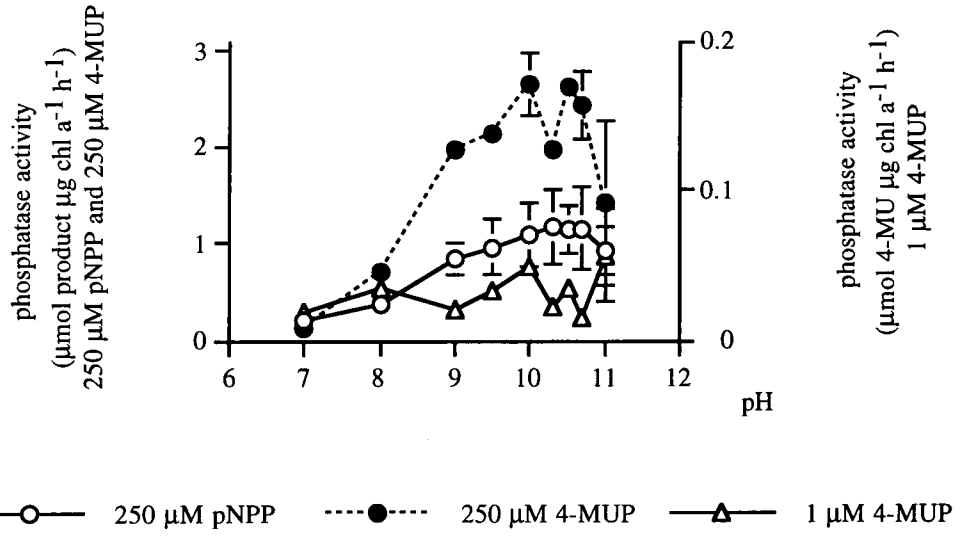


Fig. 6.3 pH spectrum of 21 d *Calothrix parietina* D550 phosphatase activity using 250 µM pNPP and 4-MUP (y axis) and 1 µM 4-MUP (yy axis) (n = 3)

6.3 Field population: *Rivularia biasolettiana*

Rivularia showed a high degree of variability in the long-term field study (see 4.42) which made interpretation of differences between substrates and substrate concentrations difficult. More detailed studies on phosphatase activity were carried out which may be helpful in illustrating features of the enzymes involved, although environmental conditions (e.g. phosphorus concentration) in the stream at time of sampling may influence the phosphatase activity of the colonies. Phosphatase activity was generally high at Red Sike, so colonies were collected when phosphorus levels were expected to be low.

Colonies collected for the experiment showing the effect of pH on phosphatase activity, were collected at the same time as those collected for time course experiments, on 17.3.1994. At the time of collection, the total P concentration of the water at Red Sike was very low (< 2 µg l^{-1}, all of which was inorganic). Colonies of *Rivularia* collected for experiments comparing teased to whole colonies, were collected from Red Sike on 13.6.1994 when phosphorus levels in the water were below detection limits.

6.31 Time course

An experiment comparing the rate of activity in whole and teased colonies was used to determine the permeability of the two substrates (Fig 6.4).

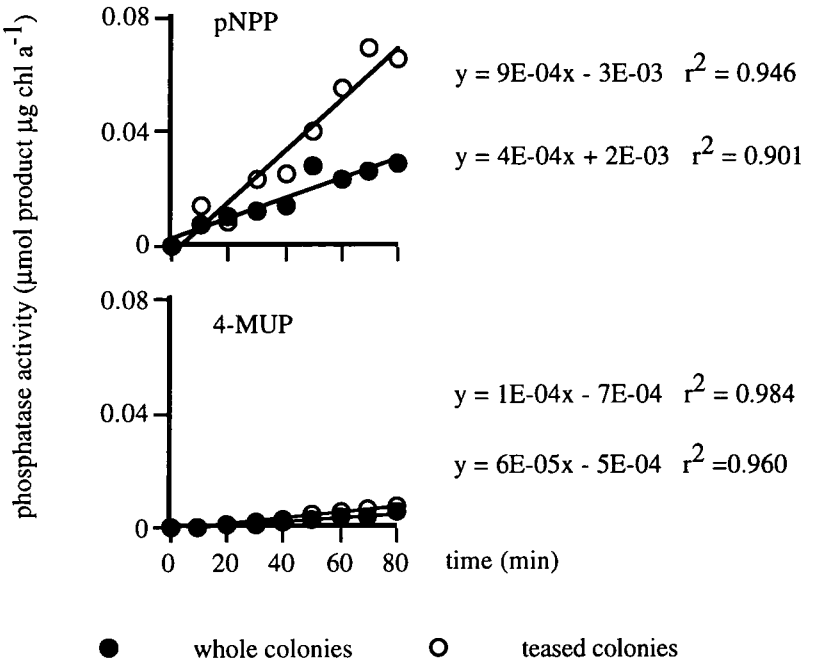


Fig. 6.4 Time course of phosphatase activity of *Rivularia biasolettiana* comparing teased and whole colonies using 250 µM pNPP and 250 µM 4-MUP, at pH 9.0

Time courses using *Rivularia biasolettiana* colonies were always linear (Fig. 6.4). Teased colonies gave a higher rate of activity than whole colonies which was significant ($p \leq 0.001$) using both substrates (Table 6.5). When comparing the rates of activity of each type of colony separately to each substrate, significantly higher rates of activity were found using pNPP than 4-MUP.

Table 6.5 Comparison of phosphatase activity using 250 µM pNPP and 250 µM 4-MUP, between teased and whole *Rivularia biasolettiana* colonies at pH 9.0; (df = 14)

Substrate and colony type	p
pNPP whole : pNPP teased	***
4-MUP whole : 4-MUP teased	***
pNPP whole : 4-MUP whole	***
pNPP teased : 4-MUP teased	***

Because of the differences between the substrates (substrate concentrations and pH) and high variability of the results in the long-term field study of *Rivularia,* different sized colonies were compared in order to find out if small differences in size would account for some of the variability (Fig. 6.5).

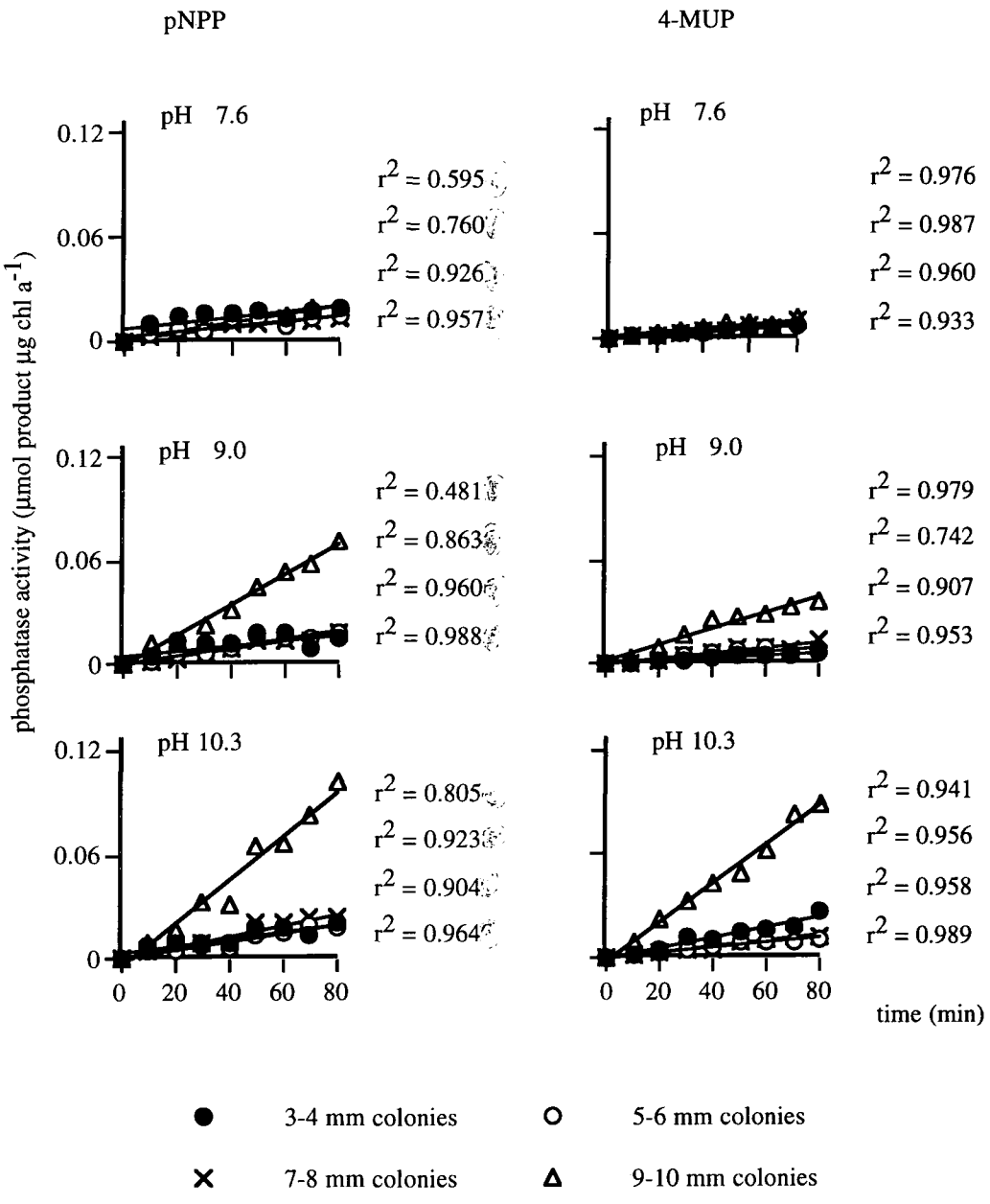


Fig. 6.5 Time course of phosphatase activity of different sized colonies (3-4, 5-6, 7-8, 9-10 mm) of *Rivularia biasolettiana* using 250 µM pNPP and 4-MUP at pH 7.6, 9.0 and 10.3

Generally, activity was much lower at pH 7.6 using both substrates, than at either pH 9 or pH 10.3. All rates of activity were linear ($p \leq 0.050$) except the 3-4 mm colonies using pNPP at pH 9. Comparing the two substrates (Table 6.6), it was found

that there was no significant difference between the rates of activity in the smaller colonies at any pH. However 9-10 mm colonies, at pH 7.6 and 9, had a significantly higher rate of activity using pNPP than 4-MUP ($p \leq 0.010$), but at pH 10.3 there was no significant difference. At pH 10.3 the 7-8 mm colonies showed a significantly higher rate of activity using pNPP ($p \leq 0.050$).

Table 6.6 Comparison of phosphatase activity of different sized *Rivularia biasolettiana* colonies (3-4 mm, 5-6 mm, 7-8 mm, 9-10 mm) between 250 µM pNPP to those using 250 µM 4-MUP at pH 7.6, 9.0 and 10.3 (df = 14)

Colony size (mm)	pH 7.6	pH 9.0	pH 10.3
3- 4	NS	NS	NS
5- 6	NS	NS	NS
7- 8	NS	NS	*
9-10	**	***	NS

When comparing the rates of activity of the different sized colonies using pNPP, at pH 7.6 there were no significant differences between colony sizes (Table 6.7). However, at both pH 9 and 10.3 there were significant differences in the rates of activity between the smaller sized colonies (3-8 mm) and the largest colonies (9-10 mm).

Table 6.7 Comparison of phosphatase activity using 250 µM pNPP and 4-MUP between different sized *Rivularia biasolettiana* colonies, at pH 7.6, 9.0 and 10.3 (df = 14)

Colony size (mm)	pNPP			4-MUP		
	pH 7.6	pH 9.0	pH 10.3	pH 7.6	pH 9.0	pH 10.3
3-4 : 5- 6	NS	NS	NS	NS	NS	***
3-4 : 7- 8	NS	NS	NS	NS	NS	***
3-4 : 9-10	NS	***	***	NS	***	***
5-6 : 7- 8	NS	NS	NS	NS	NS	NS
5-6 : 9-10	NS	***	***	NS	***	***
7-8 : 9-10	NS	***	***	NS	***	***

Using 4-MUP there was a similar pattern (Table 6.7) with the lowest pH showing no significant differences between colonies of different sizes. At higher pH there were more differences. At pH 9, as with pNPP, there were differences between the largest colonies and all the other sizes. At pH 10.3 only 5-6 : 7-8 mm colonies showed no significant difference in rate of activity.

Comparing the rates of activity of similar sized colonies at different pH using pNPP (Table 6.8), there were no significant differences between the different pH in the smaller colonies, only between pH 7.6 and 10.3 in 7-8 mm colonies and between all three combinations of pH in the largest colonies. Using 4-MUP there were more differences, especially comparing activity at pH 10.3 in the smallest colonies and when comparing all three combinations of pH in the largest colonies.

It seems unlikely that the variability in activity in the long-term study was related to small differences in colony size. From these experiments 4-MUP shows more difference related to colony size, whereas in the field study colony activity using both pNPP and 4-MUP was highly variable.

Table 6.8 Comparison of phosphatase activity using 250 µM pNPP and 4-MUP of *Rivularia* colonies (3-4, 5-6, 7-8, 9-10 mm) between pH 7.6, 9.0 and 10.3 (df = 14)

pH	p values using pNPP				p values using 4-MUP			
	3-4 mm	5-6 mm	7-8 mm	9-10 mm	3-4 mm	5-6 mm	7-8 mm	9-10 mm
7.6 : 9.0	NS	NS	NS	***	NS	NS	NS	***
7.6 : 10.3	NS	NS	**	***	***	***	NS	***
9.0 : 10.3	NS	NS	NS	**	***	NS	NS	***

6.32 Substrate concentration

Kinetic experiments were carried out on whole colonies compared to teased colonies in order to continue testing the effect of colony structure and permeability of the two substrates.

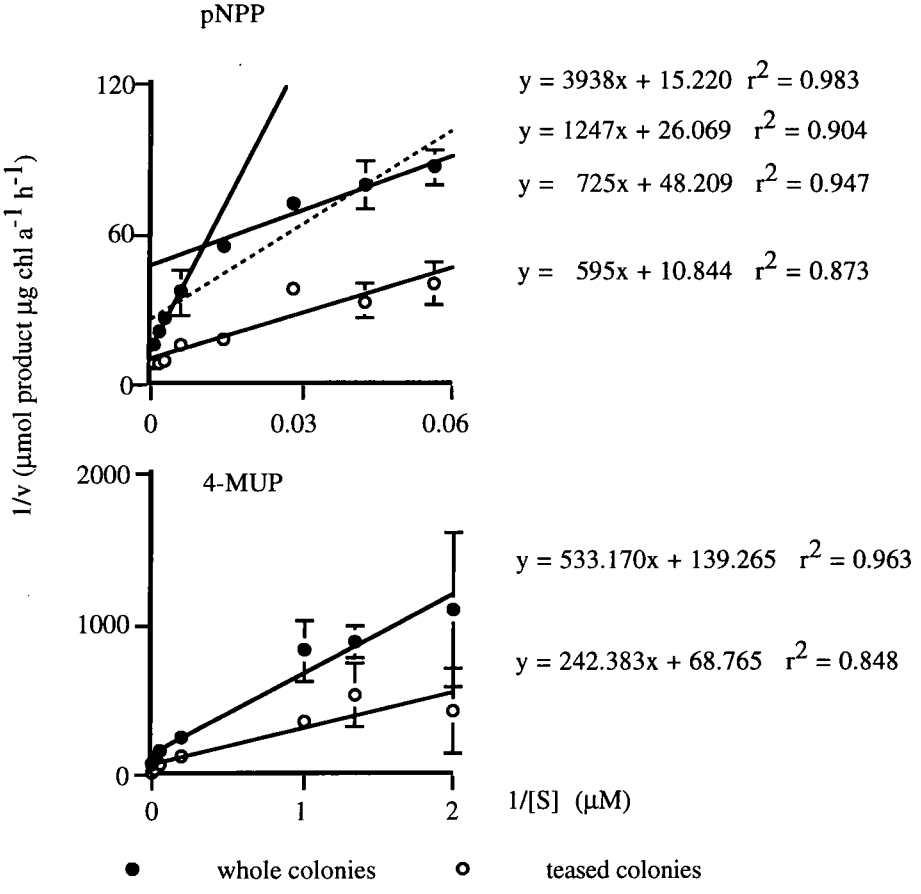


Fig. 6.6 Lineweaver - Burk plot comparing teased and whole colonies of *Rivularia biasolettiana*, using pNPP and 4-MUP, at pH 9.0 (separate regression lines for whole colonies at low and high concentrations of pNPP also shown) (n = 3)

Teased colonies showed higher activity than whole colonies using both pNPP and 4-MUP, though activity was less in both teased and whole colonies using 4-MUP (Fig. 6.6). Whole colonies showed possibly different activity at high and low substrate concentrations using pNPP, indicated by a slightly non-linear Lineweaver - Burk plot (Fig. 6.6) and an $n_H < 1$ (Table 6.9), indicating negative cooperativity. Comparing teased and whole colonies at all substrate concentrations (Table 6.9), teased colonies gave a higher V_{max} (approximately twice as high using both pNPP and 4-MUP); a similar K_m using pNPP (65, 64 µM) and a K_m nearly twice as high (9.6, 5.4 µM) using 4-MUP (Table 6.9). Both whole and teased *Rivularia* colonies gave a higher K_m with pNPP. The ratio of V_{max}/K_m was higher using 4-MUP in both whole (except at low substrate concentration where the ratio was slightly higher in pNPP) and

teased colonies. V_{max} was greater using pNPP than using 4-MUP (6.1 times with teased colonies and 7.1 times with whole colonies), Table 6.9.

Table 6.9 Kinetic values for teased and whole *Rivularia biasolettiana* colonies using pNPP and 4-MUP, assayed at pH 9.0

Colony type	V_{max}		K_m		V_{max}/K_m		n_H
	pNPP	4-MUP	pNPP	4-MUP	pNPP	4-MUP	pNPP
whole							
low [S]	0.038		13.7		0.0028		
high [S]	0.066		257.7		0.0003		
all [S]	0.050	0.007	64.1	5.4	0.0008	0.0013	0.768 ***
teased	0.092	0.015	65.4	9.6	0.0014	0.0016	

Comparing the slopes of the Lineweaver - Burk plots there was no significant difference between slopes using different substrates, indicating that the relationship of the enzyme(s) to each of the substrates was similar (Table 6.10). However, the difference between the slopes of the whole and teased colonies was just significant, using both pNPP and 4-MUP.

Table 6.10 Comparison of slopes of Lineweaver - Burk plots of teased and whole colonies of *Rivularia biasolettiana* using pNPP and 4-MUP, at pH 9.0

Substrate	df	p
whole pNPP : whole 4-MUP	11	NS
teased pNPP : teased 4-MUP	11	NS
whole pNPP : teased pNPP	10	*
whole 4-MUP : teased 4-MUP	10	*

6.33 pH spectrum

Using both substrates, the phosphatase activity of *Rivularia biasolettiana* shows a similar response to pH with maximum activity between pH 10.0 - 11.0 (Fig. 6.7). pH spectra were taken during the long-term field study; generally showing a similar pattern, though the activity was slightly different probably due to differences in the environment in which the organisms were growing.

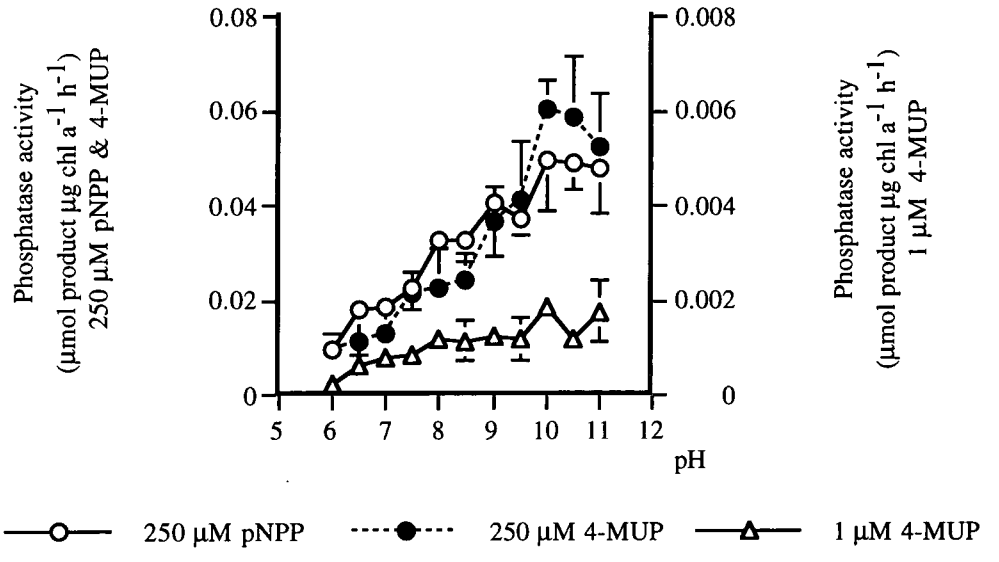


Fig. 6.7 pH spectrum of phosphatase activity of whole *Rivularia biasolettiana* colonies using 250 µM pNPP and 250 µM 4-MUP (y axis), and 1 µM 4-MUP (yy axis) (n = 4)

6.4 Summary

In *Calothrix parietina* D550 the oldest culture (28 day) showed the highest rate of activity at 1500 µM pNPP and 500 µM 4-MUP. *Rivularia biasolettiana* colony sizes did influence the rate of phosphatase activity with larger colonies (9-10 mm) showing much higher rates of activity than any of the smaller colonies at pH 9 and 10.3 though at pH 7.6 there was little difference. Teasing *Rivularia* colonies made the rate of activity significantly higher, at 250 µM pNPP and 4-MUP. pNPP gave a significantly higher activity than 4-MUP in both teased and whole colony experiments but this was not the case for experiments using different sized colonies or pH spectra.

Highest V_{max} values were in 14 d cultures of *Calothrix parietina* D550, using both substrates. K_m appeared to increase with age using pNPP and decrease with

age using 4-MUP. There may have been apparent negative cooperativity in 7 day cultures using 4-MUP. Whole colonies of *Rivularia* may also have shown apparent negative cooperativity but using pNPP rather than 4-MUP. Teased colonies showed higher activity at all substrate concentrations, higher V_{max} and generally a higher K_m. V_{max}, using both substrates, was at least one order of magnitude higher in *Calothrix parietina* D550 than in *Rivularia biasolettiana*, although K_m values were similar, especially when comparing whole colonies of *R. biasolettiana* with 7 day *Calothrix parietina* D550 cultures.

Both freshwater organisms showed higher affinity for 4-MUP, both in terms of K_m and V_{max}/K_m, than pNPP. Both organisms showed maximal phosphatase activity between pH 9.5 - 11 in both substrates at all concentrations tested and higher activity in 4-MUP than pNPP at higher pH.

CHAPTER 7

EXPERIMENTAL STUDIES ON TYNE SANDS ORGANISMS

7.1 Introduction

The long-term field study at Tyne Sands indicated differences between the phosphatase activity of the organisms using pNPP and 4-MUP (see 5.52). The aim of this chapter was to carry out more detailed experiments on the marine organisms to investigate these differences.

From the field study phosphatase activity appeared to be highest July - October (1992 and 1993) for all the organisms, although maximum activity varied between organisms and substrates. Field material was collected in July 1994, in order that phosphatase activity would be high, however, environmental phosphorus levels were high (see 7.2 and 7.3).

An axenic culture originating from Tyne Sands, *Dichothrix* D861, was grown in controlled conditions and used as a comparison. Because the substrates were assayed at 2 different pH in the long-term field study (10.3, pNPP and 7.6, 4-MUP), a pH spectrum was carried out (see 7.23 and 7.33). An intermediate pH to the long-term field study, pH 9.0, showing near optimal activity, was chosen for the more detailed studies (as in Chapter 6).

7.2 Axenic culture: *Dichothrix* D861 and field community: *"Dichothrix"*

Dichothrix was isolated from rock at the edge of R9, Tyne Sands (see 5.4). Once isolated, the culture became D861 in the Durham Culture Collection. Because *Dichothrix* D861 and the "*Dichothrix*" community originated from the same area, one being axenic the other a field population, the results are presented together in this section.

Since *Dichothrix* D861 did not show phosphatase activity until 3 weeks after subculture and material appeared unhealthy after this age, experiments were carried out 3 weeks after subculture (see 2.32). However, due to a shortage of material only 1 sample was used. "*Dichothrix*" community material was collected the day before

these experiments. The level of phosphorus in the pool on the day of collection (18.7.1994) was 19.5 μg l^{-1} inorganic P (57.4 μg l^{-1} total phosphorus) which was high and may have influenced the low phosphatase activity of the community (see 1.341).

7.21 Time course

Time courses were used to compare rates of activity in the two organisms using both substrates. The highest substrate concentrations were used to check that there was no enzyme saturation during the assay. Using both pNPP and 4-MUP the rate of phosphatase activity in homogenised *Dichothrix* D861 and the "*Dichothrix*" community was linear (Fig. 7.1).

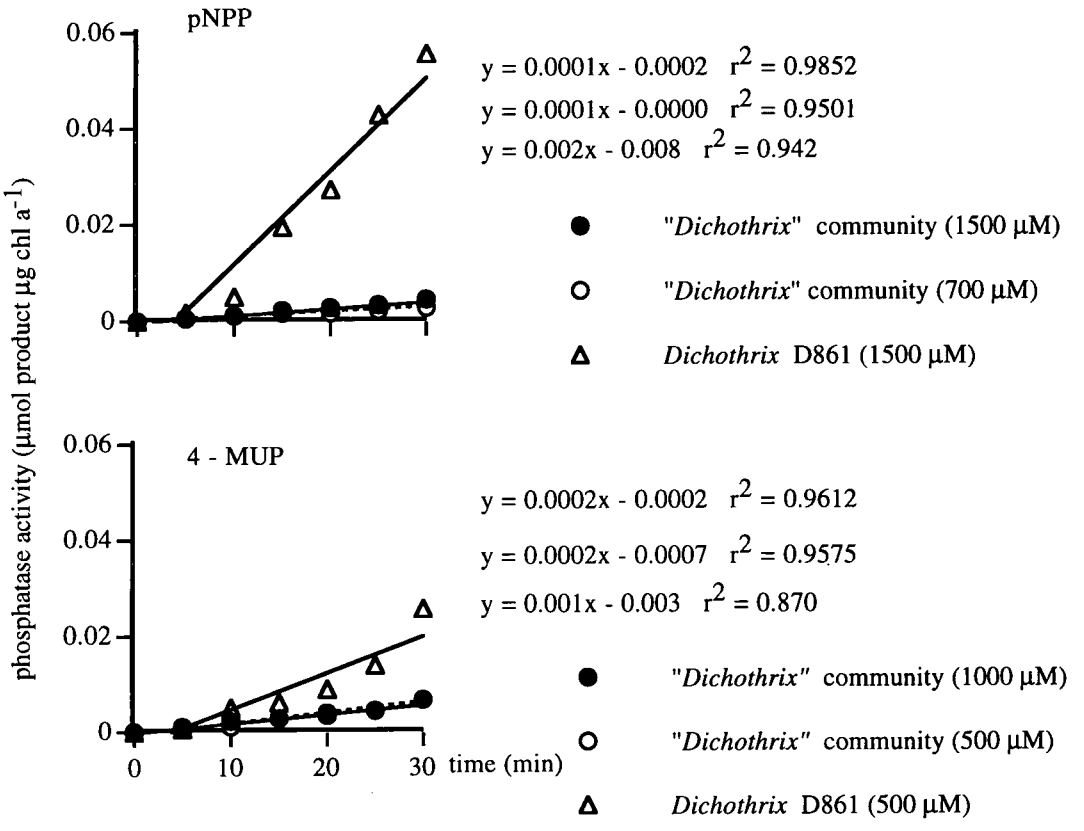


Fig. 7.1 Time course of phosphatase activity of *Dichothrix* D861 and the "*Dichothrix*" community using 1500 μM and 700 μM pNPP ("*Dichothrix*" community only) and 500 μM and 1000 μM 4-MUP ("*Dichothrix*" community only), at pH 9.0

The rates of activity of *Dichothrix* D861 and the "*Dichothrix*" community were significantly different ($p \leq 0.010$) using both substrates (Table 7.1). *Dichothrix* D861 had a higher rate of activity than the "*Dichothrix*" community, 17 times higher using 1500 µM pNPP (at 0.052 µmol pNP µg chl a^{-1} after 30 min), and nearly 5 times higher (at 0.027 µmol 4-MU µg chl a^{-1} after 30 min) using 500 µM 4-MUP (Fig. 7.1).

Table 7.1 Comparison of phosphatase activity using 1500 µM pNPP and 500 µM 4-MUP between *Dichothrix* D861 and the "*Dichothrix*" community

Organism and substrate concentration	df	p
Dichothrix : "*Dichothrix*" community 1500 µM pNPP	10	***
Dichothrix : "*Dichothrix*" community 500 µM 4-MUP	10	**

The rate of activity of the "*Dichothrix*" community was significantly higher using 1500 µM pNPP than 700 µM. Using 4-MUP there was no significant difference in the rate of activity using 1000 µM to that using 500 µM (Table 7.2). This suggests a kinetic difference between the two substrates.

Table 7.2 Comparison of phosphatase activity of the "*Dichothrix*" community using 1500 to 700 µM pNPP, and 1000 to 500 µM 4-MUP

Substrate comparison	df	p
"*Dichothrix*" community 1500 : 700 µM pNPP	10	***
"*Dichothrix*" community 1000 : 500 µM 4-MUP	10	NS

7.22 Substrate concentration

The kinetic parameters of *Dichothrix* D861 and the "*Dichothrix*" community are very different with *Dichothrix* showing higher activity at all substrate concentrations

with both substrates (Fig. 7.2). The Lineweaver - Burk plots (Fig. 7.2) of *Dichothrix* D861 and the "*Dichothrix*" community were linear.

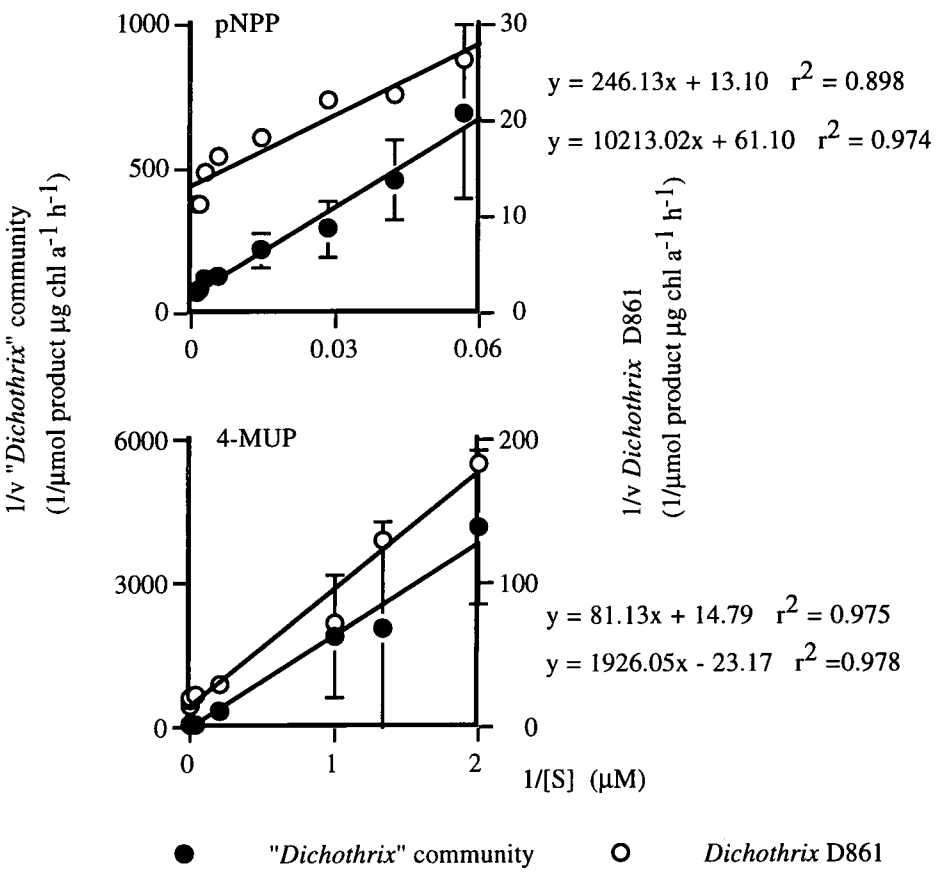


Fig. 7.2 Lineweaver - Burk plot of *Dichothrix* D861 and the "*Dichothrix*" community using pNPP and 4-MUP, at pH 9.0 (n = 4 "*Dichothrix*" community only)

The V_{max} of *Dichothrix* D861 using pNPP was slightly higher than using 4-MUP, the K_m was 5.5 times higher using pNPP and the ratio V_{max}/K_m was nearly five times higher using 4-MUP, suggesting that the phosphatase enzyme(s) in this organism have a higher affinity for 4-MUP than for pNPP (Table 7.3). Although the overall activity was much lower, the *"Dichothrix"* community gave a similar response to the two substrates, with higher affinity of the enzyme(s) to 4-MUP than pNPP. The K_m was nearly 6 times higher using pNPP and the ratio V_{max}/K_m was 23 times higher using 4-MUP. However, the *"Dichothrix"* community, in contrast to *Dichothrix* D861, showed a higher V_{max} using 4-MUP.

Comparing the two organisms, *Dichothrix* D861 had a V_{max} nearly five times higher and a K_m five times lower than the *"Dichothrix"* community using pNPP. Using 4-MUP the V_{max} of *Dichothrix* D861 was nearly twice as high and the K_m again nearly 5 times lower than the *"Dichothrix"* community. The ratio V_{max}/K_m was 36 times higher using pNPP and 7.6 times higher using 4-MUP in the axenic culture compared to the field community. The axenic organism had a higher affinity for pNPP and probably more affinity for 4-MUP than the field community, at the time of assay.

Table 7.3 Kinetic values for *Dichothrix* D861 and the "*Dichothrix*" community using pNPP and 4-MUP, assayed at pH 9.0

Organism	V_{max}		K_m		V_{max}/K_m	
	pNPP	4-MUP	pNPP	4-MUP	pNPP	4-MUP
Dichothrix D861	0.076	0.068	21.3	3.9	0.0036	0.0174
"*Dichothrix*" community	0.016	0.043	111.8	18.7	0.0001	0.0023

Comparison of slopes of the Lineweaver - Burk plots shows that *Dichothrix* D861 showed significantly higher activity ($p \leq 0.001$, Table 7.4) than the "*Dichothrix*" community. Using 4-MUP the situation was similar with *Dichothrix* D861 showing higher activity ($p \leq 0.001$). However, there was no significant difference in either organism when comparing the activity using pNPP to that using 4-MUP, suggesting that the activity was similar in each organism using both substrates.

Table 7.4 Comparison of Lineweaver - Burk plots using pNPP and 4-MUP between *Dichothrix* D861 and the "*Dichothrix*" community, assayed at pH 9.0

Organism and substrate	df	p
Dichothrix:: "*Dichothrix* " pNPP	12	***
Dichothrix:: "*Dichothrix* " 4-MUP	12	***
Dichothrix pNPP: 4-MUP	12	NS
"*Dichothrix* " pNPP: 4-MUP	12	NS

7.23 pH spectrum

Dichothrix D861 showed optimum activity (Fig. 7.3) between pH 9.0 - 11 using 250 µM pNPP (0.07 µmol pNP µg chl a^{-1} h^{-1}) and 250 µM 4-MUP (0.18 µmol 4-MU µg chl a^{-1} h^{-1}). At pH 9 activity was similar using pNPP and 4-MUP, however at higher pH activity was higher using 4-MUP.

There was a high degree of variability in the "*Dichothrix*" community especially at pH 7.5 using pNPP. Maximal activity was between pH 10.0 - 10.7 using pNPP (0.007 µmol pNP µg chl a^{-1} h^{-1}) and using 4-MUP (0.016 µmol 4-MU µg chl a^{-1} h^{-1}). Above pH 9.0, and especially between pH 10 - 11, activity was higher using 4-MUP than pNPP.

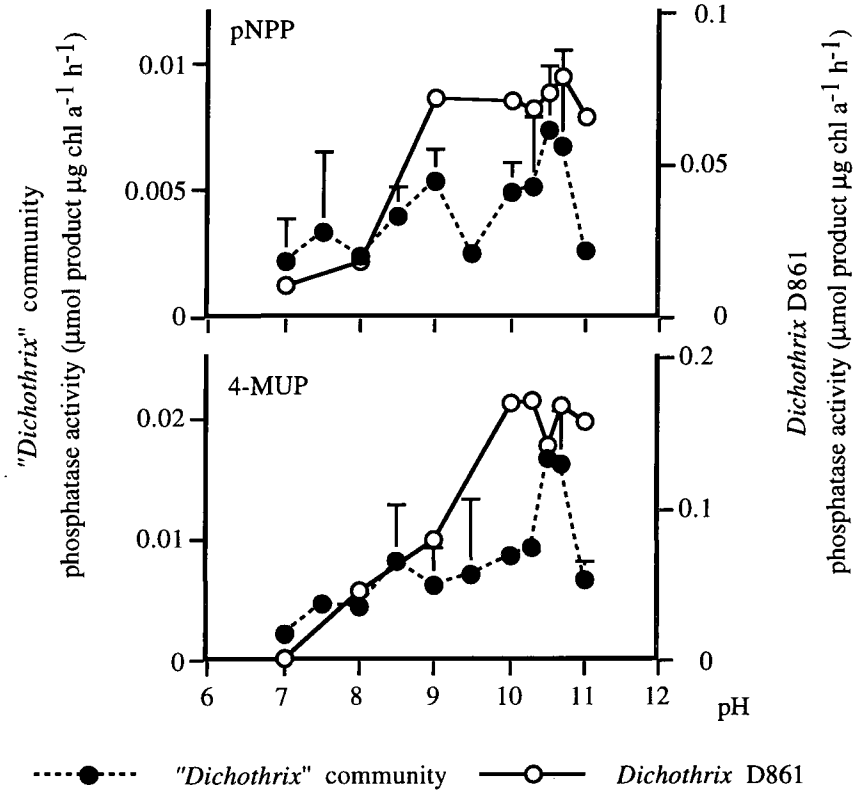


Fig. 7.3 pH spectrum of phosphatase activity of the "*Dichothrix*" community (y axis) and *Dichothrix* D861 (yy axis) using 250 µM pNPP and 4-MUP (n = 4 "*Dichothrix*" community only)

7.3 Field population: *Rivularia atra* and *Ralfsia verrucosa*

Rivularia atra and *Ralfsia verrucosa* were compared because they are potential competitors, inhabiting the same area of the rock pools and probably competing for nutrients. Both showed high levels of phosphatase activity though this fluctuated

markedly during the year (see 5.52). *Rivularia* especially, showed marked differences between the two substrates during the long-term field study. Samples for both organisms were collected on the same day (29.7.1994) from R7 where *Rivularia* was most abundant, however, it was not possible to collect enough samples for replicates to be used. The long-term field studies had indicated that this was the time of year when phosphatase activity in at least one of the substrates, was likely to be high. Inorganic P levels in R7 were 7.3 $\mu g\ l^{-1}$ (total P was 51.1 $\mu g\ l^{-1}$) at the time of collection.

7.31 Time course

Whole and teased *Rivularia* colonies and *Ralfsia* material were compared for rate of phosphatase activity using 1500 µM pNPP and 500 µM 4-MUP (Fig 7.4). In all cases the regression lines were linear.

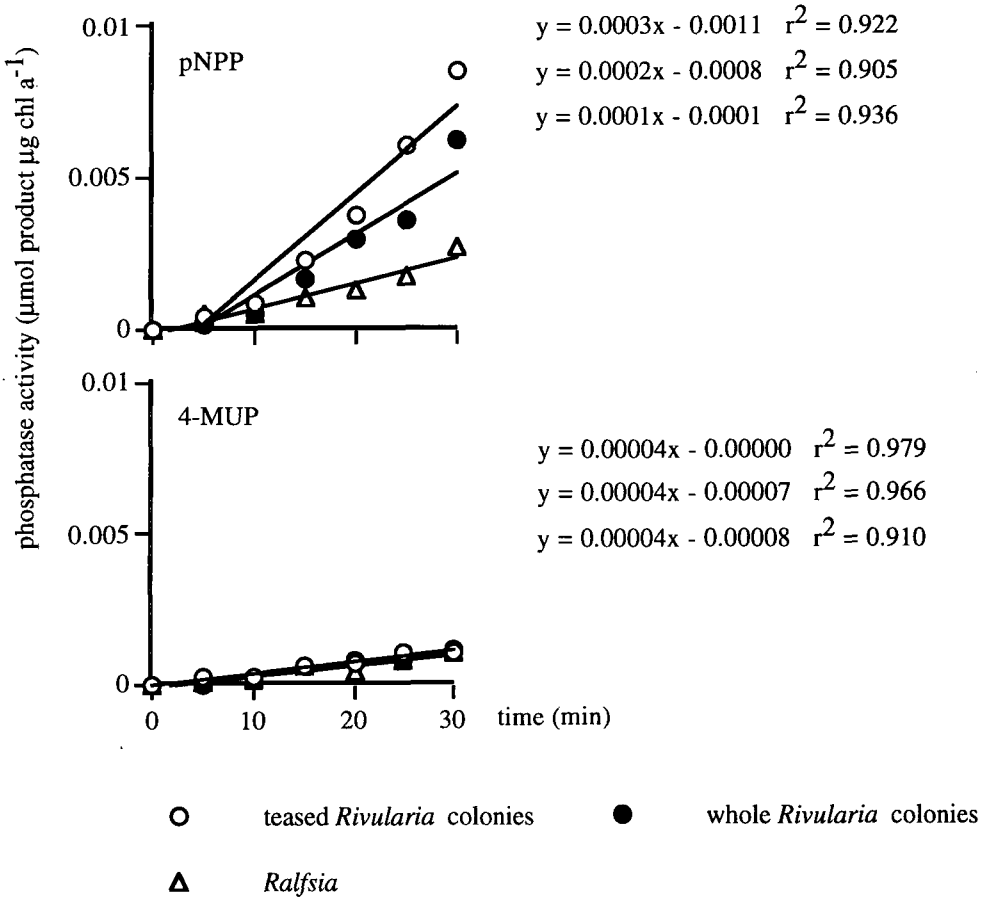


Fig. 7.4 Time course of whole and teased *Rivularia atra* colonies and *Ralfsia verrucosa* phosphatase activity using 1500 µM pNPP and 500 µM 4-MUP, at pH 9

Using pNPP, teased *Rivularia* colonies appeared to have the highest rate of activity (0.008 μmol pNP μg chl a^{-1} in 30 min) and *Ralfsia* had the lowest rate of activity (0.0029 μmol pNP μg chl a^{-1} in 30 min), Fig. 7.4. However, comparison of slopes showed that there was no significant difference between the rate of activity in whole or teased *Rivularia* but there was a significant difference ($p \leq 0.010$) in the rate of activity between *Ralfsia* and both types of *Rivularia* colonies (Table 7.5). Using 4-MUP, there was no difference in the rate of activity in any of the three types of material.

Table 7.5 Comparison of phosphatase activity using 1500 μM pNPP and 500 μM 4-MUP between whole and teased *Rivularia atra* colonies, and *Ralfsia verrucosa*

organism and substrate	df	p
Rivularia teased : whole pNPP	10	NS
Rivularia teased : whole 4-MUP	10	NS
Ralfsia pNPP : teased *Rivularia* pNPP	10	***
Ralfsia pNPP : whole *Rivularia* pNPP	10	*
Ralfsia 4-MUP : teased *Rivularia* 4-MUP	10	NS
Ralfsia 4-MUP : whole *Rivularia* 4-MUP	10	NS

7.32 Substrate concentration

Kinetics experiments were used to investigate further the differences between the two substrates, as well as the differences between the phosphatase activity of *Rivularia* and *Ralfsia*. *Rivularia* showed a higher rate of activity than *Ralfsia* at all substrate concentrations tested (Fig. 7.5).

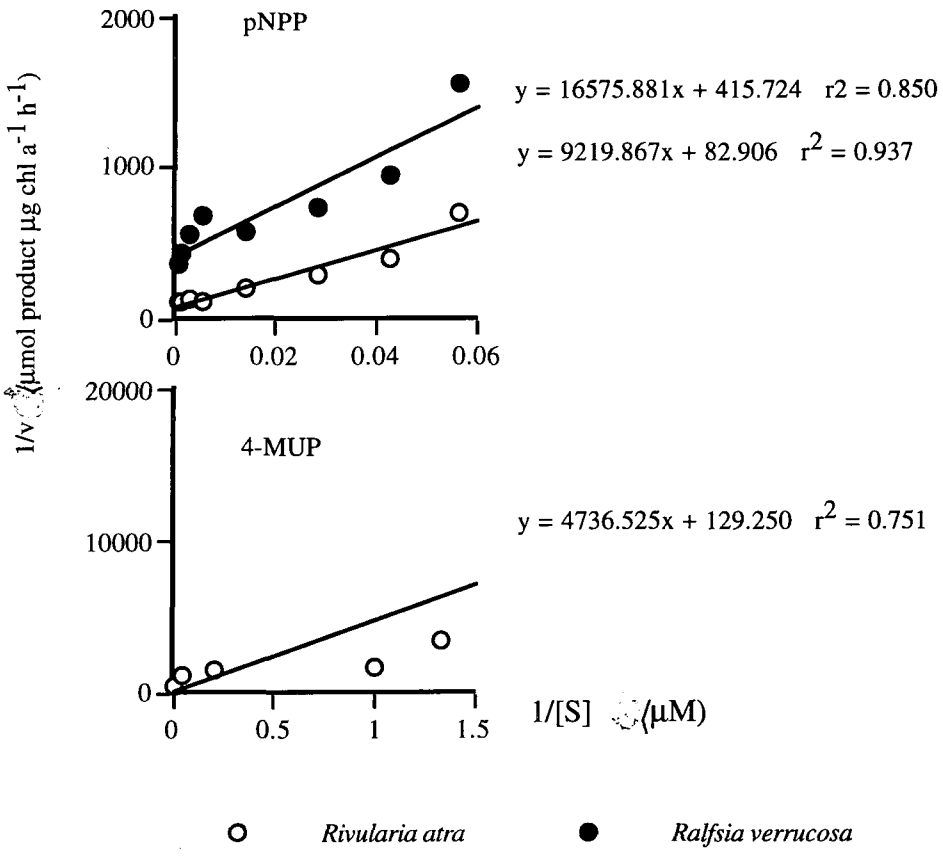


Fig 7.5 Lineweaver - Burk plot of *Rivularia atra* and *Ralfsia verrucosa* using pNPP and 4-MUP (*Rivularia* only), at pH 9.0

The V_{max} of *Rivularia* using pNPP was nearly double that using 4-MUP (Table 7.6). The K_m was over 30 times larger using pNPP and the ratio of V_{max}/K_m was 20 times larger using 4-MUP. Although the maximum activity was higher using pNPP, the affinity was much higher with 4-MUP. The V_{max} of *Ralfsia* was 5 times lower using pNPP than in *Rivularia*. The K_m and the ratio V_{max}/K_m was approximately half that of *Rivularia*. *Ralfsia* had lower maximum activity than *Rivularia*, though it had apparently higher affinity for pNPP than *Rivularia*.

Table 7.6 Kinetic values for *Rivularia atra* and *Ralfsia verrucosa* using pNPP and 4-MUP

Organism	V_{max}		K_m		V_{max}/K_m	
	pNPP	4-MUP	pNPP	4-MUP	pNPP	4-MUP
Rivularia atra	0.0121	0.0077	67.8	2.14	0.00018	0.0036
Ralfsia verrucosa	0.0024		39.8		0.00008	

There was no significant difference between Lineweaver - Burk plots comparing *Rivularia* using either substrate or comparing *Rivularia* to *Ralfsia* using pNPP.

7.33 pH spectrum

Both organisms showed optimum activity between pH 10 - 11, using both substrates at 250 µM (Fig. 7.6). Above pH 10 the phosphatase activity of *Rivularia* was higher using 4-MUP than using pNPP. The phosphatase activity of *Ralfsia* was similar using both substrates. *Rivularia* showed higher phosphatase activity at all pH except pH 8.5, where activity was similar in both organisms using both substrates.

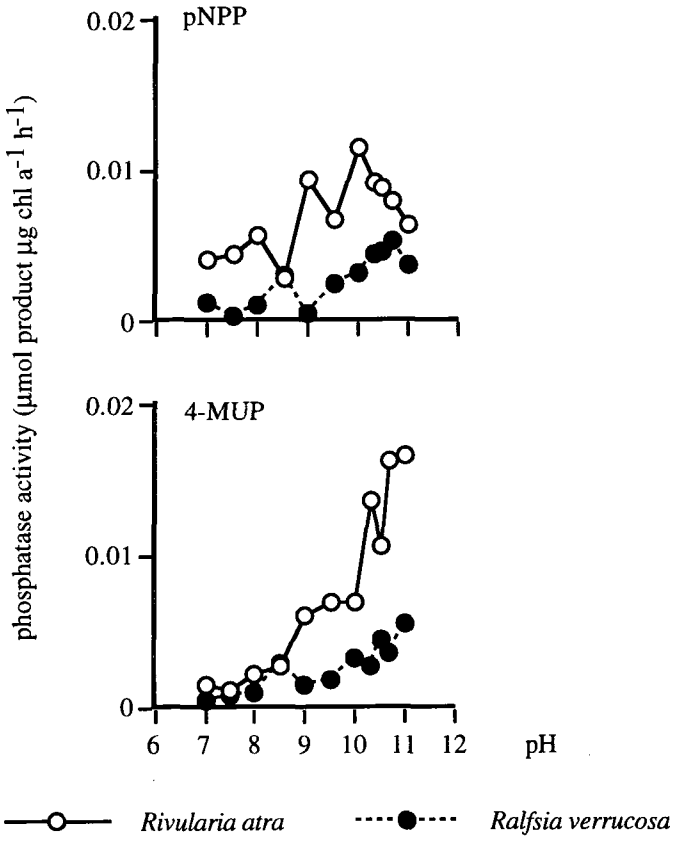


Fig 7.6 pH spectrum of phosphatase activity of *Rivularia atra* and *Ralfsia verrucosa* using 250 µM pNPP and 250 µM 4-MUP

7.4 Summary

Dichothrix D861 showed a much higher rate of activity using both substrates than the "*Dichothrix*" community. Unlike *Rivularia biasolettiana*, teased colonies of *R. atra* did not show significantly higher activity than whole colonies. *Ralfsia* showed a significantly lower rate of activity than either teased or whole *Rivularia* colonies using pNPP.

All the organisms tested had a lower K_m using 4-MUP than pNPP. pH spectra showed that organisms which had a higher V_{max} and a higher rate of activity in the time course, generally had higher activity in the pH spectrum. In *Dichothrix* D861, the "*Dichothrix*" community and whole *Rivularia atra* colonies, activity was higher using 250 μM 4-MUP than 250 μM pNPP at higher pH.

CHAPTER 8

GENERAL DISCUSSION

8.1 Introduction

The study was undertaken to test key features of environmental chemistry, especially phosphorus, in order to find out if the phosphatase activity of *Rivularia* is important in its ecology. The study has shown that *Rivularia* from each site shows some differences (Table 8.1) which relate to differences in the environmental chemistry of the two sites (Table 8.2). Each site is considered separately (sections 8.2, 8.3) and then compared (section 8.4).

8.2 Red Sike

High phosphorus concentrations occur in spring at Red Sike, although they remain low for most of the rest of the year (Fig. 4.6). The relative proportions of organic P during this spring peak differed from Livingstone and Whitton (1984). The present study found the spring peak was largely inorganic P. However, organic P probably originates from the peat at this time of year (Livingstone & Whitton, 1984, and present study Fig. 4.10, 4.12), but nothing is known of how this is transferred to the stream or to what extent it includes true organic molecules. The difference between the findings of the present study and the previous one, is perhaps related to filtration methods. The previous study used GF/C filters whereas the present study used GF/F filters. However, Fig. 4.9 shows that there is no significant difference between the two filter porosities (1.2 μm and 0.7 μm) in the concentration of organic P from bog pool water. It is also unclear to what extent the organic P present is hydrolysed by the phosphatase activity. *Rivularia biasolettiana* is effective at hydrolysing the two artificial substrates tested, and kinetic studies on colonies indicate that it can do this effectively over a wide substrate range. The non-linear double reciprocal plots of phosphatase activity in whole *R. biasolettiana* colonies (Fig. 6.6) and 7 d *Calothrix parietina* D550 (Fig. 6.2), may be the result of two or more reactive sites on the same enzyme; showing apparent negative cooperativity (Cadenas, 1978), although further work would be needed to demonstrate this. Apparent negative

cooperativity was also found in two species of *Gelidium* (Hernández *et al.*, 1995), *Zostera noltii* (Hernández *et al.*, 1994) and in the dinoflagellate *Pyrocystis noctiluca* (Rivkin & Swift, 1980), the ecological significance of this is discussed (see 8.4). The Rivulariaceae as a whole tend to be among the cyanobacteria showing the highest phosphatase activity (Whitton *et al.*, 1991).

The formation of hormogonia by *Rivularia biasolettiana* in spring, followed by many small new colonies 1-2 months later (Fig. 4.16), is probably as a response to the higher phosphorus concentrations in the water. Hormogonium formation in Rivulariaceae is brought about by the addition of phosphorus to phosphorus-limited trichomes (Livingstone & Whitton, 1983). In view of the fact that phosphatase activity of stream epilithon is known to be influenced by the ambient N : P ratio (Klotz, 1992), it is surprising that activity of *R. biasolettiana* in Red Sike shows only a moderate fall in spring when the colonies are sufficiently released from phosphorus limitation to form hormogonia (Fig. 4.18). This is perhaps because not all trichomes form hormogonia (Fig. 4.16) or because extracellular enzyme immobilised in the mucilage may persist.

The results for Red Sike show some similarities with results for streams elsewhere in northern England with abundant *R. haematites* (Pentecost, 1987). These showed high ambient N : P (based on results for nitrate) and higher phosphorus in winter than summer of which more than 85 % phosphorus was organically bound. Colony formation was reported to start in April.

In view of the fact that *Rivularia* is a N_2 fixer, *R. biasolettiana* might be expected to dominate sites where the ambient N : P ratio is low; however, the ratio tended to be high for most of the year in Red Sike (compare Fig. 4.6 with Fig. 4.7) and the streams studied by Pentecost (1987). N_2 fixation rates of *R. biasolettiana* from Red Sike, assayed in June have been found (Livingstone *et al.*, 1984) to be low and it was suggested that, in spite of being a N_2 fixer, colonies may depend on combined nitrogen for most of their nitrogen supply. However, the original theory is modified on the basis of these results to suggest that *R. biasolettiana* probably undergoes marked differences in nitrogen and phosphorus limitation throughout the year. Very new, fast

growing colonies formed in spring following a period of phosphorus uptake, are probably nitrogen limited and likely to show high rates of N_2 fixation. The ambient N : P ratio was reduced in spring at times when the ambient phosphorus was higher (compare Figs 4.6 and 4.7) and small colonies are known to show higher rates of N_2 fixation than large colonies (Livingstone *et al.*, 1984). During the rest of year, the N : P ratio was high. *R. biasolettiana* colonies are probably fairly long-lived.

The key ecological advantage of N_2 fixation for *R. biasolettiana* in Red Sike may therefore be to enhance the nitrogen supply and hence the growth rate for a period in late spring, rather than the whole year. During the summer period with favourable temperatures for growth, but very low ambient phosphorus, the trichomes revert to marked P limitation, with long hairs and high phosphatase activity. (Phosphatase activity in *R. biasolettiana* showed a positive correlation to % hair using pNPP. Temperature was positively correlated to % *Rivularia,* see Appendix iii.) This condition presumably persists in most cases until the next spring.

8.3 Tyne Sands

The large masses of drift *Laminaria* and other algae deposited at Tyne Sands at the extreme high tide level are potentially an important contributor of nutrients to the upper part of intertidal zone (Figs 5.2, 5.3). However, the very low N : P ratios of water in adjacent pools suggest that much of the nitrogen is lost to the atmosphere. This certainly occurs in warm weather as release of ammonia, but perhaps also by denitrification.

Phosphorus concentrations and ratios of inorganic P to organic P in the weed pools, *Rivularia* pools and seawater were all broadly similar to those found in 1981 (compare Khoja *et al.*, 1984 to Table 5.4), with the exception that less of the phosphate in the weed pools was organic. A possible explanation is because filtration for the early study was carried out using a GF/C filters rather than a GF/F filters (1.2 v. 0.7 μm porosity). Fig. 5.7 shows significant differences between the phosphorus concentrations of weed pool water filtered using different porosity filters. This

suggests that the phosphorus may include not only molecules in true solution, but larger materials.

The water in any particular *Rivularia* pool is probably largely replaced each time the pool is covered by an incoming tide. This water must be seawater mixed with water derived from pools further down the eulittoral initially; then, subsequently by water from the weed pools as the tide retreats. This is almost certainly the reason for the March peak in inorganic P in the *Rivularia* pools (Fig. 5.2) as it combined high tides with high concentrations of nutrients in the weed pools. High tides in June were at least 0.6 m lower so that although nutrient concentrations in the weed pools were maximal, only *Rivularia* pools (R1 and R4) which received direct drainage from the weed pools, showed similar peaks in inorganic P.

On average, *Rivularia* pools had phosphorus concentrations more or less similar to seawater in the adjacent bay, yet had much lower concentrations of inorganic nitrogen than seawater (Table 5.4), giving a very low N : P ratio. The most plausible explanation is that submerged algae remove nutrients very rapidly as the water in the pools is replaced, with this effect perhaps being especially pronounced for the most limiting nutrients: NH_4-N and NO_3-N.

Pool chemistry is certainly very dynamic, at least in warm weather. Both Khoja *et al.* (1984) and the present study found marked increases in organic P in *Rivularia* pools during intertidal studies (Fig. 5.4). The most plausible explanation is that this results from animal activity, grazing and excretion, and/or release from *Fucus spiralis*. The most consistent increase in organic P during the two studies, occurred in R5, the smallest of the *Rivularia* pools and thus with the highest cover of *Fucus spiralis* per unit volume. Any inorganic nitrogen released at the same time as organic P, was presumably taken up rapidly by algae in the pools, as nitrogen was hardly detectable during these studies (Table 5.5). Another study on the phytoplankton community and nutrients during two periods of tidal isolation, one in August the other in October, found nitrate, nitrite and benthic micrograzers decreased significantly during the period of tidal isolation in August (Metaxas & Scheibling, 1994), indicating that these nutrients may be very limited and utilised quickly. Nutrient concentrations, especially

nitrogen, being less in the *Rivularia* pools than either the weed pools or the seawater, may be accounted for by biological activity. Further studies are needed on nutrients concentrations, including organic nitrogen, during a tidal cycle, especially in summer when the larger differences appear to occur, in order to quantify the large variability that tidal cycles cause on the environment.

This explanation of pool chemistry dynamics poses a further problem. There would be a net loss of phosphorus from the pool ecosystem if organic phosphorus enriched water is removed by each incoming tide, without some compensating input of phosphorus; perhaps animals which graze algae outside the pool excrete nutrients after return to the pool, or perhaps the water is not mixed completely, below certain tide heights (e.g. 5.0 m). Table 5.6 shows that the organic P concentration in R5 and R6 is twice as high as seawater, directly following a 5.0 m tide.

A striking result of the phosphatase studies is that activity assayed with pNPP showed peaks in all the pools 1 - 2 months earlier than activity assayed with 4-MUP (Figs 5.11, 5.12). As a similar effect occurred in all the pools and with both concentrations of 4-MUP, this suggests a real difference in the phosphatase activity. The combined period of the two peaks corresponds to the period when the outermost trichomes were hairy. The presence of hairs suggests that the colonies were clearly phosphorus limited (Whitton, 1987a; 1991) during the periods when pNPP or 4-MUP were hydrolysed most actively. However, the greater affinity for 4-MUP than pNPP shown by all organisms in the kinetic studies (Tables 6.2, 6.9, 7.3 and 7.6), may mean that the switch in substrate response may reflect increased phosphorus limitation in *R. atra*. The fact that use of Spearman's rank correlation indicated greater similarity between pools when using 4-MUP (Table 5.13, 5.14) rather than pNPP (Table 5.12) as substrate also suggests that results with the former may reflect conditions when phosphorus limitation was more severe. The practical implication for phosphatase studies is that neither substrate alone is sufficient to assess the extent of phosphorus-limitation of this cyanobacterium.

The low N : P ratio in water of the *Rivularia* pools suggests an obvious advantage for a N_2-fixer. Nevertheless this is the only N_2-fixing phototroph in pools

of the *Rivularia* zone, although crust-forming N_2-fixing Rivulariaceae do occur on surfaces adjacent to R9 and dense populations of purple phototrophic bacteria (likely N_2-fixers) occur in some weed pools in hot weather. As even seawater adjacent to this shore has a relatively low N : P ratio (inorganic nitrogen : total P = 1.19 by weight), N_2-fixing organisms might be expected to be widespread further down the eulittoral. Measurements are needed of filtrable organic nitrogen to assess whether this fraction may be quantitatively important.

Elevated rates of nitrogenase activity were reported by Khoja *et al.* (1984) during warm weather in late June and July and a single phosphatase measurement (assayed with pNPP) at the same time also showed high activity. More detailed studies of N_2 fixation by *R. atra* are required over the whole period June - October, when the cyanobacterium appears to switch from a pNPP hydrolysing phosphatase system to a 4-MUP hydrolysing system, perhaps reflecting increasing P limitation.

If pool chemistry is so dynamic, the question arises whether the changes within some individual tidal cycles may be so large that they override the potential biological effects of annual changes observed by sampling at one particular time in the cycle. However, the fact that there were broadly similar patterns of change in phosphorus concentration and *R. atra* phosphatase activity in the summers of both 1992 and 1993, shows the importance of studies throughout the year. Nevertheless more detailed studies of changes in pool chemistry throughout the period they are uncovered by the tide should be a priority for study, particularly a comparison of pools exposed for differing numbers of tidal cycles.

8.4 Comparison between Red Sike and Tyne Sands

Phosphorus is known to affect the morphology of Rivulariaceae (Livingstone & Whitton, 1983). Table. 8.1 compares features of *Rivularia biasolettiana* and *R. atra*. Both species are abundant for much of the year. *R. biasolettiana* is dominant whereas *R. atra* is more seasonal, partly as a result of differences in chemistry at Red Sike and Tyne Sands as well as differences in longevity of the two species. The larger colonies of *R. biasolettiana* are likely to be longer lived.

Table 8.1 Comparison of features of *Rivularia biasolettiana* at Red Sike to *R. atra* at Tyne Sands

Features of *Rivularia*	*Rivularia biasolettiana* at Red Sike	*Rivularia atra* at Tyne Sands
Rivularia presence	dominant	abundant
Hair	colonies hairy (hair long)	restricted to summer, mainly
Hormogonia	a few % of trichomes, mainly in early spring after pulse of inorganic P	> 50 % of trichomes after increase in inorganic P
Colonies size and longevity	large (generally ≥ 2 mm) long-lived ≥ 1 year	small (generally ≤ 2 mm) survive ≤ 1 year
N_2 - fixation	low	high
Phosphatase activity	active most of year	restricted to summer, mainly
Substrate range	apparent negative cooperativity	linear
Affinity (pNPP vs. 4-MUP)	4-MUP higher	4-MUP higher
Comparison with cultures	axenic higher	axenic higher
Effect of teasing colony	significantly higher activity	no significant difference

Hormogonia formation in *R. atra* population appears to be much less seasonal than in *R. biasolettiana* (Whitton *et al.*, in press). Hormogonia were present in colonies of *R. atra* February - June. Many small new colonies were hairy (Fig. 1.5) 4 - 6 weeks after the summer peak in phosphorus (Fig. 5.2), probably as a result of active growth combined with a decrease in phosphorus concentrations once new colonies became visible.

At Tyne Sands inorganic P was negatively correlated to *R. atra* abundance, suggesting that colonies were rare when inorganic P was high, and that when inorganic P was low, colonies were more abundant (Fig. 5.8). Production of hormogonia by phosphorus deficient Rivulariaceae after addition of inorganic P, was shown by Livingstone and Whitton (1983). The resulting increase in *R. atra* abundance was visible some weeks later. By the time these new, abundant colonies became visible, inorganic P levels had dropped. These small new colonies were presumably growing fast and became phosphorus deficient, hairy and with pronounced phosphatase activity. These phosphorus deficient characteristics were positively correlated with one another and negatively correlated with colony diameter, chlorophyll *a* and hormogonia

showing that it was probably the larger, rarer colonies, with more chlorophyll which produced hormogonia. At Red Sike % cover of *R. biasolettiana* increased a few weeks after hormogonia had been seen in colonies (Fig. 4.16), at a similar time to the spring phosphorus peak in the stream (Fig. 4.6). The inorganic P peaks at both sites are probably important in the regeneration of the *Rivularia* population.

The chemical features of the two sites are compared in Table 8.2. At Red Sike inorganic P (Fig. 4.6) was usually very low and phosphatase activity, with all substrates, was high (Fig. 4.18, Table 8.1). At Tyne Sands inorganic P was always detectable (Fig. 5.2) and phosphatase activity was usually very low (Figs 5.11, 5.12), with occasional peaks after a peak in inorganic P, during maximum growth. Other studies as well as the present study have found a negative relationship between phosphatase activity to inorganic P in the environment (Velduis *et al.*, 1987; Hernández *et al.* 1995). As well as being correlated to the morphological features of *Rivularia* indicating phosphorus deficiency (hair and hair length), phosphatase activity was correlated to temperature or *Rivularia* abundance (Appendices iii, iv). At both sites organisms were phosphorus limited predominantly during the growth period, when light and temperature were higher.

Table 8.2 Comparison of chemical features at Red Sike to those at Tyne Sands (*Rivularia* pools)

Chemical variable	Red Sike	Tyne Sands *Rivularia* pools
Total P	often below detection	never below detection
Inorganic P in peak	large component of peak	large component of peak
Organic P	larger proportion of total P at other times of year	larger proportion of total P at other times of year
N:P	largely above Redfield ratio	largely below Redfield ratio
Nitrogen	never below detection, mostly NO_3-N	often below detection, mostly NO_3-N

Freshwater organisms showed non-linear enzyme kinetics with one or other substrate (see 8.2). This may be because these organisms were subjected to greater differences in phosphorus deficiency and substrate concentration than the marine

organisms. This enzyme system would maintain a constant phosphorus supply, regardless of substrate concentration (Burns & Beever, 1977; Hernández *et al.*, 1995).

Khoja *et al.* (1984) suggest that the phosphatase activity of *Rivularia atra* is a competitive advantage in this environment but *Ralfsia verrucosa* (present study) and *Fucus spiralis* (I. Hernández, pers. comm.), which is located around the edge of the pools, also showed marked phosphatase activity. Phosphatase activity in *Ralfsia verrucosa* was generally lower than *Rivularia atra* (Figs 5.14, 5.15) and the enzyme features appeared more competitive in *R. atra* (Table 7.6). So *R. atra* may have been at a competitive advantage in terms of phosphatase activity, but the organisms in the *Rivularia* pools at Tyne Sands were more likely to be nitrogen limited than phosphorus limited on most of the sampling occasions throughout the study (compare Figs 5.2 and 5.3). Cyanobacteria are well known for their high nitrogen fixation rates and possible source, through leakage (Stewart, 1963), of nitrogen in the eulittoral (Hübel & Hübel, 1974; Reed & Stewart, 1983; Wallentinus, 1991). Other studies have found marine coastal systems to be primarily nitrogen limited with an N : P ratio of approximately 3:1 (Jones & Stewart, 1969; Schramm, 1991; Dickman *et al.*, 1993; Delgado & Lapointe, 1994; Chopin *et al.*, 1995). At Tyne Sands nitrogen fixation by *Rivularia* was found to be very high (Khoja *et al.*, 1984). Khoja *et al.* (1984) suggest that this was due to the high phosphorus concentration in the pools at Tyne Sands. It is suggested that the high rates of nitrogenase activity found by Khoja *et al.* (1984) were actually the result of the low combined N in this environment rather than the high P. Mallin (1994) found that nitrogen was the principal limiting nutrient in North Carolina estuaries, but as with the present study, phosphorus was occasionally co-limiting.

At Red Sike it is the phosphatase activity of *Rivularia* that is important for most of the year. However, nitrogen fixation may be important on the rare occasions when N : P ratio may fall below the Redfield ratio or, as suggested by Fong *et al.* (1993) when combined nitrogen was < 270 $\mu g\ l^{-1}$ (Fig. 4.7). The levels of nitrogenase activity by *R. biasolettiana* at the Red Sike (Livingstone *et al.*, 1983) were found to be very low which, they suggested, was because the levels of combined nitrogen during the study were high. *R. biasolettiana* was probably obtaining nitrogen from combined

sources, for most of the year, rather than fixing it, but the present study suggests that nitrogen may be limiting also, at times. A study by Diaz and Pedrozo (1993) also suggest that although algae in an oligotrophic lake in the southern Andes were primarily phosphorus limited, nitrogen was also likely to be limiting during intermittent periods.

Generally, with both substrates, axenic organisms showed a slightly higher affinity for substrate, though younger cultures showed more similarity to field organisms (Tables 6.2, 6.9, 7.3 and 7.6). This suggests that field organisms were less phosphorus deficient than the fast growing axenic cultures which use up phosphorus sources very quickly. Culture studies of Rivulariaceae without combined nitrogen in the medium show that these organisms are able to withstand both nitrogen and phosphorus deficiency for a period of time, but it is the inclusion of inorganic P in the medium that allows the population to grow actively (Livingstone & Whitton, 1983). Although nitrogen fixation may be advantageous in a low nitrogen environment, growth rates of *Anabaena flos - aquae* were lower under nitrogen fixing conditions (Thompson *et al.*, 1994).

Clearly the interrelationship of phosphorus and nitrogen deficiency is complex, but at Tyne Sands where phosphorus is not often limiting, N_2 fixation is probably important but at Red Sike N_2 fixation is probably rare while phosphatase activity is important. These results indicate that the energetic priority is for the most limiting nutrient for most of the year, but for a period of time another limiting nutrient can be tolerated.

8.5 Concluding remarks

Both sites were subject to phosphorus limitation at some time. At Red Sike phosphorus was limiting for most of the year and the phosphatase activity of *Rivularia* was high. However, at Tyne Sands phosphorus was limiting only intermittently, when peaks in phosphatase activity occurred, but nitrogen was predominantly limiting. It seems that heterocystous forms of Rivulariaceae may occur at sites were either phosphorus or nitrogen is predominantly limiting and where the second nutrient may be occasionally limiting.

SUMMARY

1. A study of the relationship between phosphatase activity of *Rivularia* colonies, morphology and population dynamics to phosphorus fractions in the environment, was carried out at two sites: one was an upland freshwater stream in Upper Teesdale, Red Sike; the other was pools in the upper eulittoral of a North Berwickshire bay, Tyne Sands.

2. *Rivularia biasolettiana* and water samples were taken from one place in the stream at the freshwater site. Water samples were taken from 4 pools associated with decomposing seaweed (W1-4) covered by tides > 4.9 m, 9 pools containing *R. atra* (R1-9) covered by tides > 4.5 < 4.8 m, where *R. atra* colonies were also collected, and seawater. R9 was reached by tides ≥ 5.2 m. R9 contained the "*Dichothrix*" community on the rock above the pool and was at the far end of the site, as was W1.

3. Although the magnitude of the maxima varied from year to year, total P was maximum in March - May at the freshwater site and in June - July in the weed pools at the marine site; 1992 - 1994. In both these locations, the stream and the weed pools, inorganic P was the largest fraction. Only R1 and 4, probably receiving direct drainage from the weed pools, showed a peak in total P (largely inorganic) at this time. At other times, phosphorus concentrations were often near to detection limits at the freshwater site, but not at the marine site.

4. However, the *Rivularia* pools generally showed a higher peak in inorganic P in March 1993 which was associated with very high tide heights as well as high concentrations of inorganic P in the weed pools. *Rivularia* pools had generally lower concentrations of inorganic P than the weed pools and had a higher proportion of organic P, especially in the summer months.

5. Two tidal studies showed increasing organic P concentrations in summer during a tide that reached all the pools studied (5.2 m) and a tide that reached none of the pools (4.3 m).

6. Samples taken immediately the pools were uncovered by the high tide, 5.0 m in June 1994, showed inorganic P concentrations were high in the weed pools, but were only higher than seawater in R1 and R9. However, organic P was generally higher in the *Rivularia* pools than seawater.

7. Following seaweed (*Laminaria*) deposition in R7 inorganic P, which made up the larger proportion of total P, was over 1000 $\mu g\ l^{-1}$.

8. At the freshwater site in spring, total P was generally higher, approximately 50 % was organic P, in pools on top of the bog (bog pools) than in the stream or spring.

9. During a day long study in spring at three locations at Red Sike, the first spring-water sample contained more than 3 times the phosphorus concentration of any other sample during the day. It was nearly all inorganic P.

10. At the freshwater site unfiltered water from bog pools contained significantly higher total P than filtered bog pool water. At the marine site there were significant differences between phosphorus concentrations from unfiltered water and water filtered through three types of filters in weed pool water.

11. Nitrogen showed a similar pattern to phosphorus with peaks of NH_4-N and NO_3-N in April 1992 and March 1993 at the freshwater site. Nitrogen was always detectable. NO_3-N was the larger component.

At the marine site, nitrogen also showed a similar pattern to phosphorus in the weed pools, with NH_4-N the larger component. However, NO_3-N was the larger

component in all the *Rivularia* pools and showed a different pattern to that in the weed pools. Nitrogen concentrations in the *Rivularia* pools were often very low.

12. At the freshwater site trichomes of *Rivularia biasolettiana* colonies were always hairy > 90%, with some hormogonia (< 5 % of trichomes) occurring in April and May 1992 and February and March 1993. The % cover of *R. biasolettiana* in the stream increased 1-2 months after hormogonia were seen in the colonies. At the marine site, *R. atra* colonies often had hormogonia, especially June 1992 and February - June 1993. Hairs were present from July - September 1992 and from July - October 1993. *R. atra* abundance was greatest when inorganic P was below 100 $\mu g\ l^{-1}$.

13. Inorganic P showed a negative influence on water phosphatase activity in all substrates at both sites and was significant using 250 μM 4-MUP at the marine site. At both sites the phosphatase activity of filtered water was positively correlated to temperature.

14. Phosphatase activity of *R. biasolettiana* colonies (5-7 mm) was high throughout the year, dipping slightly when hormogonia were found in the colonies at the freshwater site. At the marine site, phosphatase activity of the *R. atra* colonies was generally low, although there were peaks in July/August using pNPP and September/October using both concentrations of 4-MUP, especially in 1992.

15. Using pNPP phosphatase activity of *Rivularia* colonies was always higher than using 4-MUP at the freshwater site, but this was not the case at the marine site.

16. Phosphatase activity of *Rivularia* colonies was negatively correlated to inorganic P and salinity, at the marine site. Temperature (not at Red Sike) and percentage of filaments with hairs and/or hair length was positively correlated to phosphatase activity of the colonies at both sites.

17. *Ralfsia verrucosa* phosphatase activity was positively correlated to organic P, and negatively correlated to TIN : inorganic P and salinity using 4-MUP.

18. The "*Dichothrix*" community showed a peak in phosphatase activity in all substrates in October 1992 and 1993. Activity was negatively correlated to salinity using 250 μM 4-MUP.

19. During kinetic experiments, all axenic organisms showed higher phosphatase activity than field organisms.

20. The K_m was lower using 4-MUP than pNPP in all organisms tested.

21. The freshwater organisms (*Calothrix parietina* D550, and *Rivularia biasolettiana*) both showed apparent negative cooperativity with one substrate.

22. Teased colonies of *R. biasolettiana* showed significantly higher activity than whole colonies. However, this was not the case with *R. atra*.

23. Larger colonies (> 9 mm) of *R. biasolettiana* had a significantly higher rate of activity than smaller colonies (< 9 mm) above pH 9.0.

24. The relationship of the morphology and phosphatase activity of *Rivularia* is discussed in relation to its ecology.

REFERENCES

Admiralty Tide Tables (1992-4) Vol. 1 European Waters. The Hydrographer of the Navy, Taunton.

Bedford AP, Moore PG (1984) Macrofaunal involvement in the sublittoral decay of kelp debris: The detritivore community and species interactions. Estuarine Coast Shelf Science 18: 97-111

Bellamy DJ, Tickle WN (1964) A critical limit of primary production for the survival of arctic alpine plants in the northern Pennines of England. Proceedings of the UNESCO Symposium on Subarctic Ecology: 241-246

Bendschneider K, Robinson RJ (1952) The determination of nitrate in sea water Anal. Chim. Acta 29: 272-279

Berman T (1970) Alkaline phsopahatases and phosphorus availability in Lake Kinneret. Limnol. Oceanogr. 15: 663-674

Berman T, Moses G (1972) Phosphorus availability and alkaline phosphatase activities in two Israeli fishponds. Hydrobiologia 40: 487-498

Berman T, Wynne D, Kaplan B (1990) Phosphatases revisited: analysis of particle-associated enzyme activities in aquatic systems. Hydrobiologia 207: 287-294

Bohinski RC (1987) Modern Concepts in Biochemistry. 5th edn, Allyn and Bacon Inc, Boston, USA

Boon PI (1993) Organic matter degradation and nutrient regeneration in Australian fresh waters: III. Size fractionation of phosphatase activity. Arch. Hydrobiol. 126: 339-360

Boström B, Persson G, Broberg B (1988) Bioavailability of different phosphorus forms in freshwater systems. Hydrobiologia 170: 133-155

Bradford BC (1989) A demonstration of possible links for a detrital pathway form intertidal macro-algae in the Bay of Fundy MSc. Thesis, Univ. Acadia

Broberg O, Persson G (1988) Particulate and dissolved phosphorus forms in freshwater: composition and analysis. Hydrobiologia 170: 61-90

Burns DJW, Beever RE (1977) Kinetic characterisation of the two phosphate uptake systems in the fungus *Neurospora crassa*. J. Bact. 139: 511-519

Byren BA, Davies BR (1986) The influence of invertebrates on the breakdown of *Potamogeton pectinatus* L. in a coastal marina (Zandvlei. South Africa). Hydrobiologia 137: 141-151

Cadenas E (1978) Enzimas alostéricas. H. Blume ediciones, Madrid

Carpenter SE, Harmon ME, Ingham ER, Kelsey RG, Lattin JD, Schowalter TD (1988) Early patterns in heterotroph activity in conifer logs. Proc. R. Soc. Edomn. 94B: 33-43

Castenholz RW, Waterbury JB (1989) Cyanobacteria. In Staley JT, Bryant MP, Pfennig N, Holt JG (eds), Bergey's Manual of Systematic Bacteriology, Volume 3 Williams & Wilkins, Baltimore: 1710-1727

Cembella AD, Antia NJ, Harrison PJ (1984) The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: A multidisciplinary perspective: Part 1. CRC Crit. Rev. Microbiol. 10: 317-391

Chapman ARO, Craigie JS (1977) Seasonal growth in *Laminaria logicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40: 197-205

Chappell KR, Goulder R (1992) Epilithic extracellular enzyme activity in acid and calcareous headstreams. Arch. Hydrobiol. 125: 129-148

Chopin T, Gallant T, Davison I (1995) Phosphorus and nitrogen nutrition in *Chondrus crispus* (Rhodophyta): Effects on total phosphorus and nitrogen content, carrageenan production, and photosynthetic pigments and metabolism. J. Phycol. 31: 283-293

Chróst RJ, Krambeck HJ (1986) Flourescence correction for measurements of enzyme activity in natural waters using methylumbelliferyl - substrates. Arch. Hydrobiol. 106: 79-90

Chróst RJ, Overbeck J (1987) Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Plußsee (North German eutrophic lake). Microb. Ecol. 13: 229-248

Chu SP (1942) The influence of the mineral composition of the media on the growth of planktonic algae. I. Methods and culture media. J. Ecol. 30: 284 -325

Crisp DT, Howson G (1982) Effect of air temperature upon mean water temperature in streams on the north Pennines and English Lake District. Freshwater Biology 12: 359-367

DeBoer JA, Whoriskey FG (1983) Production and role of hyaline hairs in *Ceramium rubrum*. Mar. Biol. 77: 229-234

DeBoever J, Manes A, Stans G, Bosmans E, Kohen F (1995) Comparison of chemiluminescent and chromogenic substrates of alkaline phosphatase in a direct immunoassay for plasma estradiol. Analyt. chim. Acta 303: 143-148

Delgado O, Lapointe BE (1994) Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. Coral Reefs 13: 151-159

Devito KJ, Dillon PJ (1993a) Importance of runoff and winter anoxia to the P and N dynamics of Beaver Pond. Can. J. Fish. Aquat. Sci. 50: 2222-2234

Devito KJ, Dillon PJ (1993b) The influence of hydrologic conditions and peat oxia on the phosphorus and nitrogen dynamics of a conifer swamp. Water Resources Research 29: 2675-2685

Diaz MN, Pedrozo FL (1993) Seasonal succession of the phytoplankton in a small Andean Patagonian lake (Rep. Argentina) and some considerations about the PEG model. Arch. Hydrobiol. 127:167-184

Dickman M, Stewart K, Servantvildary M (1993) Spacial heterogeneity of summer phytoplankton and water chemistry in a large volcanic spring-fed lake in Northern Iceland. Arctic Alpine Research 25: 228-239

Dring MJ (1992) The Biology of Marine Plants. Cambridge University Press, Cambridge, UK

Edgar GJ (1987) Dispersal of faunal and floral propagules associated with drifting *Macrocystis pyrifera* plants. Mar. Biol. 95: 599-610

Eisenreich SJ, Bannerman RT, Armstron DE (1975) A simplified phosphorus analysis technique. Environmental Letters 9: 43-53

Engström L (1964) Studies on bovine-liver alkaline phosphatase, purification, phosphate incorporation. Biochim. Biophys. Acta. 92: 71- 75

Fedde KN, Whyte MP (1990) Alkaline phosphatase (tissue-nonspecific isoenzyme) is a phosphoethanolamine and pyridozal-5'phosphate ectophosphatase: normal and hypophosphatasia fibroblast study. Am. J. Genet. 47: 767-775

Feder J (1973) The phosphatases. In Griffiths EJ, Beeton A, Spencer JM, Mitchell DT (eds) Environmental phosphorus handbook. Wiley & Sons. N.Y.: 475-508

Fenchel T (1972) Aspects of decomposer food chains in marine benthos. Verh. Dtsch. Zool. Ges. 65: 14-22

Field JG, Jarman NG, Dieckmann GS, Griffiths CL, Velimirov B, Zoutendyk P (1977) Sun, waves, seaweed and lobsters: The dynamics of a West Coast kelp - bed. S. Afr. J. Zool. 73: 7-10

Fitzgerald GP, Nelson TC (1966) Extractive and enzymatic analysis for limiting or surplus phosphorus in algae. J. Phycol 2: 32-37

Fong P, Zedler JB, Donohoe RM (1993) Nitrogen versus phosphorus limitation of algal biomass in shallow coastal lagoons. Limnol. Oceanogr. 38: 906-923

Franko DA (1984) Phytoplankton metabolism and cyclic nucleotides. II. Nucleotide-induced perturbations of alkaline phosphatase activity. Arch. Hydrobiol. 100: 409-421

Franko DA, Wetzel RG (1982) The isolation of cylcic adenosine 3':5'-monophosphate (cAMP) from lakes of differing trophic status: Correlation with planktonic metabolic variables. Limnol. Oceanogr. 27: 27-38

Fries L (1963) On the cultivation of axenic red algae. Physiol. Pl. 16: 695-708

Gage MA, Gorham E (1985) Alkaline phosphatase activity and cellular phosphorus as an index of phosphorus status of phytoplankton in Minnesota lakes. Freshwat. Biol. 15: 227-233

Garen A (1960) Genetic control of bacterial enzyme alkaline phosphate. In Hayes W, Clowes RC (eds), Microbial Genetics Cambridge University Press, Cambridge: 239

Geitler L (1932) Cyanophyceae. L. Rabenhorst's Kryptogamenflora XIV. Akademishe Vertagsgesellshaft, Leipzig

Gibson MT, Whitton BA (1987) Influence of phosphorus on morphology and physiology of freshwater *Chaetophora, Draparnaldia* and *Stigeoclonium* (Chaetophorales, Chlorophyta). Phycologia 26: 59-69

Godshalk GL, Wetzel RG (1978) Decomposition of aquatic angiosperms. III. *Zostera marina* L. and a conceptual model of decomposition. Aquat. Bot. 5: 329-354

Goldman JC, McCarthy JJ, Peavey DG (1979) Growth rate influence on chemical composition of phytoplankton in oceanic waters. Nature 279: 210-215

Golterman HL (1975) Chemistry. In Whitton BA (Ed) River Ecology. Blackwell, Oxford, UK: 39-80

Golterman HL, Clymo RS, Ohnstead MAM (1978) Methods for the Physical and Chemical Analysis of Freshwater. 2 nd edn, Blackwell, Oxford, UK

Grainger SLJ, Peat A., Tiwari DN, Whitton BA (1989) Phosphomonoesterase activity of the cyanobacterium (blue-green alga) *Calothrix parietina*. Microbios 59: 7-17

Griffiths CL, Stemton-Dozey JME, Koop K (1983) Kelp wrack and the flow of energy through a sandy beach ecosystem. In McLachlan A, Erasmus T (eds), Sandy Beaches as Ecosystems. Dr W Junk Pulb.: 547-556

Hanisak MD (1990) The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand nitrogen nutrition of cultured seaweeds. Hydrobiologia 204: 79-87

Hanisak MD (1993) Nitrogen release from decomposing seaweeds: species and temperature effects. J. appl. Phycol. 5: 175-181

Hargrave BT (1970) The effect of a deposit - feeding amphipod on the metabolism of benthic microflora. Limnol. Oceanogr. 15: 21-30

Harrison PG (1978) Growth of *Ulva fenestrata* (Chlorophyta) in microcosms rich in *Zostera marina* (Anthophyta) detritus. J. Phycol. 14: 100-103

Harrison PG, Mann KH (1975) Detritus formation from eelgrass (*Zostera marina* L.): The relative effects of fragmentation, leaching and decay. Limnol. Oceanogr. 20: 924-934

Healey FP (1982) Phosphate. In Carr NG, Whitton BA (eds), The Biology of Cyanobacteria. Blackwell, Oxford, and University of California Press, Berkeley. pp. 105-124

Heath RT (1992) Nutrient dynamics in Great Lakes coastal wetlands: Future directions. J. Great Lakes Research 18: 590-602

Heath RT, Cooke GD (1975) The significance of alkaline phosphatase in a eutrophic lake. Verh. int. Ver. Limnol. 19: 959-965

Hernández I, Niell FX, Fernández JA (1992) Alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. J. exp. Biol. Ecol. 159: 1-13

Hernández I, Pérez-Llorens JL, Fernández JA, Niell FX (1994) Alkaline phosphatase acitivty in *Zostera noltii* Hornem. and its contribution to the release of phosphate in the Palmones River Estuary. Estuar. coast. Shelf Sci. 39: 461-476

Hernández I, Fernández JA, Niell FX (1995) A comparitive study of alkaline phosphatase activity in two species of *Gelidium* (Gelidales, Rhodophyta). Eur. J. Phycol. 30: 69-77

Holmes NTH, Whitton BA (1984) Phytobenthos of the River Tees and its tributaries. Freshwat. Biol. 11: 139-168

Horiuchi T, Horiuchi S,. Mizuno D (1959) A possible negative feedback phenomenon controlling formation of alkaline phophomonoesterase in *E. coli.* Nature (London) 183:1529-1530

Hoshaw RW, Rosowski JR (1979) Methods for microscopic algae. In Stein JR (ed.) Handbook of phycological methods: Culture methods and growth measurements. Cambridge University Press, Cambridge, UK: 53-67

Hübel H, Hübel M (1974) *In situ*-Messungen der Stickstoff-Fixierung an Mikrobenthos der Ostseeküste. Arch. Hydrobiol. 46: 39-54

Hull WE, Halford SE, Gutfreund H, Sykes BD (1976) ^{31}P nuclear magnetic resonance study of alkaline phosphatase: The role of inorganic phosphate in limiting the enzyme turnover rate at alkaline pH. Biochemistry 15: 1547-1561

Hurd CL, Galvin RS, Norton TA, Dring MJ (1993) Production of hyaline hairs by intertidal species of *Fucus* (Fucales) and their role in phosphate uptake. J. Phycol. 29: 160-165

Ihlenfeldt MJA, Gibson J (1975) Phosphate utilisation and alkaline phosphatase activity in *Anacystis nidulans* (Synecoccus). Arch. Microbiol. 102: 23-28

Islam MR, Whitton BA (1992) Phosphorus content and phophatase activity of the deepwater rice-field cyanobacterium (blue-green algae) *Calothrix* D764. Microbios 69: 7-16

Jaag O (1945) Untersuchungen über die Vegetation und Biologie der Algen des näckten Gesteins in den Alpen, in Jura und im schweizerischen Mittelland. Beiträge zur Kryptogamenflora der Schweiz IX (3). Kommissionsverlag Buchdrukerei Bückler, Bern

Jansson M (1975) Phosphatases in the Koukkel lakes. In Progress Report from the Koukkel project 4: 119-131 Institute of Limnology, Uppsala, Sweden

Jansson M (1981) Induction of high phosphatase activity by aluminium in acid lakes. Arch. Hydrobiol. 93: 32-44

Jansson M, Olsson H, Pettersson K (1988) Phosphatases; origin, characteristics and function in lakes. Hydrobiologia 170: 157-175

Johnson GAL (1978) Geology. In Clapham AR (ed), Upper Teesdale. Collins, London: 122-129

Jones K, Stewart, WDP (1969) Nitrogen turnover in marine and brackish habitats. III. The production of extracellular nitrogen by *Calothrix scopulorum.* J. mar. biol. Ass. U.K. 49: 475-483

Jones RI (1992) Phosphorus transformations in the epilimnia of small humic forest lakes. Hydrobiologia 243/244: 105-111

Kain JM (1971) Synopsis of biological data on *Laminaria hyperborea.* F.A.O. Fisheries synopsis 87

Kaushik NK, Hynes HB (1968) Experimental study on the role of autumn shed leaves in aquatic environments. J. Ecol. 56: 229-243

Kelly MG, Onyeka JOA (1992) Introduction to Statistics and Experimental Design for the Life Sciences. ABIC Publications, Enugu, Nigeria

Khoja TM, Livingstone D, Whitton BA (1984) Ecology of a marine *Rivularia* population. Hydrobiologia 108: 65-73

Kirkby SM, Whitton BA (1976) Uses of coded data in the study of *Calothrix* and *Rivularia.* Br. phycol. J. 11: 407-416

Klotz RL (1992) Factors influencing alkaline phosphatase activity in stream epilithon. J. Freshwat. Ecol. 7: 233-242

Kobori H, Taga N (1979) Phosphatase activity and its role in the mineralization of organic phosphorus in coastal sea water. J. exp. mar. Biol. Ecol. 36: 23-39

Koop K, Lucus MI (1983) Carbon flow and nutrient regeneration from the decomposition of macrophyte debris in a sandy beach microcosm. In: Sandy Beaches as Ecosystems. In McLachlan A, Erasmus T (eds), Dr W Junk Pulb.: 249-262

Kuenzler EJ, Perras JP (1965) Phosphatases of marine algae. Biol. Bull. Woods Hole 128: 271-284

Kuhnemann O (1970) Alguras considraciones sobre les bosques de *Macrocystis pyrifera.* Physis 29: 273-296

Lavery PS, McComb AS (1991) Macroalgal sediment nutrition interactions and their importance to macroalgal nutrition in a eutrophic estuary. Est. coast. shelf Sci. 32: 281-295

Little ECS (1953) The decomposition rates and manurial value of some common brown seaweeds. 7th Pacific Science Congress 5: 1-9

Livingstone D, Whitton BA (1983) Influence of phosphorus on morphology of *Calothrix parietina* (Cyanophyta). Br. phycol. J. 18: 29-38

Livingstone D, Khoja TM, Whitton BA (1983) Influence of phosphorus on the physiology of a hair-forming blue-green alga (*Calothrix parietina*) from an upland stream. Phycologia 22: 345-350

Livingstone D, Whitton BA (1984) Water chemistry and phosphatase activity of the blue-green alga *Rivularia* in Upper Teesdale streams. J. Ecol. 72: 405-421

Livingstone D, Pentecost A, Whitton BA (1984) Diel variations in nitrogen and carbon dioxide fixation by the blue-green alga *Rivularia* in an upland stream. Phycologia 23: 125-133

Mahasneh IA, Grainger SLJ, Whitton BA (1990) Influence of salinity on hair formation and phosphatase activities of the blue-green alga (cyanobacterium) *Calothrix viguieri* D253. Br. phycol. J. 25: 25-32

Mahasneh IA, Kumar A, Tiwari DN (1993) The switch on time of phosphomonoesterase activity in relation to cellular phosphorus in 6 strains of blue-green algae (cyanobacteria). Phycologia 32: 180-183

Mallin MA (1994) Phytoplankton ecology of North Carolina estuaries. Estuaries 17: 561-574

Mann KH (1972) Macrophyte production and detritus food chains in coastal waters. Mem. Ist. Ital. Idrobiol. 29. Suppl. 353-383

Marker AFH, Crowther CA, Gunn RJM (1980a) Methanol and acetone as solvents for estimating cholrophyll *a* and phaeopigments by spectrophotometry. Arch. Hydrobiol. Beih. Ergebn. Limnol. 14: 52-69

Marker AFH, Nusch EA, Riemann B (1980b) The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusions and recommendations. Arch. Hydrobiol. Beih. Ergebn. Limnol. 14: 91-106

Marker AFH, Jinks S (1982) The spectophotomic analysis of chlorophyll *a* and phaeopigments in acetone, ethanol and methanol. Arch. Hydrobiol. Beih. Ergebn. Limnol. 16: 3-17

Metaxas A, Scheibling RE (1994) Changes in phytoplankton abundance in tidepools over a period of tidal isolation. Bot. mar. 37: 301-314

Minderman G (1968) Addition, decomposition and accumulation of organic matter in forests. J. Ecol. 56: 355-362

Moss B (1988) Ecology of Fresh Waters: Man and Medium. 2nd edn, Blackwell, Oxford, UK

Mulholland PJ (1992) Regulation of nutrient concentrations in a temperate forest stream: Roles of upland, riparian and instream processes. Limnol. Oceanogr. 37: 1512-1526

Murphy J, Riley J (1962) A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27: 31-36

McComb RB, Bowers GN, Posen S (1979) Alkaline Phophatases. Plenum Press, N.Y., 986 pp.

McGarrigle ML (1993) Aspects of river eutrophication in Ireland. Annls Limnol. 29: 355-364

McGarrigle ML, Kilmartin L (1992) UV- Sensitive phosphate in Irish peaty waters: A study of potential effects on freshwater ecosystems. Environmental Research Unit, Dublin, Ireland

McLachlan A (1983) Sandy beach ecology: a review. In McLachlan A, Erasmus T (eds), Sandy Beaches as Ecosystems. Dr W Junk. Publ.: 321-380

McLachlan A, Erasmus T, Dye AH, Woolridge T, Van der Horst G, Rossouw G, Lasiak TA, McGwynne L (1981) Sandy beach energetics: an ecosystem approach towards a high energy interface. Estuar. coast. Shelf Sci. 13: 11-25

Newman PJ (1988) Classification of Surface Water Quality: Review of Schemes Used in EC Member States. Heinemann Professional Publishing, Oxford, UK

Olsson H (1983) Origin and production of phosphatases in the acid Lake Gårdsjön. Hydrobiologia 101: 49-58

Olsson, H. (1990) Phosphatase activity in relation to phytoplankton composition and pH in Swedish lakes. Freshwat. Biol. 23: 353-362

Parsons TR, Maita Y, Lalli CM (1984) A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, U.K.

Patterson G (1983) Effects of Heavy Metals on Freshwater *Chlorophyta*. Ph.D. Thesis, University of Durham, England

Pentecost A (1978) Blue-green algae and freshwater carbonate deposits. Proc. R. Soc. Lond. B. 200: 43-61

Pentecost A (1987) Growth and calcification of the freshwater cyanobacterium *Rivularia haematites* Proc. R. Soc. Lond. B 232: 125-136

Pettersson K (1980) Alkaline phosphatase activity and algal surplus phosphorus as phosphorus - deficiency indicators in Lake Erken. Arch. Hydrobiol. 89: 54-87

Piggot CD (1978) Climate and Vegetation. In Clapham AR (ed), Upper Teesdale, the area and its Natural History. Collins, London: 102-121

Price NC, Stevens L (1982) Fundamentals of Enzymology. Oxford University Press, Oxford

Redfield AC (1934) On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnstone Memorial Volume, Liverpool University Press, Liverpool: 176-192

Reed RH, Stewart WDP (1983) Physiological responses of *Rivularia atra* to salinity: Osmotic adjustment in hyposaline medium. New Phytol. 95: 595-603

Rhee G-Y (1980) Continuous culture in phytoplankton ecology. In Droop MR, Jannasch HW (eds) Advances in Aquatic Microbiology, Vol. 2. Academic Press N.Y.:151-203

Rigler FH (1956) A tracer study of the phosphorus cycle of lake water. Ecology 37: 550-562

Rigler FH (1961) The uptake and release of inorganic phosphorus by *Daphnia magna* Straus. Limnol. Oceanogr. 6: 165-174

Rippka R, Deruelles JB, Waternury JB, Herdman M, Stanier RY (1979) Genetic assignments, strain histories and properties of pure cultures of cyanobacteria. J. gen. Microbiol. 111: 1-61

Rivkin RB, Swift E (1980) Characterization of alkaline phosphatase and organic phosphorus utilization in the oceanic dinflagellate *Pyrocystis noctiluca*. Mar. Biol. 61: 1-8

Robertson AI, Lenanton RCJ (1984) Fish community structure and food chain dynamics in the surf zone of sandy beaches: The role of detached macrophyte detritus. J. exp. mar. Biol. Ecol. 84: 265-283

Ross MH, Ely JO, Archer JG (1951) Alkaline phosphatase activity and pH optima. J. Biol. Chem. 192: 561-568

Ruttner F (1953) Fundamentals of Limnology. 2nd edn, University of Toronto Press, Toronto.

Ryther JH, Corwin N, DeBusk TA, Williams LD (1981) Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26: 107-115

Sæbo S (1968) The autecology of *Rubus chamaemorus* L. 1. Phosphorus economy of *Rubus chamaemorus* in an ombrotrophic mire. Meldinger frå Norges Landbrukshøgskole 47: 1-67

Sanden P, Danielsson A (1995) Spacial properties of nutrient concentrations in the Baltic sea. Environmental monitoring and Assessment 34: 289-307

Satchell JE (1974) Litter - interface of animate/inanimate matter. In Dickinson CH, Pugh GJF (eds), The Biology of Plant Litter Decomposition. Academic Press, NY: xiii-x1iii

Schindler DW (1975) Whole - lake eutrophication experiments with phosphorus, nitrogen ad carbon. Verh. int. Ver. Limnol. 19: 3221-3231

Schindler DW (1977) Evolution and phosphorus limitation in lakes. Science, NY 195: 260-262

Schindler DW, Nighswander JE (1970) Nutrient supply and primary production in Clear Lake, eastern Ontario. J. Fish. Res. Bd Can. 27: 2009-2036

Schopf JW, Walter MR (1982) Origin and evolution of cyanobacteria: The geological evidence. In Carr NG, Whitton BA (eds), The Biology of Cyanobacteria. Blackwell, Oxford, and University of California Press, Berkeley 543-564

Schramm W (1991) Chemical characteristics of marine littoral ecosystems. In Matheieson AC, Nienhuis PH (eds), Ecosystems of the World, Vol 24. Intertidal and littoral ecosystems. Elsevier, London: 27-42

Schwabe GH, Mollenhauer R (1967) Über den Einfluss der Begleitbakterien auf das Lagerbild von *Nostoc sphaericum*. Nova Hedwigia 13: 77-80

Shapiro J (1988) Introductory lecture at the international symposium "Phosphorus in Freshwater Ecosystems", Uppsala, Sweden in October 1985. Hydrobiologia 170: 9-17

Sinclair C, Whitton BA (1977) Influence of nutrient dificiency on hair formation in the Rivulariaceae. Br. phycol. J. 12: 297-313

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799-801

Stainton MP, Capel MJ, Armstrong FAJ (1977) The Chemical Analysis of Freshwater. 2nd edn, Fish. Mar. Serv. Misc. Spec. Publ. 25.

Stewart WDP (1963) Liberation of extracellular nitrogen by two nitrogen-fixing blue-green algae. Nature 200: 1020-1021

Sutcliffe DW (1979) Some notes to authors on the presentation of accurate and precise measurements in quantitative studies. Freshwat. Biol. 9: 397-402

Tenore KR (1977) Growth of *Capitella capitata* cultured on various levels of detritus derived from different sources. Limnol. Oceanogr. 22: 936-941

Tenore KR (1983) What controls the availability to animals of detritus derived from vascular plants: Organic nitrogen enrichment or caloric availability? Mar. Ecol. Prog. Ser. 10: 307-309

Thompson P-A, Oh H-M, Rhee G-Y (1994) Storage of phosphorus in nitrogen fixing *Anabaena flos-aquae*. J. Phycol. 30: 267-273

Torriani A (1960) Influence of inorganic phosphate on the formation of phosphatase by *Escherichia coli*. Biochim. Biophys. Acta 38: 460-469

Veldhuis MJW, Venekamp LAH, Ietswaart T (1987) Availability of phosphorus sources for blooms on *Phaeocystis pouchetii* (Haptophyceae) in the North Sea: impact of the river Rhine. Neth. J. Sea Res. 21: 219-229

Velimirov B, Field JG, Griffiths CL, Zoutendyk P (1977) The ecology of kelp bed communities in the Benguela upwelling system. Helgol. wiss. Meeresunters 30: 495-518

Wallentinus I (1991) The Baltic sea gradient. In Matheieson AC, Nienhuis PH (eds), Ecosystems of the World, Vol 24. Intertidal and littoral ecosystems. Elsevier, London: 83-108

Whitton BA (1987a) Survival and dormancy of blue-green algae. In Henis Y (ed.), Survival and Dormancy of Microorganisms. Wiley, New York: 109-167

Whitton BA (1987b) The biology of Rivulariaceae. In Fay P, Van Baalen C (eds), Cyanobacteria: Current Research. Elsevier, Amsterdam: 513-534.

Whitton BA (1988) Hairs in eukaryotic algae. In Round FE (ed.), Algae and the Aquatic Environment. Biopress Ltd., Bristol, UK: 446-460

Whitton BA (1991) Use of phosphatase assays with algae to assess phosphorus status of aquatic environments. In Jeffery DW, Madden B (eds), Bioindicators and Environmetal Management. Proc. 6th International Bioindicators Symposium. Academic Press London: 295- 310

Whitton BA (1992) Diversity, Ecology and Taxonomy of Cyanobacteria. In Mann NH, Carr NG (eds), Photosynthetic Prokaryotes. Plenum Press, New York: 1-51

Whitton BA, Potts M (1982) Marine littoral. In Carr NG, Whitton BA (eds), The Biology of Cyanobacteria. Blackwell, Oxford, and University of California Press, Berkeley: 515-542

Whitton BA, Potts M, Simon JW, Grainger SLJ (1990) Phosphatase activity of the blue-green alga (cyanobacterium) *Nostoc commune* UTEX 584. Phycologia 29: 139-145

Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb. Ecol. 21: 85-98

Whitton BA, Yelloly JM, Christmas M, Hernández I (in press) Surface phosphatase activity of benthic algae in a stream with highly variable ambient phosphate concentration. Ver. int. Ver. Limnol. 25:

Wilkins AS (1972) Physiological factors in the regulation of alkaline phosphatase synthesis in *Escherichia coli.* J. Bact. 110: 616-623

Wynne D (1977) Alterations in activity of phosphatase during the *Peridinium* bloom in Lake Kinneret. Physiol. Pl. 40: 219-224

Zar JH (1984) Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, New Jersey.

Zehnder A, Hughes EO (1958) The anti-algal activity of acti-dione. Can. J. Microbiol. 4: 399-408

ZoBell CE (1971) Drift seaweeds on San Diego county beaches. In North WJ (ed.), The Biology of Giant Kelp Beds (*Macrocystis*) in California. Nova Hedwigia, Verlag von J Cramer Beih., Germany: 269-314

APPENDICES

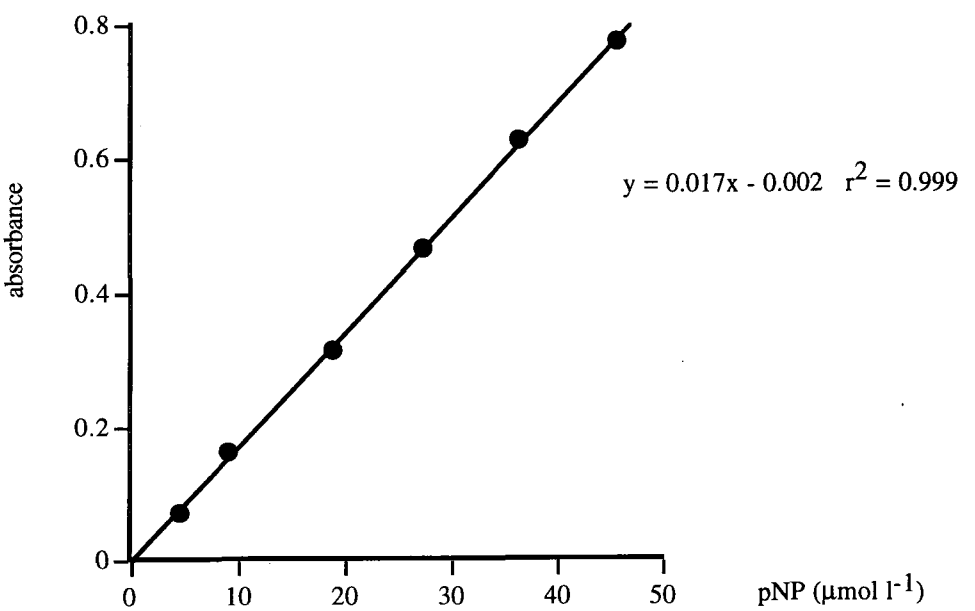
i Uptake of product by *Rivularia* biasolettiana colonies

Absorbance (pNP) or fluorescence (4-MU) readings with colonies in product compared to product without colony (control). 100 µmol 4-MU reading after 101 x dilution.

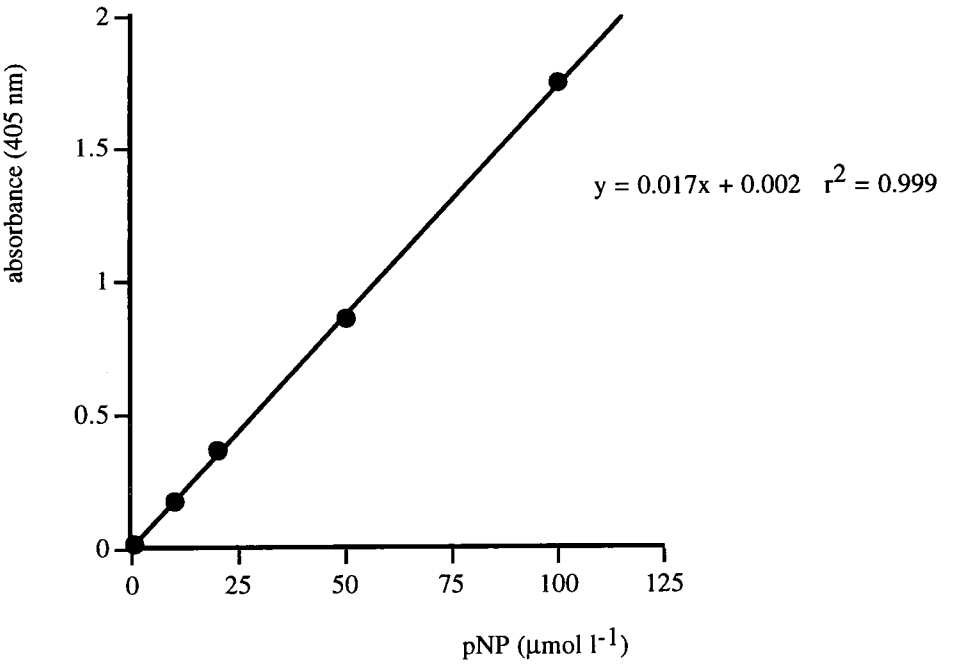
100 µmol l^{-1} pNP	control	100 µmol l^{-1} 4-MU	control	0.5 µmol l^{-1} 4-MU	control
1.491	1.595	84	69	29	30
1.590	1.591	80	81	30	31
1.553	1.607	86	99	29	29
1.574	1.592	76	52	29	30
NS		NS		NS	

ii Calibration curves

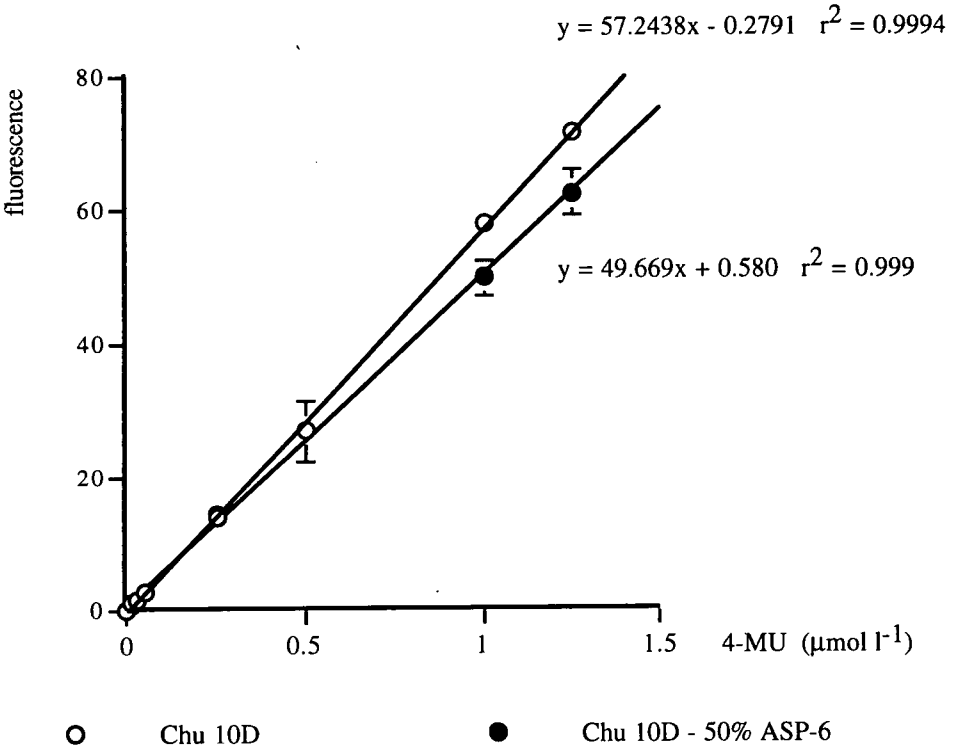
Freshwater pNP calibration curve using Chu 10D assay medium



pNP calibration using Chu 10D-50 - % ASP-6 medium



Calibration curve of 4-MU using Chu 10D assay medium and Chu 10D - 50 % ASP-6



iii Correlation of variables from Red Sike

Variable	Positive correlation		Negative correlation	
	***	**	***	**
absorbance (OD)		Fe tot		
Ca	cond	Mg, pH	current, width	
conductivity	pH, Ca	Mg, alk	current, width	
current speed	width		Mg, cond, Ca, alk	pH
Fe filt	Fe tot			
Fe tot	Fe filt	OD		
inorganic P (Pi)				
Mg		cond, Ca	current	width
Mn filt	Mn tot			
Mn tot	Mn filt			
NH_4-N	TIN, NO_3-N			
NO_3-N	TIN, NH_4-N			
organic P (Po)	Pt			TIN : Pt
% hair			% hormogonia	
% hormogonia			% hair	R250P
% *Rivularia* (% R)		temperature		
Rivularia phosphatase				
250 µM pNPP (R250P)		% hair		% hormogonia
250 µM 4-MUP (R250M)		R1M		
1 µM 4-MUP (R1M)		R250M		
pH	cond	Ca		current
temperature		% R		
TIN : Pi		TIN : Pt		
TIN : Pt		TIN : Pi		Pt, Po
total alkalinity (alk)		conductivity	current	width
total inorganic N (TIN)	NO_3-N, NH_4-N			
total P (Pt)	Po			TIN : Pt
water phosphatase activity				
250 µM pNPP (W250P)		W250M		
250 µM 4-MUP (W250M)		temp, W250P		
1 µM 4-MUP (W1M)		temp		
width	current		Ca, cond	alk, Mg

iv Correlation of variables from Tyne Sands

Variable	Positive correlation	Negative correlation
Rivularia abundance (abun)	Ri 250M, TIN : Pi	Pi, Po, Pt
Chl a (in *Rivularia* colonies)	Pi, Po, Pt, weed, diam	NH_4-N, TIN : Pi, %hair, hair leng, Ri 250P, Ri250M, Ri1M
Colony diameter (diam)	chl a, weed	%hair, Ri1M
Conductivity	pH, W250P	NH_4-N, NO_3-N, TIN : Pi
Salinity	%horm, tide, weed	%hair, hair leng, temp, Ri250P, Ri250M, Ri1M, Ra250M, Ra1M, W250M, W1M, D250M
Temperature (temp)	%hair, hair leng, Po, Pt, Ri250P, Ri250M, Ra250M, Ra1M, W250P, W250M, W1M	%horm, NO_3-N, salinity, tide
Tide height (tide)	NH_4-N, NO_3-N, TIN : Pi, salinity, weed	%hair, Po, Pt, temp, Ra250M, W250M, W1M
Weed deposits present (weed)	chl a, diam, salinity, tide, Ra250M	%hair, Ri250M, Ri1M, W250M, W1M
% Hair (%hair)	temp, hair leng, Ri250P, Ri250M, Ri1M	chl a, diam, %horm, NO_3-N, tide, salinity, weed
Hair length (hair leng)	temp, %hair, Ri250P, Ri250M	chl a, horm, salinity
Hormogonia (%horm)	salinity	%hair, hair leng, temp, Ri250M, Ri1M
Inorganic P (Pi)	chl a, Po, Pt, NO_3-N	abun, TIN : Pi, Ri250P, Ri250M, W250M
Organic P (Po)	chl a, Pi, Pt, temp, Ra250M, Ra1M, W250M, W1M	tide, abun, TIN : Pi
total P (Pt)	chl a, Pi, Po, temp, Ra250M, Ra1M, W250M	abun, TIN : Pi, tide, Ri250P
NH_4-N	TIN : Pi, Ri250P, tide	cond, chl a, W1M
NO_3-N	Pi, TIN : Pi, tide	cond, %hair, temp, W250M
Total inorganic Nitrogen : inorganic P (TIN : Pi)	NH_4-N, NO_3-N, tide, Ri250P, abun	chl a, cond, Pi, Po, Pt, Ri250M, Ra250M, Ra1M, W250M
pH	conductivity	

iv Correlation of variables from Tyne Sands (cont.)

Variable	Positive correlation	Negative correlation
Rivularia phosphatase activity 250 µM pNPP (Ri250P)	%hair, hair leng, NH_4-N, TIN : Pi, temp, Ri250M, Ri1M, W250P, W1M	chl a, Pi, Pt, salinity
Rivularia phosphatase activity 250 µM 4-MUP (Ri250M)	abun, %hair, hair leng, temp, Ri250P, Ri1M, Ra250M, Ra1M, W250M, W1M, D250M	chl a, %horm, Pi, TIN : Pi, salinity, weed
Rivularia phosphatase activity 1 µM 4-MUP (Ri1M)	%hair, Ri250P, Ri250M, Ra250M, Ra1M, W1M	chl a, diam, %horm, salinity, weed
Ralfsia phosphatase activity 250 µM pNPP (Ra250P)	Ra250M, Ra1M,	
Ralfsia phosphatase activity 250 µM 4-MUP (Ra250M)	Po, Pt, temp, weed, Ra250P, Ra1M, Ri250M, Ri1M, W250M, W1M, D1M	TIN : Pi, salinity, tide
Ralfsia phosphatase activity 1 µM 4-MUP (Ra1M)	Po, Pt, temp, Ra250P, Ra250M, Ri250M, Ri1M, W250M, W1M, D250P	TIN : Pi, salinity
Filtered water phosphatase activity 250 µM pNPP (W250P)	cond, temp, W250M, Ri250P	D1M
Filtered water phosphatase activity 250 µM 4-MUP (W250M)	Po, Pt, temp, W250P, W1M, Ri250M, Ra 250M, Ra1M	Pi, NO_3-N, TIN : Pi, salinity, tide, weed
Filtered water phosphatase activity 1 µM 4-MUP (W1M)	Po, temp, W250M, Ri250P, Ri250M, Ri1M, Ra250M, Ra1M	NH_4-N, salinity, tide, weed
"*Dichothrix*" community phosphatase activity 250 µM pNPP (D250P)	Ra1M	
"*Dichothrix*" community phosphatase activity 250 µM 4-MUP (D250M)	D1M, Ri250M	salinity
"*Dichothrix*" community phosphatase activity 1 µM 4-MUP (D1M)	D250M, Ra250M	W250P