Phosphorus dynamics in the swale - Ouse River system

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in the

Swale – Ouse River System

By

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

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Abstract

A study was made of the phosphorus ecology of the Swale – Ouse river in northern England. It ranges from a stream draining a peat moorland to a mature river influenced by urbanisation (estimated population 250 000) and intensive agriculture. The aims were to assess the concentration and variability of aqueous N and P on spatial and temporal scales, and the response of two common mosses, *Fontinalis antipyretica* and *Rhynchostegium riparioides*, to those changes. Key aspects included analysis of water chemistry, internal nutrient contents of mosses, and 'surface' phosphatase activity. The rate of activity of *in situ* plants was measured over an annual period, in conjunction with short-term studies of transplanted populations. Studies of phosphatase activity in the water also were carried out to assess the biological cycling of phosphorus.

Aqueous total phosphorus and total dissolved nitrogen concentration increased on passing down the river. In the upper reaches, total phosphorus comprised equal contributions of dissolved organic and inorganic phosphorus, which were almost certainly derived from diffuse sources. Further downstream, total phosphorus was almost entirely comprised of inorganic phosphorus from point source inputs.

The nitrogen and phosphorus content of *Fontinalis antipyretica* and *Rhynchostegium riparioides* increased on passing downstream, consistent with the water chemistry. The rate of phosphomonoesterase activity of both mosses was high in the upper reaches of the river, and was inversely related to nutrient content. *Fontinalis antipyretica* sampled from streams draining peat moorland was shown to have a high phosphodiesterase : phosphomonoesterase ratio. A possible explanation for this is that peat is a potentially rich source of phosphodiester substrate, although increased phosphodiesterase activity may be a response to extreme phosphorus limitation. Transplantation of *F. antipyretica* showed that internal nutrient content and phosphatase activity respond to changes in ambient nutrients.

Aqueous phosphomonoesterase activity was studied over a 12-month period. Laboratory and field studies suggest it plays an important role in the phosphorus dynamics of the Swale – Ouse river system.

List of abbreviations

a	annum
A320	absorbance of U.V. light at 320 nm
ANOVA	analysis of variance
AONB	area(s) of outstanding natural beauty
APA	alkaline phosphatase activity
bis-pNPP	bis-p-nitrophenyl phosphate
BOD	biological oxygen demand
°C	degrees Celsius
C.V.	coefficient of variation (%)
CSO	combined sewer outfall
DAPI	4', 6-diamidino-2-phenylindole
DMG	3, 3-Dimethyl-Glutaric acid
DNA	deoxyribonucleic acid
DOE	Department of the Environment
d. wt	dry weight
EDTA	Ethylenediaminetetra-acetic acid
ESA	environmentally sensitive area
FRP	filtrable reactive phosphorus
FOP	filtrable organic phosphorus
FTP	filtrable total phosphorus
g	gramme
GQA	general quality assessment
h	hour
HEPES	(N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid])
Km	Michaelis constant (strict usage)
Ks	apparent half-saturation constant for enzymatic activity
L	litre
LOIS	Land Ocean Interaction Study
ma	million years (old)
meq	milliequivalent
MF	3-o-methylfluorescein
MFP	3-o-methylfluorescein phosphate
min	minute
mL	millilitre
mM	millimolar
M	molar
moll	mole
MTR	Mean Trophic Rank
MU	4-methylumbelliferone
MUP	4-methylumbelliferyl phosphate
n	number
nmol	nanomole
NNED	N-1-napthylethlenediame dihydrochloride

NRA	National Rivers Authority
p	probability
PAR	photosynthetically available radiation
Pi	phosphate (any form of orthophosphate)
PDE	phosphodiester
PDEase	phosphodiesterase
PME	phosphomonoester
PMEase	phosphomonoesterase
pNP	p-nitrophenol
pNPP	p-nitrophenyl phosphate
PP	particulate phosphorus
RACS[R]	Rivers sub-group of LOIS
s	second
S.D.	standard deviation
S.E.	standard error
SSSI	Site(s) of Special Scientific Interest
STW	sewage treatment works
t	tonnes
TDI	Trophic Diatom Index
TIN	total inorganic nitrogen
TP	total phosphorus
WTW	Wissenschaftliche-Technische Werkstätten
w/v	weight/volume
µg	microgramme
µM	micromolar
µmol	micromole

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CHAPTER 1

Introduction

1.1 Preamble

This project was undertaken within the broader confines of the Land Ocean Interaction Study (LOIS), detailed below. The subject of study was P dynamics in the Swale - Ouse system, with respect to the influence of P on the biota. This introductory chapter reviews work concerning the sources of P to lotic systems, and the availability of P to the biota within freshwaters. This includes the importance of P fractions, and the processes which, mediate changes in various "pools". The focus of this is the role of enzyme catalysed hydrolysis of organic P.

1.2 LOIS

The Natural Environmental Research Council (NERC) funded LOIS, their largest project to date, which focussed on the export of dissolved and particulate material out into the North Sea from the east coast of the U.K. (see Wilkinson *et al.*, 1997). The Humber catchment rivers form the major source of material output from the estuary, and were selected for detailed investigation by the LOIS community (Leeks *et al.*, 1997). The Rivers, Atmosphere, and Coastal Studies (RACS) was a subgroup of LOIS comprising freshwater and marine biologists, chemists, hydrologists and atmospheric scientists.

RACS [R], the river sub-group, was composed of the core chemistry and the special topics. The core chemistry laboratory located at York, collected a range of determinants at designated sites for the benefit of the LOIS community. Two sites were sampled weekly on the Swale and Ouse. Groups tasked with describing the spatial and temporal changes of

certain determinants, and modelling river function used these data. The special topics were set up to assess processes in more detail. This study was one special topic concerned with nutrients, in particular P, in the Swale-Ouse tributary of the Humber. Other topics focussed on N (Ainsworth & Goulder, 1998; Pattinson *et al.*, 1998) as well as P (House & Warwick, 1998a, 1998b) in the Swale-Ouse and were conducted over the same period as this study, 1994 - 1997.

1.3 Nutrients

Nutrients are one of the driving forces behind primary productivity in freshwaters. The three primary macro-nutrients are C, N and P. It is generally considered that P, due to its scarcity, is the element which, limits primary productivity in most temperate rivers and lakes. Therefore, P has been the focus of much research in freshwater biology over the past four decades.

Enrichment of waters by high concentrations of P is generally considered to be a primary factor responsible for eutrophication (Harper, 1992). In view of the problems this causes, monitoring of P is a statutory remit of the Environment Agency, and chemical assessment of P concentration in rivers and lakes is commonplace worldwide. However, the variability of P means that chemical sampling programmes are often poor predictors of the biological consequences of P enrichment. Therefore, limnologists have long sought for answers to this problem and increasingly have turned to biological solutions. Dillon & Rigler (1974) working on 19 lakes in North America, demonstrated the strong relationship between P and chlorophyll a. Indeed, the authors suggested it would be possible to estimate the algal biomass from one measurement of P at the spring overturn. Would it also be possible to assess concentrations of P from biotic measures?

A review of biological monitoring of eutrophication by Kelly & Whitton (1998), outlines some of the methods currently in use. The Mean Trophic Rank (MTR), (Holmes, 1995) and the Trophic Diatom Index (TDI), (Kelly & Whitton, 1995; Kelly 1998a) attempt to assess critical concentrations of nutrients by changes in macrophyte and diatom communities. Both are numerical indices based on the sensitivity of species present in the community to the availability of nutrients, in particular N and P. The MTR is criticised due to its slow response to changes in nutrients, and the influence of nutrients in sediments on rooted macrophytes. The TDI is more sophisticated, and has recently been subject to rigorous testing and development (Harding & Kelly, 1998). However, as with all floristic based monitoring systems, the communities are subject to a range of parameters not just P. Kelly (1998b) has recommended the use of specific physiological tests, such as phosphatase assays, to enhance the predictive power of the relationship between plant communities and nutrients.

1.4 Sources of P

The inputs to lotic ecosystems may be generally put into two classes, diffuse and point sources, both of which may be organic or inorganic. Diffuse sources occur throughout a catchment, and soil leachate is generally thought to provide the major contribution of diffuse inputs. Point sources, such as sewage treatment works (STW), and storm overflows (CSO) are concentrated anthropogenic inputs, and most of the P input to rivers is from STW (Harper, 1992). As well as STW, Carr & Goulder (1990b) point out fish farms can be the source of significant inputs of nutrients. Point sources have much higher concentrations of nutrients and have a greater influence on receiving waters than diffuse sources, and early catchment studies focussed on the influence of point sources. However, current research is changing the previously dismissive attitude to the importance of diffuse sources (Whitton & Kelly, 1998).

The impact of diffuse inputs, particularly to upland catchments, or zones of intensive agriculture, is now becoming apparent.

1.41 Geological

Although P is of great biological importance, geologically, by mass it is globally of much less significance. All P is derived from igneous rocks, but is by no means an abundant constituent of the lithosphere. It forms only 0.1% of the rocks that make up the bulk of the earth's crust, and is thus geochemically classed as a trace element (McKelvey, 1973). It is of mineral origin, and the most common species is fluorapatite, $Ca_5(PO_4)_3F$. P commonly replaces silicon in the SiO_4 teterahedra of igneous silicates and is common in both igneous and metamorphic rocks. Nockolds (1954) provides detailed element analyses of 2507 igneous rock types, including P_2O_5, which ranges from undetectable to 1.68%.

Through weathering and erosion, P is liberated from igneous source rocks and apatite minerals. The solubility of apatite is Eh dependant, and breakdown is accelerated under acid conditions, including organic acids (Kpomlekou & Tabatabai, 1994). P can then be accumulated in sedimentary rocks, particularly those rich in calcium such as limestones. Ronov & Yaroshevsky (1969) estimate that sedimentary rocks comprise only 7.9% of the total lithosphere, and this may lead one to believe they are of less importance as P sources. However, sedimentary rocks occupy a much greater surface area than igneous rocks, and therefore exert a greater influence on soil properties. The weaker lattices, greater permeability and porosity of sedimentary rocks, exposes them to increased weathering potential. Dillon & Kirchner (1975) showed an increased export of P from sedimentary compared to igneous rocks, in Canadian catchments. Therefore, the most concentrated sources of geological P, such as phospho-silicate igneous rocks, may not be those most likely to export P.

1.42 Soils and agricultural runoff

Direct geological inputs to freshwaters are negligible when compared to the soils which overly them. Soils are more dynamic than the lithosphere, and contain a biological component virtually absent in the underlying strata. The organic component, and nutrient processing, means soils contain organic and inorganic P, compared with just inorganic P in rock strata. Current estimates of the total P in soils suggests the organic component forms between 30-60% (Dalal, 1977). In some organic soils (Harrison, 1987) or soils of a hydromorphic nature (Zech *et al.*, 1987), this may be as much as 90%. In upland soils, particularly peat, organic P is often in the diester form (Bedrock *et al.*, 1994; Zech *et al.*, 1987) suggesting less processing than agricultural mineral soils, which results in higher concentrations of organic monoesters, such as phytates.

Organic P often forms a significant component of total P exported from soils in surface runoff (Haygarth & Jarvis, 1997) and via subsurface pathways (Turner & Haygarth, 1998). Inorganic P is rapidly immobilised by binding with calcium (House *et al.*, 1986), iron (Stumm & Morgan, 1970), and aluminium (Jansson, 1981; Joseph *et al.*, 1995), preventing its export from soils. Organic P, which appears to bind to metals by a different mechanism to inorganic P (Klotz, 1991), is often more vulnerable to transfer than inorganic P (Chardon *et al* 1997).

1.43 Atmospheric

Although largely ignored in the literature, the input of atmospheric P, particularly to upland areas, must be of quantitative importance. In upland catchments, it is improbable to assume that all the soil P is derived from the underlying rocks, therefore, inputs must occur from precipitation. With the current concern about N deposition in upland areas, is it logical

to assume that P is re-introduced to upland areas in a similar fashion? The literature on the contribution of P in rainfall is scant, but has been reviewed by Gibson *et al.*, (1995) and values of atmospheric P deposition range from 5 to over 100 kg P km^{-2} a^{-1}. Results from their study of an upland site in Ireland estimated an atmospheric input of 22 kg P km^{-2} a^{-1}. The direct input of P to upland streams in the U.K. is quite low, as the ratio of terrestrial to aquatic surface area is always high. However, P deposited on terrestrial surfaces may be quickly exported by leaching to surface waters. The rate at which this occurs will be a function of sorption capacity of the soils comprising the terrestrial surface.

1.5 P in freshwaters

The vast number and variety of carbon based molecules is well known, however, even many chemists do not realise that a similar great variation and wide extent is to be found in the chemistry of compounds based on P (Van Wazer, 1973). The best known of the vast quantity of P compounds is phosphate. In freshwaters, P occurs in a fully oxidised state with four oxygen atoms in a tetrahedral structure. Only very rarely will natural conditions allow the formation of reduced P compounds, such as trialkylphosphines (R_3P).

Phosphates are readily combined to form organic or inorganic compounds in aquatic environments. Inorganic P, commonly known as ortho-phosphate, may have one or two hydrogen atoms covalently bonded to the oxygen atoms, dependent on pH (Stumm & Morgan, 1970). The oxygen atoms of the tetrahedra may form O-P bonds with more than one P atom and these structures are referred to as condensed phosphates. The simplest of these "polyphosphates", pyrophosphate (P_2O_7) combines just two P atoms. However, long chains with many P atoms are known and Van Wazers (1973) comparison with C may well be justified.

As well as covalent bonds such as the hydrogen oxygen bond or the P-O bond, the PO_4^{3-} ion bonds strongly to ions of opposing charge. Of interest in freshwaters are the metal cations, Al^{2+}, Fe^{2+}, Mg^{2+} and Ca^{2+}, which have a strong affinity for PO_4^{3-} anions. Although most clay has a net negative charge, positively charged surfaces of clays and colloids readily adsorb phosphates.

Although inorganic orthophosphate is the principal form in which P is interchanged among components of aquatic systems, a major share of the P in surface waters is organic (Hooper, 1973). In spite of the importance of organic P, few have attempted the difficult task of identifying organic P compounds, let alone relating them to processes within waterbodies. This difficulty is not restricted to organic compounds; the standard method of analysis of both inorganic and organic P in aquatic samples is far from ideal.

1.6 P analysis

The standard method of P analysis is based on the phospho-molybdenum blue method of Murphy & Riley (1962). Filtration through a 0.45 μm membrane separates the dissolved from the particulate fractions. Fractions capable of forming the heteropoly blue complex are termed reactive (Cembella *et al.*, 1984). Fractions requiring prior treatment, such as UV irradiation (Strickland & Parsons, 1972), or hot persulphate digestion (Jeffries *et al.*, 1979), may be termed unreactive. The filtrable reactive fraction is generally thought to be equivalent to dissolved inorganic P, ortho-phosphate. However, such classification is only as good as the method determining it, and doubts about the accuracy of the molybdenum blue method have been raised. Inaccuracy arises from the hydrolysis of acid labile compounds, as well as complexation between molybdate and silica, germanium and arsenic ions (Broberg & Pettersson, 1988). Radiochemical (Rigler, 1956, 1966; Lean, 1973) and enzymatic (Pettersson, 1979; Stevens, 1979) studies give weight to the argument that the actual

orthophosphate concentration is much smaller than that determined by the molybdenum blue method. This overestimation has been attributed to the measurement of colloidal and acid-labile organic fractions as orthophosphate.

1.7 Importance of P to the biota

Dissolved inorganic P is considered directly available to primary producers, and uptake into the biota occurs rapidly (Bostrøm *et al.*, 1988). As well as total concentrations of P, the relationship between N and P is of importance in biological systems. Following studies of marine organisms, Redfield (1934) suggested an ideal ratio of C:N:P of 42:7:1 under which, organisms would not be nutrient stressed. Assuming N and P to be the major limiting nutrients in freshwaters, at N:P ratios < 7:1 potential growth would be N-limited. This situation would favour organisms capable of deriving N from sources other than dissolved N, N-fixers for example (Schindler, 1975; 1977). N:P ratios > 7:1 would favour organisms adapted to P limited conditions.

P stored within the biota is often released in an organic form. Abiotic breakdown of stable organic P compounds in waters with neutral or weakly alkaline pH is slight (Shen & Morgan, 1973). A rapid mechanism must therefore exist to reverse this inorganic to organic transformation. Phosphatase enzymes are one such catalyst, and several reviews of their ecological importance have been made (Siuda, 1982; Cembella, 1984; Jansson *et al.*, 1988; Chróst, 1991). The term phosphatase is commonly used for enzymes which catalyse the hydrolysis of esters and anhydrides of phosphoric acid (Feder, 1973). This biochemical pathway is important in the regeneration of phosphate in freshwater ecosystems, and a key parameter in aquatic ecology (Hoppe, 1991) . Phosphatase activity is present in a large number of organisms and is moderated by the presence, or absence, of dissolved inorganic P.

Therefore, as well as being an important part of the P cycle, Petterson (1980) outlines its use as an indicator of P status in freshwaters.

1.8 Phosphatases

Many aquatic and soil organisms are capable of hydrolysing organic phosphates in their environment due to the presence of 'surface' phosphatase enzymes, and can then utilise part or all of the phosphate released. In their comprehensive review, Cembella *et al.* (1984) cite studies where activity was enhanced when the organisms were P-limited. A study of fungal mycorrhizae (Leake & Miles, 1996), suggests the enzyme activity may be de-repressed as a direct response to the presence of a suitable external substrate.

Fitzgerald & Nelson first demonstrated in 1966, using filamentous algae and blue-green algal bloom samples, that the de-repression of phosphatase activity had potential for monitoring purposes in freshwater environments. Subsequently, there have been many studies on phosphatase activities in freshwater environments, and a number of researchers have repeated the proposal that these can be adapted for monitoring purposes. Whitton (1991) reviewed some proposals on the use of phosphatase for monitoring. There are, however, obvious difficulties to be resolved before it is possible to standardise methods for practical use.

1.81 Phosphatases and phosphoesters

Phosphatases are enzymes with the ability to hydrolyse phosphoesters, which include phosphomonoesters, phosphodiesters and phosphotriesters (McComb *et al.*, 1979). Each of the enzymes is substrate specific, although there are rare exceptions (Yoshida *et al.*, 1989). There is also evidence that some phosphatases may aid the uptake of inorganic phosphate under certain conditions, but detailed studies are lacking and this probably has little relevance

to the development of a practical methodology for assaying the P status of organisms or communities.

There have been a few bacterial studies on hydrolysis of phosphotriesters, but these would appear to be largely of academic interest. It is the ability to mobilise phosphomonoesters and phosphodiesters, which is of importance to organisms short of phosphate and unable to make use of particulate materials. Phosphomonoesters range from single sugar phosphates, which are likely to be metabolised rapidly in nature, to highly insoluble metal phytates, which dominate the P fraction of some soils. Phosphodiesters, include nucleic acids, which are among the first molecules released during breakdown of biological materials. There is evidence that phosphodiesters are especially important P sources within soils and soil organisms (Browman & Tabatabai, 1978; Leake & Miles, 1996; Myers & Leake, 1996).

The majority of reports in the literature concern alkaline phosphatase activity (APA), which is essentially a synonym for phosphomonoesterase (PMEase) activity at high pH values, typically in the range pH 7.5 to 10.0. However, a number of studies have shown acid phosphatase to be dominant in lake water (Cotner & Heath, 1988; Jansson *et al.*, 1981), stream epilithon (Chappell & Goulder, 1992, 1994a) and bryophytes (Al-Shehri, 1992, Christmas & Whitton, 1998a, 1998b; Press & Lee, 1983). This variation in pH optimum suggests that either surface PMEase is not the same in all organisms, or has the ability to adapt to external pH conditions. Indeed, it is very probable that the activity observed in some organisms is in fact due to more than one enzyme.

Alkaline PMEases usually include zinc in the molecule, and also require free Zn^{2+} for optimum activity, thus, their development may perhaps be hindered at times by lack of zinc, and their activity is likely to be influenced by chelating agents in the environment (see

below). PMEase activity of populations of several terrestrial lichens taken from old mine spoil wastes, has been shown to be more resistant to elevated zinc in the medium than populations from sites without zinc contamination (Stevenson, 1994).

Both PMEases and nucleotidases can release the phosphoryl group of ATP (Ammerman & Azam, 1991). Siuda & Güde (1994) attempted to resolve their relative contribution by using inhibitors of PMEases, chiefly inorganic phosphate. However, it remains unclear whether studies using ATP are examining the significance of PMEases, nucleotidases or both (Hernández *et al.*, 1996a).

The location of 'surface' phosphatases is usually reported to be on the cell membrane (Bieleski, 1974), in the periplasm of prokaryotes, or immobilised somewhere in the cell wall (Hernández, *et al.*, 1994). It is often present in the mucilage excreted by many bacteria and algae, presumably in most cases it is immobilised, but perhaps also released. Phosphatase activity is sometimes high in water, so presumably there is a possibility that phosphatases might become trapped by the surfaces of organisms, which did not form it, but there has never been any report of this. Another possibility is that some organic phosphates might pass via the cell membrane and that hydrolysis actually takes place inside the cell.

1.82 Measurement of activity

Activity is usually measured by the hydrolysis of simple analogue phosphate esters, which release moieties easily quantified using a spectrophotometer or fluorometer. The standard substrates for spectrophotometry are p-nitrophenyl phosphate (p-NPP) and bis-p-nitrophenyl phosphate (bis-pNPP), both of which release p-nitrophenol (p-NP: measured at 405 nm). These are used to assay phosphomonoesters and phosphodiesters, respectively. These substrates are soluble over the whole pH range and relatively non-toxic, although the p-

nitrophenol released is toxic to some organisms at the concentrations likely to be released in some assays requiring high levels of substrate. Because APA assays are conducted very widely in hospitals to screen for a range of cancers and other tissue disorders, p-NPP is relatively cheap. Key practical details of how to conduct assays are well known (Petersson & Jansson, 1978; Huber & Kidby, 1984), but surprisingly, there is no published account which brings these all together, or answers some obvious queries such as the stability of the molecule under various environmental conditions.

Cembella *et al.* (1984) emphasise that p-NPP can be hydrolysed by a range of hydrolases, and not just PMEases in the strict sense, which raises the problem of enzyme specificity for this substrate. However, it is unclear whether this is of practical relevance for monitoring purposes, because organisms are presumably capable of taking up phosphate from whatever source it originates.

PMEase activity can also be assayed fluorometrically with several fluorogenic substrates, 4-methylumbelliferyl phosphate (MUP) being by far the most widely used. This is hydrolysed to 4-methylumbelliferone (MU) and detection limits can be increased using HPLC (Kang & Freeman, 1987). Another substrate, 3-*o*-methylfluorescein phosphate (Perry, 1972) is hydrolysed to 3-*o*-methylfluorescein, although high background fluorescense has reduced its popularity (Petersson & Jansson, 1978). These substrates have the advantage over the spectrophotometric method in that their use can be made almost a hundred times more sensitive, and so it is possible to miniaturise assays.

It is usually assumed that the results of assays with p-NPP and MUP can be treated as interchangeable and this has been shown for dissolved and particulate PMEase (Boon, 1993) and bryophytes (Christmas & Whitton, 1998b). However, at least one study (for an intertidal

population of the blue-green alga *Rivularia atra*: Yelloly & Whitton, 1996) showed differing patterns of seasonal change in PMEase activity, depending on which substrate was used.

How analogue substrates relate to naturally occurring organic P is a matter of some debate. The problem inherent in the measurement of P in waters by the molybdenum blue method has already been discussed (1.6). However, the popularity of this method means that comparison between filtrable organic P (FOP) and enzyme utilisable substrate has been carried out by a number of workers. Estimates of the biologically labile proportion of FOP range from 32 – 75 % (Chróst & Overbeck, 1987; Hino, 1988; Taft *et al.*, 1977). Combinations of phosphatases and nucleases increase the availability of dissolved organic P (Hino, 1989). The source of the FOP and its molecular weight can reduce its suitability for hydrolysis (Hino, 1988, 1989; Paerl & Downes, 1978)

Some studies using analogue phosphomonoester substrates (pNPP) reported in the literature have been conducted over periods of many hours e.g. 120 h, 72 h, 96 h, (Berman, 1969; Jones, 1972; Reichardt *et al.*, 1967). However, a number of errors may arise in doing this, and it is suggested that assays should be restricted to no more than one hour. For many materials it should be possible to restrict the assays to 20 - 30 min. In the case of very short studies, it is important to ensure that all the moiety to be used for measurement has been released by the organism. It has been demonstrated for seaweeds that retention of the moiety can introduce significant errors (up to 20%) unless allowance is made (Hernández & Whitton, 1996).

Several studies have used radiolabelled substrates (see Hernández *et al.*, 1996a), and with glucose-6-phosphate it is possible to label the phosphoyl moiety with ^{32}P, or the glucose with ^{14}C, thus providing a means of following the post-hydrolysis fate of either part of the

substrate. Radiolabelled [γ-^{32}P]ATP was used by Siuda & Güde (1994) as a specific substrate for 5'nucleotidase activity. This activity is thought to represent a pathway of P regeneration separate to that of phosphomonoesterase activity.

1.9 Influence of environmental conditions

1.91 pH

This is probably the most important factor, because phosphatases always show a marked response to pH. In some cases the response is especially sharp, suggesting that a single enzyme is involved, while in other cases it is less so, suggesting either that several enzymes are involved or that several associated factors influence the observed activity. Many rooted plants show peak activity in the acid range (Tarafdar & Claassen, 1988), often at about pH 5.5. However, some show alkaline activity and occasionally there are maxima in both ranges. Cyanobacteria mostly show maxima well into the alkaline range, sometimes above pH 10.0.

A problem that has long confused the study of phosphatases, especially interpretation of the ecological significance of the results, is the fact that the pH value for maximum activity shown in laboratory assays often differs markedly from the pH value at the site from which the organism was taken. Typically, APA shows a higher pH optimum than that likely to be found in the field, while bryophytes and roots taken from an environment with slightly alkaline pH values usually show the presence of an acid phosphatase. However, studies of Swedish acid lakes suggest that in acidified waters, the pH optima may adapt to reflect the environment (Jansson *et al.*, 1981). A study by Fedde & Whyte (1990) on mammalian tissue has shown that the substrate concentration used in assays was responsible for the apparent discrepancy between pH optima and ambient pH. A reduction in the substrate concentration

used for APA assays led to a drop of almost two pH units in the optimum pH. Rather similar results have been found for the freshwater diatom *Synedra acus* (Hantke & Melzer 1993) and several freshwater strains of *Calothrix* (Whitton, pers. comm.). In these cases, a decrease in substrate concentration also led to a reduction in the pH optimum towards values the organisms are likely to encounter in the field.

1.92 Other elements and compounds

A number of studies have been conducted on the influence of other ions on APA. Providing that a standard medium (e.g. Gibson & Whitton; 1987a, 1987b) is used for assays, the measure of activity appears to be relatively insensitive to shifts in most ions. Not surprisingly, phosphate is the most important. As this is omitted from assay media, the only problem likely to arise is if assays are conducted at high substrate concentrations. This probably does not matter in short-term assays, but time-course studies are needed if assays with high substrate concentration are run for long period. Because of the requirement of many phosphatases for Zn^{2+}, the concentration of chelating agent in the assay medium should be no more than that required to chelate key trace metals.

Laboratory studies of purified enzyme showed that alkaline phosphatases are metallo-enzymes, *E. coli* has 2-3 g-atoms of Zn^{2+} per mole of enzyme. The metal is essential for catalytic activity and possibly for the maintenance of native enzyme structure (Reid & Wilson, 1971). Unlike most alkaline phosphatases, those of *Haloarcula marismortui* require Ca^{2+} and not Zn^{2+} for its activity (Golman *et al.*, 1990). Activation of various tissue phosphatases is observed with Co^{2+}, Mg^{2+} and Mn^{2+} (Fernley, 1971). These essential metals may be complexed with aquatic compounds causing reduction in phosphatase activity in field studies. Polyphenols affect P regeneration in freshwaters, not only by binding directly to the phosphatase enzyme, but also by complexing metals required by the enzyme for activity

(Serrano & Boon, 1991). Complexation of the phosphatase substrate by metals such as aluminium (Jansson, 1981), is thought to lead to increased enzyme production in competition for the substrate. Acid phosphatases have no cation requirement, but are specifically inhibited by fluoride (Hollander, 1971)

1.93 Light

The APA of many algae, particularly seaweed, shows a marked response to light, even in short-term studies. A brief summary of the results for marine algae is therefore included here. Some species, such as the red algae *Porphyra umbilicalis* (Hernández *et al.*, 1993) and *Gelidium latifolium* (Hernández *et al.*, 1994) and the blue-green *Rivularia atra* (Yelloly & Whitton, 1996) show no short-term dependence of APA on light flux. In *Ulva lactuca*, however, APA was higher in the light than the dark (Weich & Granéli, 1989).

Suppression in the dark is independent of the external phosphate concentration. Similarly, APA in *Gelidium sesquipedale and* the seagrass *Zostera noltii* increased with photon irradiance (Hernández *et al.* 1994b, 1995). In *Z. noltii*, APA showed a saturation curve with maximum activity at a flux value greater than 130 μmol photon m^{-2} s^{-1} and this pattern was similar that of the influence of irradiance on direct phosphate uptake. Rivkin & Swift (1979) found that the marine dinoflagellate *Pyrocistis noctiluca* had a diel pattern of APA, with the highest levels under highest irradiance. The light quality has also been shown to influence APA in several freshwater eukaryotic phytoplankton, with higher activity under blue light (Wynne & Rhee, 1988). This dependence of APA on light suggests a dependence on metabolic energy from phosphorylation. However, more critical studies (e.g. use of photosynthetic uncouplers/inhibitors) are needed to establish the underlying mechanisms involved.

In contrast, Stewart & Wetzel (1982) found enhanced phosphatase activity in a pelagic lake mixed community with high concentrations of dissolved humic materials, particularly under low light regimes. The low light regime of a shaded stream was also consistent with increased alkaline phosphatase activity in the green alga *Selenastrum capricornutum* (Klotz, 1985). The red alga *Corallina elongata* achieved maximum APA in the dark, with minimum activity at relatively low irradiance (Hernández *et al.*, 1996a). APA in this species may balance the P requirements when the direct uptake of orthophosphate is reduced under conditions of limiting reducing power. However, the dependence of APA on darkness may in some cases be indirect, resulting from the fact that APA is inversely related to internal phosphate. A long pre-incubation in darkness may stimulate the enzyme synthesis due to a decrease in phosphate uptake, which is an energy-dependent reaction. Overall, the response to light seems to be species-specific. Temporal variations in APA may be a factor in the co-occurrence of species in a phosphate-depleted environment, with the PME fraction made available to different species at different times of the day (Klotz, 1985).

1.94 Temperature

The effects of temperature on enzyme activity are (likely to be) complex and include the effect on stability of the APA, the effect on the actual velocity of breakdown of the complex, and the effect on the enzyme-substrate affinity. In many tests, the optimum temperature for APA has been found to be higher than temperatures likely to occur in the field. The moss *Hydrogonium fontanum* grown in culture had optimum PMEase and PDEase of 60 and 65 °C (Al-Shehri, 1992), when assayed for short incubation periods (90 min), although the organism itself is likely to have been damaged.

Although a rise in assay temperature leads in most cases to an increase in APA, it should not be assumed that activity for a particular species is necessarily highest at the time of

year when field temperatures are highest. There have apparently been no studies for freshwater organisms to establish whether or not this applies. However, it is known that it does not apply to the intertidal brown alga, *Fucus spiralis*. The activity of a population at Tyne Sands, Scotland, was higher in April (when mean tidepools temperature during low tide = 14 °C) than in August (mean tidepools temperature during low tide = 20 °C) (Hernández *et al.*, 1997). In fact APA during the winter was always higher than during the summer, in spite of assays being conducted at field temperatures.

If the influence of temperature on a particular population has been studied in advance, then it may be suitable to conduct assays on any one day at temperatures higher than those occurring in the field. The response to temperature at different times of year needs to be established for organisms sampled at different seasons, especially if routine assays are conducted at one standard laboratory temperature. Although any difference in response seems unlikely for slow-growing river organisms like bryophytes, some faster-growing algae might well be represented by different populations at different times of year or show obvious differences in physiological responses.

1.10 Phosphatase activities of freshwater organisms

Ecological studies on phosphatase followed from the studies by Torriani (1960), who showed that *E. coli* developed phosphatase activity when cultured in medium with low concentrations of phosphate. There have subsequently been numerous studies relating phosphatase activity of water, bacteria, algae and submerged bryophytes to the chemistry of the water. Some early lake studies (e.g. Berman, 1970) showed an inverse relationship between the total APA content of the water column and the P content of the water. However, the relationship is not always so clear, based on results for Lough Neagh, Stevens & Parr (1977) warned that the contribution of allochthonous material may diminish the value of total

APA as reliable indication of algal P-limitation. Where both bacteria and algae influence APA, several attempts at discriminating the individual components have been made. This has been attempted statistically (relationship between APA and chlorophyll content or bacterial density: Jones, 1972; Boon, 1990), by differential separation, filtration, or centrifugation (Stewart & Wetzel, 1982), or differential inhibition (Boon, 1994).

A number of studies (Reichardt *et al.*, 1967; Berman, 1970; Jones, 1972; Heath & Cooke, 1975) have shown that dissolved enzymes contribute to total PMEase activity in the water. The source of this enzyme has been attributed to algae (Pettersson, 1980), zooplankton (Jansson, 1976), bacteria, fish-farms (Carr & Goulder, 1990a) and other anthropogenic sources (Chappell & Goulder, 1994b). There are few data on possible activity by dissolved PDEase, though it is sometimes detectable. However, experimental studies on 50 blue-green algal strains have never shown extracellular PDEase activity, although many strains show surface PDEase activity, and most can release extracellular PMEase (Whitton *et al.*, 1991).

One of the most successful studies was done before many of the practical problems in assessing the value of data on APA were realised. Fitzgerald & Nelson (1966) found that a range of algae (4 blue-green algae, 3 greens, 2 diatoms) from three lakes in the Wisconsin area which were P-limited had as much as 25 times more APA than algae with surplus available P. Petersson's (1980) study of Lake Erken confirmed the relationship between internal P concentration of algae and APA. Gage & Gorham (1985) suggested a combination of cellular P content and APA, as good diagnostic criteria for phytoplankton P status in Minnesota lakes.

The majority of more recent studies have focussed on the relationship between activity and P content of attached organisms, or parts of their tissue. However, it is clearly important

to establish the relationship between phosphatase activities and the nutrient composition of the water, if phosphatase activity is to be used for monitoring. One obvious problem is the variability of nutrient concentrations in the ambient water, especially in flowing waters. This is likely to influence fast-growing organisms differently from slow-growing ones. Furthermore, some attempts to relate plant phosphatase to the composition of the water have considered only its P content, whereas in many cases it may be the N : P ratio which is more important. Under conditions combining high levels of combined N and high light flux, it seems likely that P-limitation can show at relatively high ambient levels. This is clearly an important consideration when considering the possible impact of nutrient removal on a river system. Another difficulty in assessing the relationship between phosphatase activities and the ambient water is that some algae and bryophytes may utilise organic N sources, yet these are hardly ever included in surveys of water chemistry.

The difficulty in removing allochthonous material in samples taken from the water column has already been mentioned. This can largely be overcome by using attached organisms, but this still does not overcome the problem that natural materials include epiphytic bacteria and algae and sometimes also inorganic deposits. Some studies ignore this problem; some wash the material thoroughly in an attempt to deal with a single species, while others deliberately measure the phosphatase activity of the whole community. Among the latter are a few measurements of PMEase activity of biofilms on rocks.

Environmental chemistry, morphology, N and P composition and APA were all brought together in a study of 32 samples of Chaetophorales (filamentous green algae) in rivers of N-E. England (Gibson & Whitton, 1987a). This group of organisms is characterised by the formation of long multicellular hairs under conditions of P limitation (Whitton & Harding, 1978; Gibson & Whitton, 1987b). Algal P ranged from 0.097 - 1.87% dry weight, and the N : P ratio (by weight)

from 3.65 to 35.6. There was a significant negative relationship between algal N : P ratio and aqueous P, amount of hair formation and aqueous P, and between APA and algal P. There was a significant positive correlation between algal P and aqueous P and N, amount of hair formation and N : P ratio, and between APA and hair formation. Among rivers in the region showing APA were the West Allen, the Nent and several sites on the South Tyne, though not Hayden Bridge, the most downstream one. There was no activity in the lower Skerne or the Tees at Hurworth. However, material of *Stigeoclonium* showing obvious hairs was sampled in the eutrophic River Wiske, suggesting that even at such a site it seems likely that there may be occasional periods during which the aqueous P is sufficiently reduced for a fast-growing organism to show P-limitation.

1.11 Bryophytes

1.111 Ecology and distribution

Smith (1978) recognises 175 genera of bryophytes in Britain and Ireland. Of these, 9 genera of moss are commonly associated with lotic freshwater environments. Not all of these are wholly aquatic; genera such as *Bryum* have both aquatic and terrestrial species. Indeed, those that are considered aquatic have varying habitat preference (Vitt *et al.*, 1986). Species of *Fissidens* and *Philonotus* are commonly found in the splash zone of streams, whereas *Fontinalis*, *Rhychostegium* and *Amblestygium* are more commonly found fully submerged in slow flowing rivers. Two of the most commonly occurring mosses in British aquatic habitats are *Fontinalis antipyretica* and *Rhynchostegium riparioides*, indeed, *F. antipyretica* is the only moss to have a common name, Willow moss. *Fontinalis* is fairly ubiquitous worldwide; Glime (1968) has shown it to be the dominant vegetation in many mountain streams in North America.

Zonation of mosses along the length of streams suggests that particular species have preferences for specific habitats. Watson (1968) has tabulated various species by habitat in his British flora. Specific studies have been undertaken in north east England on a number of rivers (Holmes & Whitton, 1975, 1977a, 1977b, 1977c); Whitton *et al.*, 1998), north west England (Harding *et al.*, 1981; Tutin, 1949) and the Anglo-Welsh borders (Merry *et al.*, 1981). These studies had common trends: in upper reaches of the rivers *Hygrohypnum ochraceum* and *H. luridum* were common; further downstream, *Amblystegium riparium* was more common, whereas, *R. ripariodes*, *F. antipyretica* and *Cinclidotus fontinaliodes* were fairly ubiquitous throughout much of the river. An increased tolerance for pollution may explain the abundance of *A. riparium* in the lower reaches of rivers. Frahm (1975) tested the tolerance of five mosses to several ions, in all cases, *A. riparium* tolerated concentrations as great or greater than *F. antipyretica* and *F. crassipes* (supposedly pollution tolerant species). Indeed, its adaptation to polluting conditions has caused it to be a nuisance in both sewage filter beds and brewery effluent channels (Hussey, 1983; Kelly & Huntley, 1987)

Bryophyte communitites are is a constant state of flux, due in part to environmental change. Of the ten changes in macrophyte community predicted to occur in the River Wear following a survey in 1966 (Whitton & Buckmaster, 1970), eight were realised (Whitton *et al.*, 1998), including the marked increase of *F. antipyretica* upstream. In recent years, *F. antipyretica* has been found in abundance in upland parts of R. Swale (Christmas & Whitton, 1998a, 1998b; Holmes & Whitton, 1977), whereas, in an extremely detailed study of North Yorkshire approximately a century earlier, it was restricted in the low country (Baker, 1906). Its presence was not mentioned in the detailed transects made of upper Swaledale where it is now common, and it is extremely unlikely to have been overlooked.

1.112 Nutrients

The zones in which lotic bryophytes are found have greatly varying ambient nutrient chemistry, which in turn affects the internal nutrient concentration of the mosses present. Much of the research on internal chemical contents of mosses has tended to focus on cations, and less attention has been given to negatively charged ions such as nitrate and phosphate. This may be due to a bias toward terrestrial rather than aquatic mosses in the history of bryophyte research. However, mosses adapt rapidly to anthropogenic manipulation of inorganic anion concentration. Press & Lee (1983) reported an order of magnitude difference in P contents of species of *Sphagnum* from areas of variable P supply, which suggests that internal contents reflect ambient P availability. The uptake of P caused significant decreases in the tissue N:P ratio of moss after the addition of inorganic P in woodland streams in Tennessee (Steinman, 1994). Both *Hygrohypnum* sp. and *Fontinalis neomexicana* from reaches of an arctic river artificially fertilised with phosphoric acid, had P contents twice that of mosses from control sites (Finlay, 1994). The suggestion is made that mosses are dominant sinks for P in such rivers. Indeed, Meyer (1979) reported that mosses very rapidly sequestered inorganic P introduced to a river with low concentrations of P, and may act as buffers against rapid changes in P chemistry. The location of N and P within moss fronds tends to be toward the tips, they are usually acquired as anions and are present intracellularly in forms associated with cell metabolism (Brown, 1982). This is certainly the case with *F. antipyretica* and *R. riparioides*, both mosses having approximately twice the contents of N and P in the tips compared to the base of the frond (Christmas, unpublished data). In studies of aquatic mosses for both phosphatase and heavy metal accumulation, terminal tips were used to reflect the most recent streamwater chemistry.

1.113 Bryophytes as monitoring tools

Terrestrial and aquatic mosses may be used as indicators of their environment. In contrast to vascular plants, which derive nutrients from the soil, terrestrial mosses derive their nutrients from the air, and therefore serve as indicators of air quality (Furr *et al.*, 1979). In aquatic ecosystems, mosses, unlike rooted macrophytes are reliant on water alone for the supply of elements and often reflect the ambient water chemistry. In view of the fact aquatic mosses are: tolerant to pollution, non-motile, easy to sample, fairly easy to identify, evergreen (Mouvet, 1985; Say *et al.*, 1981) and simple to transplant (Kelly *et al.*, 1987) they are ideal as biomonitors. Siebert *et al.* (1996) draw the distinction between bioindicators and biomonitors, the latter give quantitative rather than just qualitative information concerning the environment.

The use of mosses as bioindicators has been almost exclusively restricted to studies of the accumulation of metals. Studies have been made on the bio-accumulation of copper (Hartman, 1969), lead, zinc and cadmium (Whitton *et al.*, 1982) to indicate pollution in freshwaters. Say *et al.* (1981) attempted to develop biomonitoring methodologies and found good correlation between moss contents and concentrations of metals in the water. However, attempts to use mosses to assess sediment contamination proved to be less successful.

1.12 Aims

The project set out to assess biological aspects of P dynamics along a continuum of contrasting sites within the Swale – Ouse catchment. Studies of bryophytes have shown they respond to nutrient concentrations in the water and play a significant role in phosphorus ecology. Therefore, mosses were chosen for a focussed study with the aim of assessing how

their physiology changed in relation to ambient water chemistry, on passing down the river. In addition, studies of enzyme activity in the water was carried out to assess the biological recycling of P. It was planned to combine analysis of field material from a range of sites with laboratory experimental studies.

The key areas of study are:

i. To quantify the concentration and variability of dissolved and particulate nutrients on spatial and temporal scales, along a river continuum. (Chapter 4)

ii. To carry out laboratory studies into the factors influencing the surface phosphatase activity of *Fontinalis antipyretica* and *Rhynchostegium riparioides.* (Chapter 5)

iii. Carry out field studies over a one year period of internal nutrient content and surface phosphatase activity of moss along a river contiuum. (Chapter 6)

iv. Conduct field experiments to assess factors within the catchment which influence surface phosphatase activity on *in situ* and transplanted moss populations (Chapter 7, 8)

v. Assess the role of phosphatase in the cycling of P along the river continuum (Chapter 9)

CHAPTER 2

Materials and methods

2.1 Safety

Field visits to river sites were made during daylight hours and preferably with two field operatives. The distance driven in one day was always less than 500 km and driving was shared between operatives. No attempt was made to sample during conditions of standing snow. For the initial part of this study (up to December 1995) sampling safety equipment for entering the water were waders, lifevest and wading stick. However, access to the river in anything greater than low to moderate flow conditions proved problematic. From December 1995 onwards, a valve-less drysuit and diving gloves were worn whenever entering the river.

2.2 Standard field techniques

On each sampling occasion various parameters were recorded at each site. Readings made while standing in the river, were taken before collection of water samples. Care was taken to collect water upstream of the immediate zone of disturbance. Conductivity, temperature, dissolved oxygen and current speed were measured with the appropriate meter and probe in the centre of the river or stream. A Wissenschaftliche-Technische Werkstätten (WTW) (model LF 191) was used to measure conductivity and temperature. Calibration with British Drug Houses (BDH) standards (36 & 1413 $\mu S\ cm^{-1}$) was carried out monthly. The thermistor was calibrated at 0 °C and room temperature (approximately 22 °C). A mercury in glass thermometer was used for reference.

Dissolved oxygen was measured with a WTW meter (model OXI 191). Calibration was carried out before each measure using a WTW Oxical –S sleeve and field barometer.

Checks for membrane damage or air bubbles under the membrane were made before sampling. The meter was switched to standby an hour before use. The electrode was cleaned and the ion solution changed every six months.

Current speed was estimated using an Ott meter with the empella blades facing into the direction of flow, at $^1/_3$ total water depth. Revolutions min^{-1} were converted to m s^{-1} as for Patterson (1983).

The alkalinity and pH of 50 mL of quiescent river water was measured in the field with a WTW meter (model pH 192), in conjunction with a Russell glass bodied combination electrode (CW76). The meter was field calibrated using standard pH buffers: 4.0, 7.0, 9.22 supplied by BDH, and the probe checked every three months following the protocol of Davison (1990). Alkalinity was estimated by single end point titration to pH 4.2 with 0.02 M HCl (APHA 1992), where alkalinity was particularly high, 0.1 M HCl was used. The equations used were as follows:

$$\text{Alkalinity, } CaCO_3 \text{ mg } l^{-1} = \frac{A \times N \times 50\,000}{S}$$

Where;

A = Vol. of HCL (mL)
N = normality HCL (M)
S = Sample vol. (mL)

The ionic form of bicarbonate alkalinity was calculated using sample pH thus;

$$HCO_3^- \text{ as } CaCO_3 \text{ mg } l^{-1} = \frac{T - 5.0 \times 10^{(pH - 10)}}{1 + 0.94 \times 10^{(pH - 10)}}$$

X
Where;

T = $CaCO_3$ mg L^{-1}

Water samples for nutrient (N and P) analysis were collected in acid washed 250-mL Nalgene plastic bottles. For the estimation of bacterial numbers, water was collected in sterile glass 250-mL medicine bottles. All water samples were taken from the middle of the river or stream, at two thirds the total depth, except at Naburn weir (km 145.0), where for reasons of safety, samples were taken from a jetty on the east bank. All samples were returned to the laboratory, on ice, in a cool box.

Daily flows were recorded by the Environment Agency at three locations along the river: km 49.9, km 145.0, km -1.9, 86.1 (the first number indicates distance up the tributary, the second the confluence with the main river). Data from Crakehill km 100.5 was used for Thornton Manor km 107.9, 7.4 km downstream. River stage (water depth) measured by hydrograph, was converted to flow using rating equations calculated for each site. The rating equations account for current speed and the cross section of the site. Crakehill km 100.5 and km -1.9, 86.1 were sites with V-notch weirs, whereas km 145.0 and km 49.9 were natural riverbeds.

Flow was calculated on several occasions at two upstream sites, km -2.5 and km 10.9. The cross sectional area of the river was estimated using averaged depth and width measurements, and current speed measured using an Ott current metre. The results were plotted against flows at km 49.9 and a linear relationship obtained. This allowed calculation of flows at these upstream sites from flows at km 49.9 during sampling occasions when no direct measures were made.

2.3 Standard laboratory techniques

2.31 General labware

All chemicals used were of AnalR grade. Stock solutions were made up using MilliQ water. Glassware and plastics for nutrient analysis were rinsed three times in distilled water

prior to soaking in 10 % H_2SO_4 for at least 20 min. They were then rinsed three times in distilled water and oven dried at 105°C for standard glassware and 40° for plastics. Volumetric glassware was air dried at room temperature. Heavily soiled glassware was soaked overnight in hot tap water and 10% non-ionic surfactant, prior to acid washing.

2.32 Water samples

Samples were filtered under weak (< 100 mm Hg) vacuum immediately after returning from the field. An autoclaveable Nalgene filter tower housing sterile cellulose nitrate Whatman filters was used. All water samples for estimates of dissolved nutrient analysis were passed through 0.45-µm membranes.

The absorbance of light at 320 nm (A320) by water samples gave an indication of dissolved organic carbon in upland streams (Edwards & Cresser, 1987). Samples were placed in 1-cm quartz cuvettes; the light source was a deuterium lamp.

2.4 Nutrient analysis

As part of the LOIS harmonisation programme, quality control checks on all fractions of N and P analysis were carried out by another laboratory (IFE, Wareham), see House and Frickers (1995). Detection limits were calculated as three times the standard deviation of blank samples (n = 10).

2.41 Nitrogen

Water samples for nitrogen fractions were filtered in the laboratory on the day of sampling and then stored frozen (-20 °C) for up to two months prior to analysis. Reagents were made fresh on the day of analysis and stock solutions were stored for up to 3 months (Table 2.1).

Table 2.1. Stock solutions and reagents for the analysis of nitrogen fractions: NO_3^-, NO_2^- and NH_4^+

Stock Solution	Reagent
Alkaline stock. Dissolve 100 g of sodium citrate and 5 g NaOH in 250 mL distilled water. Leave to cool and dilute to 500 mL. Stored for up to 3 months	Oxidising reagent. Mix 400 mL alkaline stock with 100 mL sodium hypochlorite (12% w/v available Cl).
Buffer stock. Dissolve 100 g Ammonium chloride, 20 g sodium tetraborate and 1 g EDTA into 500 mL distilled water and dilute to 1000 mL Stored for up to 1 month.	Phenol reagent. Dissolve 100 g phenol in 1000 mL of 95% ethanol
Sulphanilamide stock. Dissolve 5 g sulphanilamide in 200 mL of distilled water. Add 100 mL 11.63 M HCl and dilute to 500 mL. Stored for up to 1 month	Sodium nitroprusside reagent. Dissolve 1 g sodium nitroprusside in distilled water and dilute to 200 mL
NNED (N-1-napthylethlenediame dihydrochloride). Dissolve 0.5 g NNED in 500 mL distilled water. Stored for up to 1 month	

2.411 Nitrate

In the presence of cadmium, soluble nitrate (NO_3^-) is reduced to nitrite (NO_2^-). To analyse water samples for nitrate the first step is to convert all the nitrate to nitrite by passing the sample through a cadmium column as for Stainton *et al.*, (1977). The column was packed with freshly activated cadmium with an average grain diameter of approximately 0.5 mm. The cadmium grains were stored in 2 N HCl and activated by rinsing in distilled water, followed by swirling in 2% w/v $CuSO_4$ $5H_2O$.

5 mL of alkaline stock, 25 mL of distilled water, 25 mL of filtered sample were mixed and passed through the column at approximately 6 mL min^{-1}, the last 25 mL was retained for analysis. Reagents (Table 2.1) were then added to analyse for nitrite (see below). Blanks were distilled water and standards were diluted from 1000 mg L^{-1} KNO_3-NO_3 stock solutions. Nitrate was calculated as the difference between reduced nitrate-nitrite and sample nitrite. The detection limit was 2 μg L^{-1} NO_3-N.

2.412 Nitrite

Nitrite may be acidified to nitrous acid, which couples with the addition of two amines to form a pink azo dye. 2.5 mL of buffer, 25-mL filtered sample and 0.5 mL of sulphanilamide were added to a 100-mL Erlenmeyer flask. After 5 min, 0.5 mL of N-1-napthylethlenediame dihydrochloride (NNED) was added. The samples were read using a spectrophotometer after 10 min at 543 nm, and were stable for 2 h. The detection limit was 2 μg L^{-1} NO_2-N.

2.413 Ammonium

Ammonium was determined spectrophotomically by the absorbancce of indophenol blue, as for Solórzano (1969). 1 mL of phenol reagent, 25-mL filtered sample, 1 mL

nitroprusside then 2.5 mL of oxidising reagent were added to a 100-mL Erlenmeyer flask, in a fume cupboard. The colour was read after 1 hour using a spectrophotometer set to 640 nm, and was stable for up to 24 h. The detection limit was 3 $\mu g\ L^{-1}$ NH_4-N.

2.42 Phosphorus

Analysis for phosphorus in freshwater was done broadly following the ascorbic acid method of Eisenreich *et al.*, (1975) after Murphey & Riley (1962). The phosphorus present forms a heteropoly acid-phosphomolybdic acid complex, which is reduced to form a deep blue colour. The colour is proportional to the phosphorus present and is measured spectrophotomically. Prior treatment by filtration and or acid digestion partitions phosphorus into fractions. The fractions are estimates of chemical species (Table 2.2).

The following fractions: filtrable reactive phosphorus (FRP), filtrable total phosphorus (FTP) and total phosphorus (TP) were determined directly. Filtrable organic phosphorus (FOP) and particulate phosphorus (PP) were calculated from these direct measures (Table 2.2).

Analysis was done on the day of collection and reagents were made up fresh on the day from stock solutions. The stock solutions were renewed every three months or if notable changes in solutions occurred (Table 2.3).

For the analysis of FTP and TP, 5 mL of digestion reagent were added to 25-mL samples (filtered or unfiltered) in 100-mL Erlenmeyer flasks. The flasks were covered with aluminium foil and autoclaved at 1 bar for 60 min. The flasks were cooled to room temperature and 5 mL of mixed reagent I added. The blue colour developed and was read after 20 min, on a spectrophotometer at 882 nm, using 5-cm cuvettes. The colour was stable for approximately 2 h. Samples with absorbance > 0.7, beyond the linear range of the spectrophotometer, were re-analysed after dilution with de-ionised water. Standards were

Table 2.2. Phosphorus fractions in common usage in freshwater studies.

Name	Acronym	Filtration	Digestion	P Species
Soluble reactive phosphorus	SRP	None	None	Orthophosphate and colloidal phosphorus
Filtrable reactive phosphorus	FRP	0.45 µm	None	Orthophosphate and colloidal phosphorus
Filtrable Total phosphorus	FTP	0.45 µm	Acid Persulfate	Orthophosphate, colloidal and organic phosphorus
Organic phosphorus	FOP	0.45 µm	Acid Persulphate	FTP – FRP
Particulate phosphorus	PP	None	Acid Persulphate	TP – FTP
Total phosphorus	TP	None	Acid Persulphate	All phosphorus species

Table 2.3. Stock solutions and reagents for the analysis of phosphorus fractions: FRP, FTP, TP.

Stock solution	Reagent
Digestion acid. 100 mL of H_2SO_4 mixed with 500 mL of distilled water, left to cool, then made up to 1000 mL. Final concentration 1.8 M. Stored in the dark at 4°C	Digestion reagent. 10 mL of Digestion acid was added to 50 mL of distilled water. 6g of $K_2S_2O_8$ (potassium persulfate) was dissolved and the solution diluted to 100 mL.
Antimony stock. 53.3 mL of H_2SO_4 mixed with 500 mL of distilled water and left to cool. Dissolve 0.748 g of K(SbO) $C_4H_4O_6$ ½ H2O (potassium antimonyl tartrate) and dilute to 1000 mL. Filter any precipitate that forms. Stored in the dark for up to 3 months at 4°C.	Mixed reagent I. 25 mL of Antimony stock was added to 25 mL Molybdate stock. 0.2 g of ascorbic acid was dissolved and the solution diluted to 100 mL.
Molybdate stock. Dissolve 10.839 g of Na_2MoO_4 $2H_2O$ in 500 mL of distilled water and dilute to 1000 mL. stored in the dark for up to 3 months at 4°C.	Mixed reagent II. 25 mL of Antimony stock, 10 mL of Digestion acid and 25 mL Molybdate stock were mixed. 0.2 g of ascorbic acid was dissolved and the solution diluted to 100 mL.

diluted from 1000 mg l^{-1} KH_2PO_4-P stock solution, supplied by BDH, to produce a standard curve. Blanks and standards were subject to the same treatment as the river water samples.

To determine FRP, 5 mL of mixed reagent II was added to 25-mL samples of filtered water, standards and blanks and measurement of colour was as above. Problems were occasionally encountered while attempting to measure FRP in peaty waters with high colour. In these circumstances, absorbance of acidified water (5 mL of 0.18 M H_2SO_4 was added to 25 ml of river water) at 882 nm was measured. If the absorbance of the acidified river water was greater than distilled water, that value was taken as the blank.

Detection limits were calculated as three times the standard deviation of the blank sample. For FRP (distilled water blanks only), FTP and TP this was 2 $\mu g\ L^{-1}$ PO_4-P using 5-cm cuvettes. The detection limit for all fractions was 2 $\mu g\ L^{-1}$.

From August 1995 to March 1996, at km -2.5, 10.9, 49.9, 107.9, 145.0 and -1.9, 81.9, replicate (n=3) samples for P analysis were collected at points ¼, ½, ¾ the width of the river from left to right facing upstream with a 30 s interval between samples. Details of mean, S.E. and C.V. are given in appendix I. Due to the low S.E. and C.V. at all sites for all fractions the sites were considered "well mixed" chemically, therefore, from March 1995 a single sample from midstream was analysed.

2.5 Biological samples

2.51 Bacterial counts

Bacterial numbers were estimated by epi-fluorescence microscopy. The reagents (Table 2.4) were preserved with fixative (final concentration 2.5 % w/v), stored at 4 °C in the dark and filter sterilised (0.2 μm) and brought to room temperature (22 °C) prior to use

(Kepner and Pratt 1994). All glassware, filter apparatus and pipette tips were autoclaved prior to use.

Water samples were collected in the field in 250-mL sterile glass medicine bottles facing into the flow of the river at about 1/3 depth. If analysis was not carried out on the day of collection then a 19-mL sub-sample was mixed with 1 mL of fixative and stored in a sterile 30-mL screw cap glass bottle at 4 °C in the dark. Analysis was done within 1 month of collection.

Filters were 25 mm diameter polycarbonate, 0.2 μm membranes obtained from Nucleopore. These were stained with irgalan black reagent for 24 h, rinsed with diluent and air dried prior to use. The filters were wetted with diluent before use and placed shiny side up in a sterile, glass, Millipore filter tower, mounted above a Buchner flask.

Table 2.4. Reagents used in the enumeration of bacteria by epi-fluorescence microscopy.

Reagent	Contents
Fixative 10% (w/v) P_i-buffered formalin	0.40 g NaH_2PO_4 1.23 g Na_2HPO_4 25 mL 40% Formalin 75 mL distilled water
Dispersant. 0.1 M tetrasodium PP_I	44.61 g $Na_4P_2O_7$ $10H_2O$ 1000 mL of distilled water
DAPI fluorochrome 100 μg mL^{-1}	10 mg 4', 6-diamidino-2-phenylindole (DAPI) 95 mL distilled water 5 mL 40 % Formalin
Surfactant 5 % Teepol	5 mL Teepol 90 mL distilled water 5 mL 40 % Formalin
Irgalan black solution 0.2 % irgalan black (w/v)	0.2 g of irgalan black 2.02 g of acetic acid 95 mL distilled water 5 mL of 40 % formalin
Diluent	500 mL filter sterilised (0.2 μm) and autoclaved Distilled water 25 mL 40 % Formalin

Sample and diluent were mixed in a sterile snap cap, the final volume of which was 9.9 mL, and mixed with a vortex stirrer. If the cells were found to aggregate and were not randomly distributed, then 1 mL of phosphate buffer was added and the sample stirred once more. 0.1 mL of DAPI fluorochrome was added and the snap cap sealed and placed in the dark for 10 min.

2 mL of surfactant was added to the filter tower and drawn through the membrane with a weak vacuum (< 100 mm Hg). After staining, the sample was placed in the filter tower and drawn through with a vacuum < 30 mm Hg so as not to disrupt the cells. If filtering took longer than 5 min, the sample was diluted. Just prior to the last of the sample passing through the filter, 1 mL of diluent was added to rinse away excess fluorochrome. A final 1 mL of diluent was added when the sample had passed, and the filter tower disassembled. The filter paper was placed sample side up on tissue paper to air dry for 5 min. A drop of immersion oil was placed on a clean slide, and the filter on top of this, sample side up. A second drop of oil was placed in the centre of the filter and a cover slip placed on top. The oil was spread evenly by light pressure on the cover slip with forceps. Slides were stored at 4 °C in the dark for up to two weeks with minimal loss of fluorescence.

Slides were observed using a Nikon fluorophot microscope incorporating a Nikon HB-202AN high-pressure mercury light source. The combination excitation filters were set to UV on a standard turret. An eye piece fitted with a 10x10 Whipple style graticule, was calibrated with a stage micrometer. The objective was a Ziess neofluar flat field x100 oil immersion lens.

Counts were made from randomly located fields of view, with the sample not observed between fields to avoid bias. The edges of the filter were ignored. A minimum of

400 cells were counted from at least 20 fields of view on duplicate slides giving a 95 % confidence interval of mean ± 10 % assuming Poisson's distribution.

2.52 Moss

Moss plants were collected from flowing parts of the river or stream, only submerged plants were taken. Plants exposed during low flows were ignored for routine analysis. Three handfuls of moss were taken on each sampling occasion, which provided between 50 and 100 fronds. Each fist-sized clump was washed thoroughly in flowing river water at the site for about 20 s. The three clumps were then placed in a sterile plastic bag and stored in a coolbox, on ice, for return to the laboratory.

Moss samples were analysed and prepared for long term storage on the day of collection. Plants were placed in a polypropylene tray and immersed in distilled water. Basal sections of the plants most contaminated with manganese oxide were removed with scissors, and the fronds replaced in the plastic bag for washing (see below). Tips were selected randomly for assays; the rest were put aside for nutrient analysis.

2.53 Treatment of epiphytes

Mosses form semi-permanent substrates within rivers and are colonised by epiphytes. The abundance of epiphytes in freshwater varies seasonally. Chapell & Goulder (1994c) found over one order of magnitude difference in epiphytic bacterial abundance between winter (January 1991; ~1 x 10^7 cells cm^{-2}) and early summer (May 1991; ~20 x 10^7 cells cm^{-2}) on *Phragmites australis* and *Elodea canadensis* from a gravel pit in N.E. England. Algal and bacterial epiphytes can express phosphatase activity (Burkholder & Wetzel, 1990; Chappell & Goulder 1994c), therefore, their contribution to the surface phosphatase activity of mosses needed to be assessed. Two options were considered: sampling mosses with intact epiflora

and relating epiphyte abundance to total phosphatase activity, or removal of epiphytes to assess the phosphatase activity of the moss alone.

Attempts to sample mosses with intact populations of epiphytes proved impossible, even slight disturbance of the plants dislodged notable quantities of loosely attached populations. In view of this inability to sample the intact community, it was decided to standardize the populations of epiphytes on moss samples by attempting to remove them. A number of methods have proven successful, including: brushing (Chappell & Goulder, 1994c), scraping (Burkholder & Wetzel, 1990), mechanical stomaching (Fry *et al.*, 1985) and agitation (Wehr *et al.*, 1983; Zimba *et al.*, 1997).

To remove all but the most stubborn adnate epiflora, a simple technique combining stomaching and agitation was employed. Terminal 2-cm tips were retained in a sterile plastic bag, de-ionised and UV-irradiated water added, and the bag shaken vigorously for approximately 2 min. The water was drained and replaced with fresh and the procedure repeated 4 or 5 times, however, the first wash removed the majority of epiphytes.

The influence of epiphytes on phosphatase activity was carried out using washed and unwashed mosses. Results are presented in table 2.5 from samples taken from km 10.9 and km 107.9 when summer epiflora was abundant, 8 July 1996.

Table 2.5 Comparison of PMEase activity before and after washing 2-cm tips of *F. antipyretica* and *R. riparioides* sampled on 8 July 1996. Values for moss expressed as μmol MU g d. wt^{-1} h^{-1}, supernatant normalized to biomass of moss utilized. n = 7 ± S.D.

Species	Unwashed		Washed		Supernatant		Washed + Supernatant
	mean	S.D.	mean	S.D.	mean	S.D.	
km 10.9							
F. antipyretica	44.9	7.6	39.0	6.7	3.22	0.99	42.2
R. riparioides	35.6	0.9	33.2	7.1	4.17	1.12	37.3
km 107.9							
F. antipyretica	8.73	1.84	6.40	1.2	1.52	0.30	7.92
R. riparioides	9.23	0.98	7.82	1.1	2.45	0.74	10.27

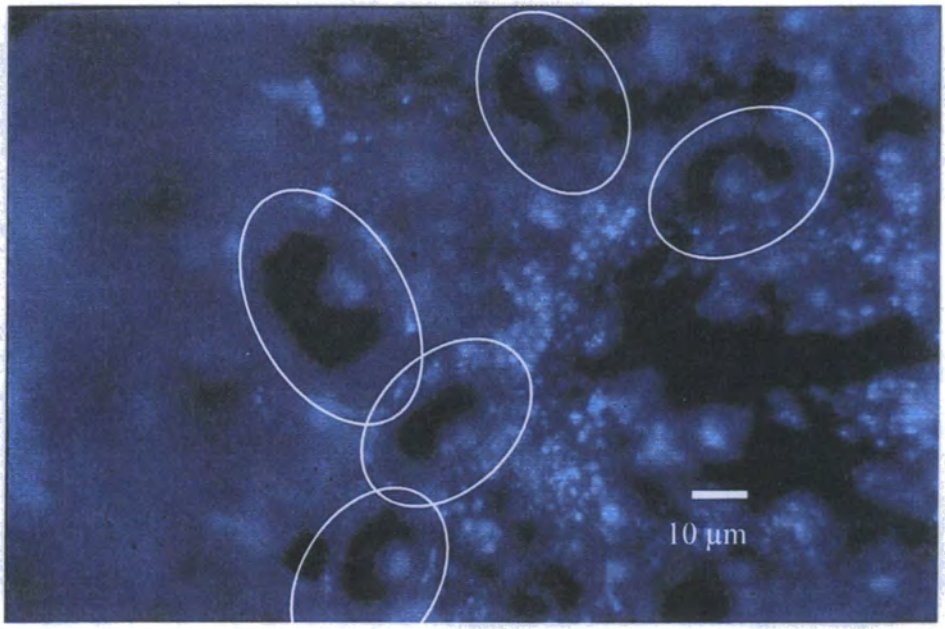

Figure 2.1 Fluorescence photomicrograph of the surface of a leaf of *F. antipyretica* taken from a mid-section (4 - 6 cm from tip) of a frond and stained with DAPI. Elipses mark the presence of individual *Cocconeis* sp., bacteria appear as light blue/white.

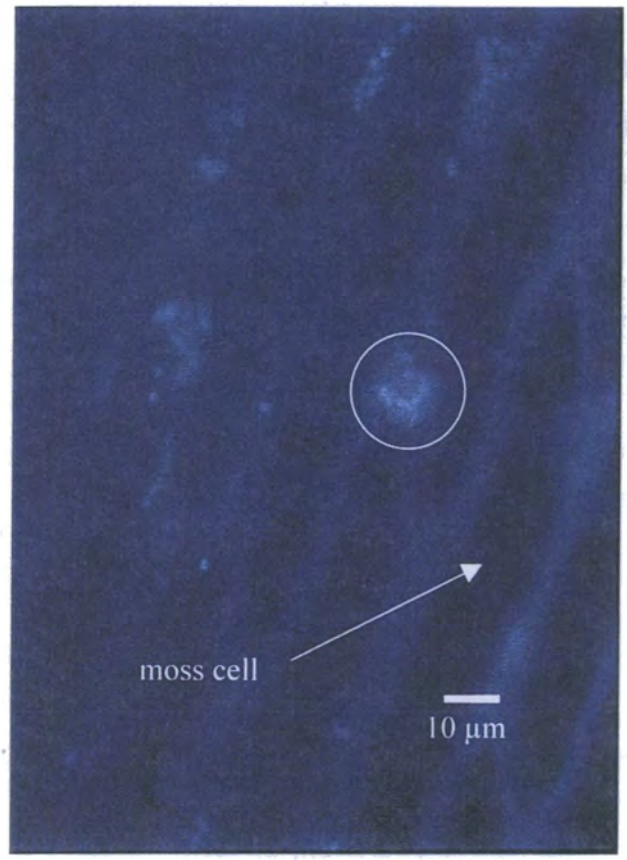

Figure 2.2 Fluorescence photomicrograph of the surface of a leaf of *F. antipyretica* taken from the tip (0 - 2 cm) of a frond and stained with DAPI. Bacteria are circled and appear light blue/white.

An initial wash reduced PMEase activity of both mosses by approximately 10 % at the two sites studied, further washing had no effect. Epifluorescence and light microscopy revealed that washing of terminal 2-cm tips removed approximately 75% of bacterial and 70 % of diatom flora. However, analysis of sections further from the tips found washing to be less efficient at removing epiphytes. Examples of unwashed leaves show (Figure 2.1, 2.2) show the difference in colonisation between basal and tip moss leaves. All studies were therefore made on terminal 2-cm tips, to reflect the properties of the moss itself and considered most likely to reflect the most recent ambient chemical conditions (Say & Whitton, 1983)

2.6 Nutrient analysis of plant material

Nutrient analysis of moss tips was carried out using oven dried, ground material. For mosses, 30 to 50, 2-cm tips were rinsed in distilled water and blotted dry on tissue paper. The paper was left overnight at room temperature to air dry. Air dried material was placed into aluminium foil envelopes and dried at 105 °C for 24 h. The envelopes were then placed in a dessicator and left to cool. Dried material was ground to a fine powder using a mortar and pestle, dried once more, stored in stoppered glass tubes and placed in a desiccator.

P content was determined by acid-persulfate digestion following the APHA method. C and N content was determined using a Carlo Erba CHN-OS elemental analyser. During all analyses with either method, unknown samples were run alongside known standards. These standards included: KH_2PO_4, Acetinilide and Sulphanalamide (Analar grade supplied by BDH and Microanalysis); and environmental standards of: chlorella, pond sediment, pepper bush leaf and tea leaf supplied by N.I.E.S.

2.7 Phosphatase activity

Material was assayed for phosphatase activity by modifying the method of Grainger *et al.* (1989). A range of biological material was analysed; both autotrophs and heterotrophs

from field material and axenic cultures. Minor modifications were necessary for the different types of material assay and the substrate used.

2.71 Assay medium

Assays were performed in an artificial freshwater medium, a modified version of the number 10 medium of Chu (1942). The modifications to the medium included; halving of the original EDTA concentration and removing inorganic phosphorus as they inhibit alkaline phosphatase activity. Combined nitrogen was also removed from the medium. All reference to assay medium refers to this medium. Salt and element concentrations are given in Table 2.6.

Table 2.6 Chemical and element concentrations in phosphatase assay medium.

Chemical	mg L^{-1}	µM	Element	mg L^{-1}	µM
$CaCl_2 + 2H_20$	35.83	243.7	Cl	20.43	576.3
$MgSO_4 + 7H_20$	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 + $2H_20$	1.67	4.20	Mg	2.46	101.4
$FeCl_3 + 6H_20$	1.21	4.50	K	2.24	57.3
H_3B0_3	0.715	11.56	Fe	0.250	4.50
$ZnSO_4 + 7H_20$	0.056	0.190	B	0.124	11.50
$MnCl_2 + 4H_20$	0.045	2.280	Zn	0.013	0.190
$NiSO_4 + 7H_20$	0.038	0.135	Mn	0.012	0.218
$CuSO_4 + 5H_20$	0.020	0.078	Ni	0.008	0.030
$COSO_4 + 7H_20$	0.010	0.035	Cu	0.005	0.078
$Na_2M00_4 + 2H_20$	0.007	0.028	Mo	0.003	().013
			Co	0.002	0.037

2.72 Substrates

Phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) were measured using analogue substrates supplied by Sigma Co., U.S.A. PMEase was measured using two substrates, methylumbelliferyl phosphate (MUP) and paranitrophenyl phosphate disodium salt (pNPP). PDEase was measured using bis-paranitrophenyl phosphate sodium salt (bis-pNPP). Substrates were filter sterilised (0.2 μm) prior to use, autoclaving hydrolysed both MUP and pNPP.

2.73 Buffer

All chemicals used in the buffer solutions were supplied by Sigma Co., U.S.A. Buffers were dissolved in assay medium and the pH adjusted with 5M NaOH, the buffer final concentration during the assay was 50mM. When not used immediately, buffers were kept in the dark at 4°C for up to 48 h, and were filter sterilised (0.2 μm) on all occasions before use.

Table 2.7 The pH range and pKa at 20°C of the buffers used in the phosphatase assay.

Buffer	pH range	pKa at 20°C
3, 3-Dimethyl-Glutaric acid (DMG)	5.0 - 6.5	3.66 and 6.20
(N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]) (HEPES)	7.0 - 8.0	7.5
Glycine	8.5 - 9.5	9.6

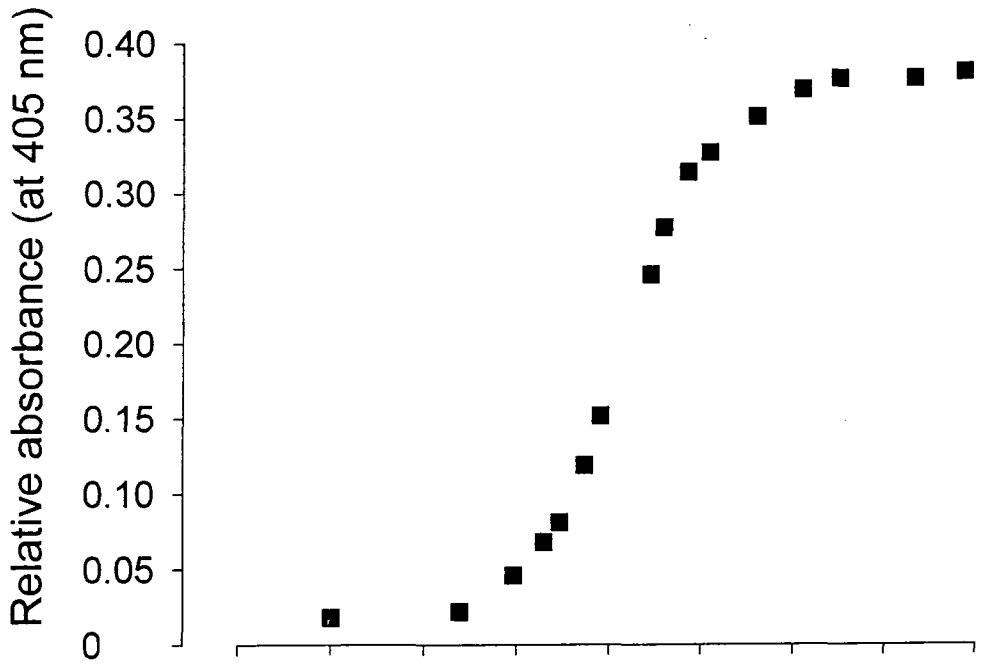

Figure 2.1 The influence of pH on the relative absorbance of pNP (final concentration 20 μmol) at 405 nm.

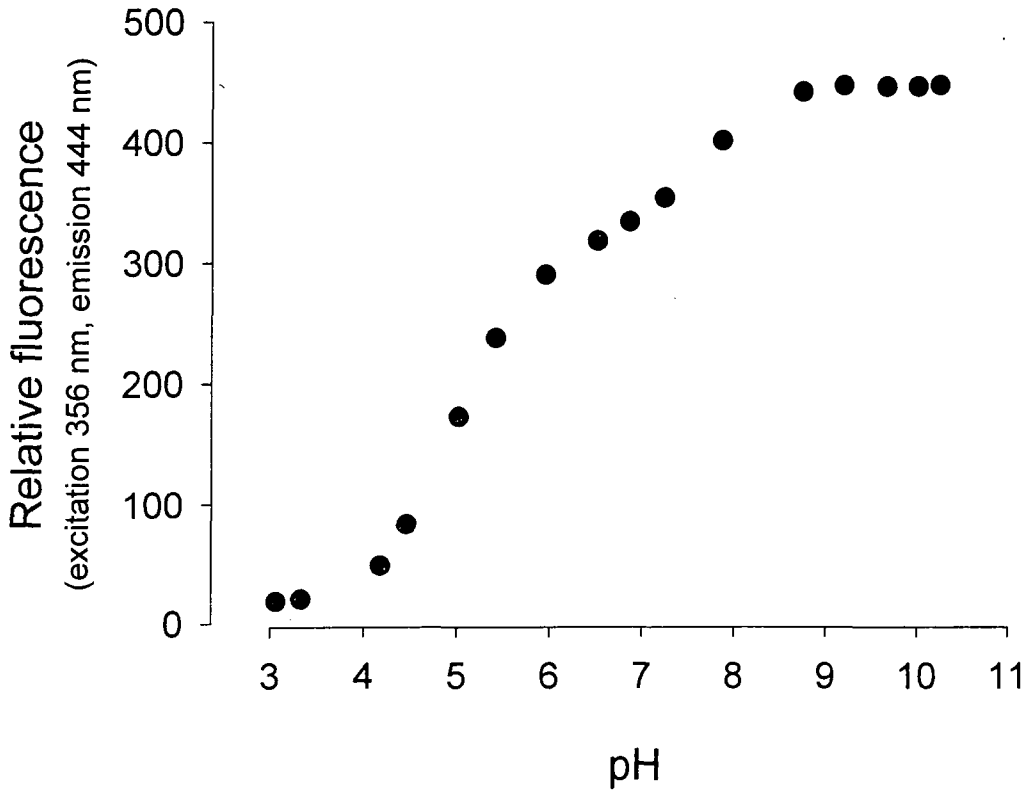

Figure 2.2 Influence of pH on the relative fluorescence of MU (final concentration 2 μmol); excitation 356 nm, emission 444 nm.

2.74 Terminator

Terminator was added to an aliquot from the phosphatase assay after an allotted time period for two reasons. Firstly, to halt the reaction by raising the pH of the assay media, lysing any enzyme present. Secondly, to increase the pH to facilitate maximum fluorescence or colour development from the substrates. Figure 2.3, 2.4 show the influence of pH on the relative absorbance and fluorescence of the substrate products, pNP and MU. Both products have low absorbance or fluorescence at pH < 4.0, but increase steeply between 6.0 and 8.0 to reach an asymptote when pH > 9.5. Reading the extinction coefficient at maximum deflection increases the sensitivity of the method and means the standard need only be read at values of > 9.5.

For assays using pNPP as a substrate, 0.25 mL of 5 M NaOH added to a 2.5 mL aliquot of assay medium was used to terminate the reaction. The pH was raised to > 12.5 and the solution was stable at room temperature for at least three hours. However, this was not the case with bis-pNPP and MUP, which were unstable at pH > 12.0. The fluorescence of MU decreased at a rate of 0.2 μmol h^{-1} at pH 11.6, whereas, the absorbance increased at 0.3 μmol h^{-1} when bis-pNPP was kept at pH 12.5. In view of the unstable nature of these substrates at high pH values, an alternative to the 5 M NaOH terminator was devised. It consisted of: 2.5 mM EDTA, 50 mM K_2HPO_4 and 50 mM NaOH. Both EDTA and K_2HPO_4 act as inhibitors of many phosphatases and 50 mM NaOH raised the buffer pH > 9.5, but not to levels where auto-hydrolysis would occur.

2.75 Moss Phosphatase

2.751 MUP method

Each assay used two 2-cm tips, with three replicates for each measurement. The tips were assayed in sterile snap-cap vials containing 5.9 mL buffered, 0.2 µm filter-sterilised medium (modification of No. 10 medium of Chu, 1942, without combined N or P: Grainger *et al.*, 1989).

Three different buffers (all 50 mM final concentration) were used to test for the optimum pH: pH 5.5, dimethylglutaric acid; 7.5, HEPES; 9.5, glycine; pH adjustments were made with 5 M NaOH. The vials were placed in a shaking water bath at 25 °C and left for 20 min to equilibrate. The assays were carried out using 0.1 mL 4-methylumbelliferyl phosphate (4-MUP) to give a final concentration of 100 µM. The assay was terminated after 20 min by transferring a 5-mL aliquot to 0.5 mL of terminator in a cuvette. 20 min was found to be short enough to obtain fluorescence without the need for dilution, and long enough to avoid problems of substrate retention (Hernández & Whitton, 1996). No significant difference was found between assays under high light (100 µmol PAR m^2 s^{-1}) and darkness, therefore, assays were conducted under laboratory light conditions (30 – 60 µmol PAR m^2 s^{-1})

2.752 pNPP and bis-pNPP

The method using colourimetric substrates was identical to the MUP method with the following exception, 2.9 mL instead of 5.9 mL final assay volume, the larger volume required to fill the fluorometer cuvettes was unnecessary for spectrophotometer cuvettes.

2.76 Water phosphatase

Assays for water phosphatase were primarily conducted using the sensitive fluorometric substrate MUP although pNPP and bis-pNPP were also used. The water was vacuum filtered (< 10 cm Hg) to give two fractions: filtered and particulate. Filtration was carried out using a sterile Whatman filter tower housing 0.2-μm polycarbonate membranes. The PMEase activity of filtered and total water was determined separately using four replicate sub-samples. The particulate fraction was calculated as the difference between mean total and filtered samples. Blanks were sterilised river water taken from each site. Samples were sterilised initially by autoclaving at 1.0 bar for 15 min. However, boiling the river water for 15 min was as effective and more rapid.

2.9 mL of sample were added to 3.0 mL of filter sterilised buffer (see above) in a snap cap vial. The vials were placed in a shaking water bath at 25 °C and left for 20 min for the temperature to stabilise. 100 μL of substrate added and samples incubated for 2 h. The water bath was covered to maintain a stable temperature. The reaction was halted with the appropriate terminator (see above) and the absorbance or fluorescence recorded immediately.

2.8 Phosphorus utilisation

Prior to the assay, mosses were washed as for the phosphatase assay, then rinsed with distilled water and blotted on tissue paper. Substrate was added (final concentration of 1.0 mg L^{-1} P) to three replicate vials containing 10 ml of buffered (pH 5.5) medium. Six tips were placed into each vial and incubated at 25 ° C for approximately 18 h. 10-ml aliquots were then removed from the vials and analysed for P.

Three substrates were tested on this occasion: inorganic phosphate supplied as KH_2PO_4, DNA (degraded free acid type IV obtained from herring sperm) and phytic acid (inositol hexaphosphoric acid type V sodium salt obtained from maize).

Both organic P substrates were obtained from Sigma Chemical Co. Although 1.0 mg L^{-1} P is far above the typical level found in the stream, it corresponds to the uppermost concentration recorded during a study of streams in an adjacent catchment (Livingstone & Whitton, 1984).

2.9 Computing and statistics

Pentium computers with the Windows 95 operating system were used throughout the study. The most widely used applications were those within the Microsoft Office 97 suite of programs. Word processing was done using Word 97 and the bulk of the data handling with Excel 97. Graphs were produced using Sigmaplot for Windows v3.0. Linear and non-linear regression equations and best-fit lines were calculated with Sigmaplot. Statistical analysis of data was carried out using Minitab for windows (release n.11).

Access 97 was used for long term storage of data, the Swale – Ouse relational database (SWORD97) held all data from the University of Durham's involvement with LOIS.

UNIX machines were used to access the LOIS database at the Institute of Hydrology, Wallingford. Data supplied by the Environment Agency and the LOIS core chemistry program was accessed and downloaded using the Institute of Hydrology's WIS software.

CHAPTER 3

Swale-Ouse study area

3.1 Introduction

The biology and chemistry of a river system are dependent on a number of geographic factors, both natural and anthropogenic, which have varying degrees of influence. This chapter reviews the factors likely to influence the chemistry and biology of the Swale – Ouse. Detailed descriptions and photographs of sites visited as part of two seasonal studies are included.

3.2 The Swale - Ouse catchment

3.21 The river system

The Swale rises in the Pennines in North Yorkshire and flows east towards the Vale of York (Figure 3.1). The upper part of the catchment includes the Mallerstang and Swaledale Head, Site(s) of Special Scientific Interest (SSSI). It is one of approximately 50 SSSI and two Area(s) of Outstanding Natural Beauty (AONB), in the Swale, Ure, Ouse catchment, which covers 3200 km^2. The upper Swale cuts through beds of limestone and descends in a series of steps leading to dramatic staircases of waterfalls. The gradient of the river is steeper than other neighbouring catchments, Figure 3.2 shows the river profile of the Swale - Ouse. The gradient and high rainfall cause the river to be exceptionally "spatey" (Environment Agency, 1997). Annual rainfall in the upper catchment is approximately 2000 mm a^{-1} compared to 600 mm a^{-1} at Selby. A more detailed description of the geography of the study area is provided by Jarvie *et al.* (1997) and Law *et al.* (1997).

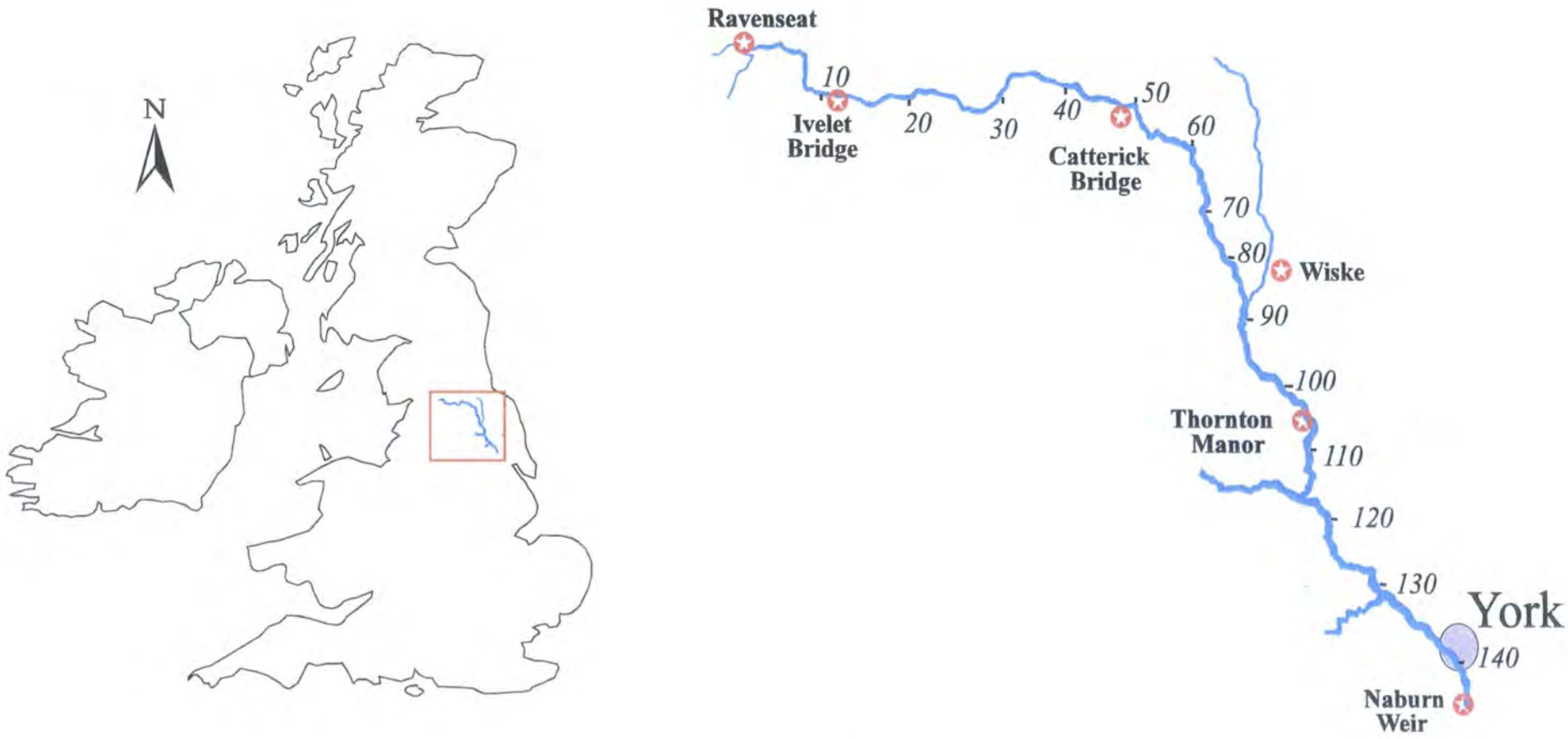

Figure 3.1 Location of the Swale - Ouse river system within the U.K., distance (km) from the source is shown.

The major sampling points used by LOIS and this study.

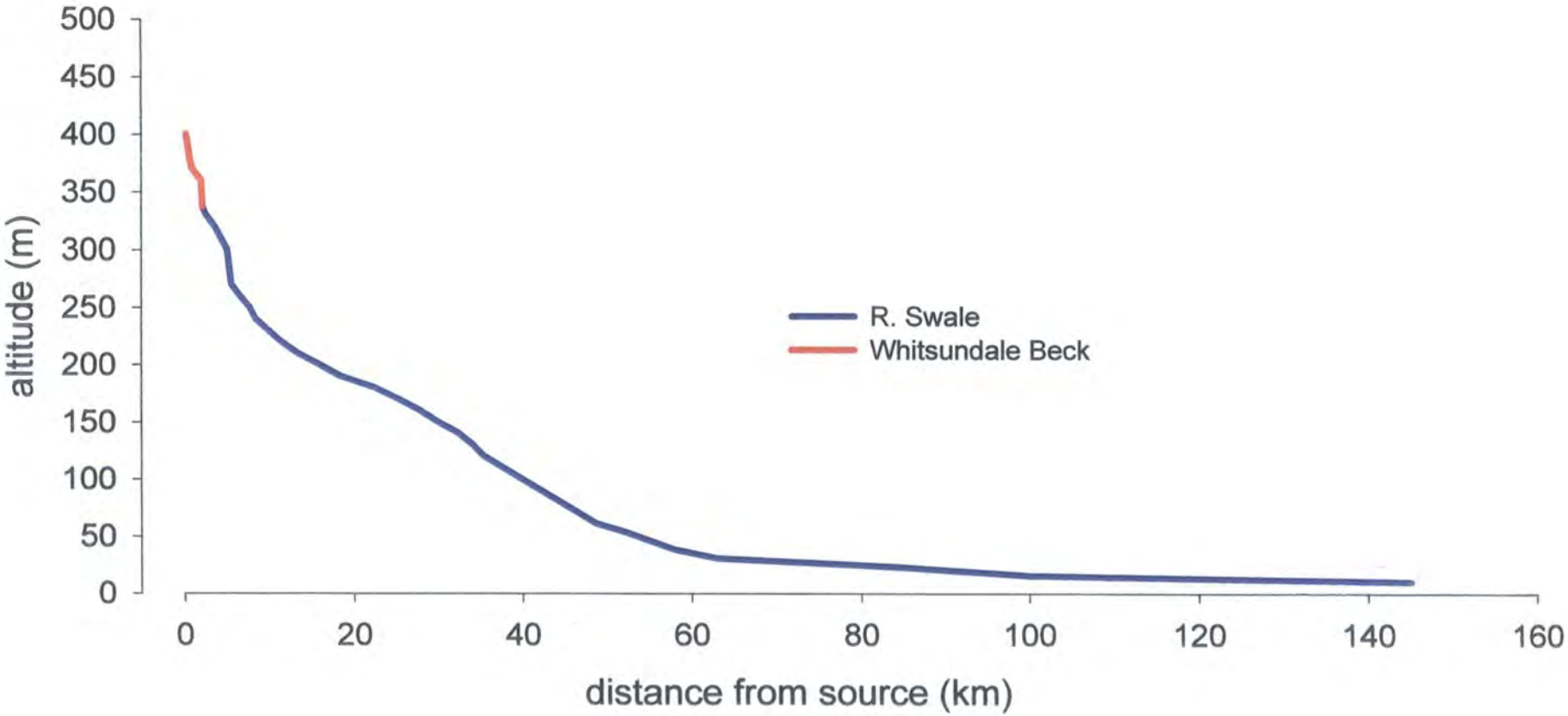

Figure 3.2 Profile of the Swale and its upstream tributary Whitsundale Beck

The upper Swale supports populations of native brown trout and grayling. Below Richmond, coarse fish occur in large numbers and include barbel, roach, chub, and species introduced by angling clubs, including common bream (Environment Agency, 1997).

A number of major tributaries join the Swale, Arkle beck at km 23.5, Bedale Beck at km 71.6, R. Wiske at km 86.1 and Cod beck at km 100.3. At the confluence with the Ure, approximately 2 km downstream of Boroughbridge, both rivers form the Ouse. The Humber is formed at the confluence of the Ouse and R. Trent (Jarvie *et al.*, 1997). It forms the largest contribution of freshwater to the North Sea, and drains over one-fifth of the land area of England (24 000 km^2).

3.22 Geology

The upland regions of western Swaledale have abundant outcroppings of rock, whereas, further east toward the Vale of York, much of the solid geology is covered by drift deposits from the last ice age. In general, the rocks are not ancient, the oldest being lower carboniferous (350 ma). The regional dip is to the east, courtesy of the great Pennine anticline and the rocks therefore become younger in an easterly direction (Figure 3.3).

Upper Swaledale has outcroppings of Millstone Grit and Limestone of Upper and Lower Carboniferous age. The oldest rocks are limestones of the Lower Carboniferous, the Yoredale and Scar limestone series. These were formed in a quiet marine setting with abundant corals, suggesting a warm tropical climate. The massive limestone beds were significantly folded and faulted and the fissures created provided pathways for hydrothermal fluids which deposited metalliferous minerals (Pb and Zn), which have been of commercial interest since Roman times. Mining was carried out on a large scale in the last century. and Whitaker (1823) describes the process of "hushing" the overburden and ore as, detrimental to the spawn (of fish) by impregnating the water with filth, poisonous minerals and particles of lead. For many miles in its descent so pollutes and discolours the river. Lead and zinc are

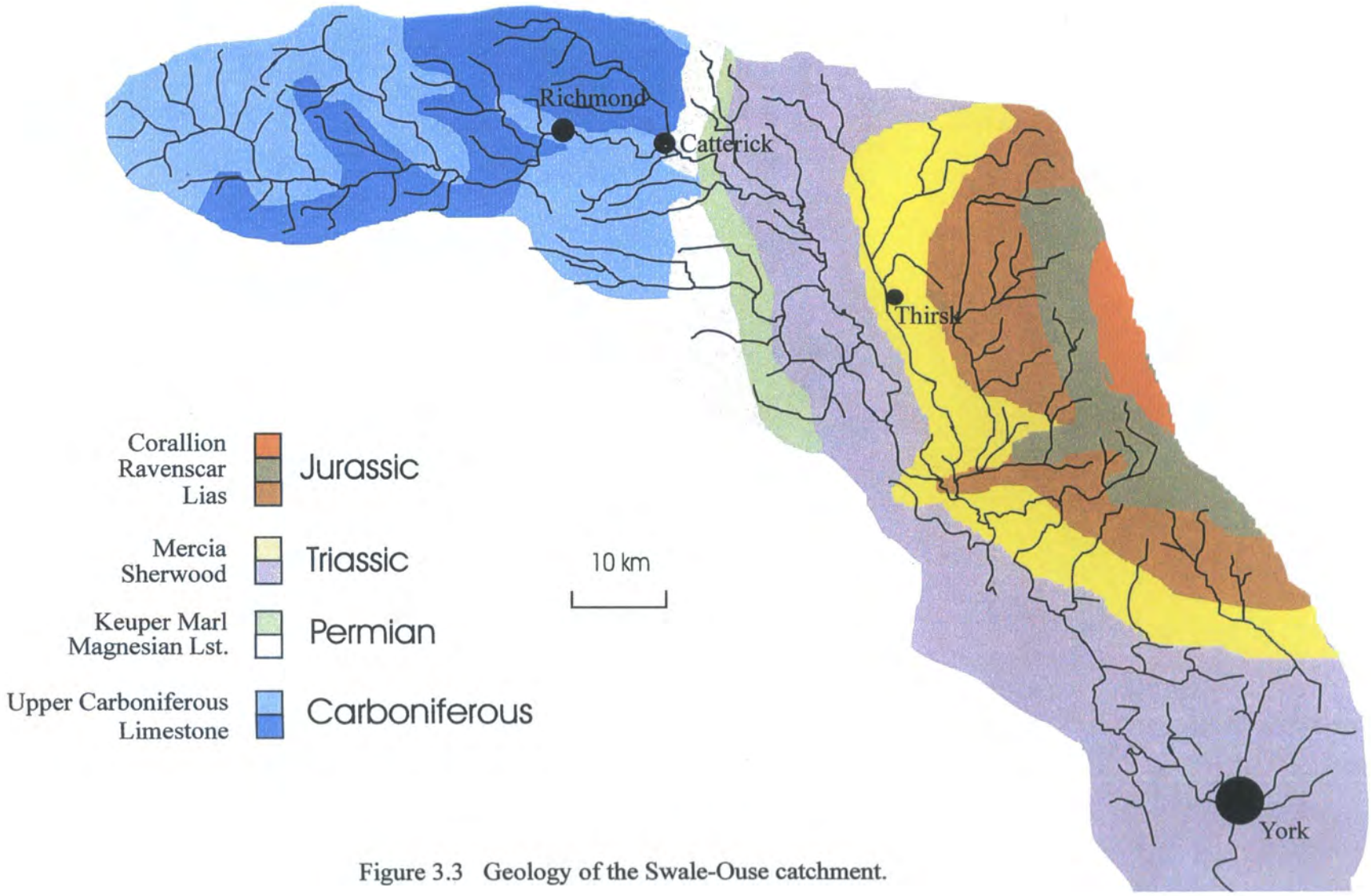

Figure 3.3 Geology of the Swale-Ouse catchment.

still present in the water and sediments from the disused mines and contaminated alluvium within the river catchments (Macklin *et al.*, 1997; Neal *et al.*, 1997).

Overlying the limestone is the Upper Carboniferous, predominantly Millstone Grit, a coarse, quartz grained sandstone, the felspathic matrix and current bedding suggest rapid deposition, probably during the formation of a large delta. Millstone Grit has been quarried extensively throughout Swaledale for building materials as well as millwheels. The Upper Carboniferous was named because of the coal measures, although outcrops of coal are present in Swaledale, they are rare compared with South Yorkshire. Tan Hill public house (Figure 3.4) was once the site of an adit coal mine, other seams have been worked at Downhlome and Hudswell (Baker, 1906). The river passes through Carboniferous rocks until Catterick where the Permo-triassic strata is exposed.

The Permo-Trias uncomformably overlies the Carboniferous (Dakyns, 1891), and consists of Magnesian Limestone, Keuper Marl (calcareous mudstones), the Bunter Sandstone and Mercia and Sherwood mudstones. The Magnesian Limestone is marine in origin, whereas, the sandstones and mudstones overlying them are of mixed terrestrial and lacusturine environments. The Bunter sandstones are composed of frosted grains and contain wind shaped stones (driekanters), indicative of aeolian deposition. The mudstones and marls include salt psuedomorphs and veins of gypsum, indicative of a frequently evaporating salt lake, both the gypsum and sandstone have been of commercial value in the past. Present day quarrying is limited to small-scale extraction of sand from the Bunter Sandstone near Ainderby Steeple, but gravel, sand and clay were formerly taken from many sites in the district, and gypsum was once mined at Brompton (Allison & Hartnup, 1981). The Rhaetic beds are present throughout Yorkshire (Baker, 1906), these thinly bedded shales and sandstones mark the transition between the Triassic and Jurassic.

The Lower Jurassic or Lias is composed of shales and mudstones, the best exposures of which are found on the Yorkshire coast at Whitby. The Lias mudstones are of marine origin and the stratigraphy sub-divided by characteristic ammonite fauna. The Ravenscar and Corallion group are a mixture of sandstones, shales, ironstones and oolitic limestones. These are characteristic rocks of the North York Moors and as such influence the eastern most tributaries rather than the Swale or Ouse.

3.23 Soils and land use

The soils vary greatly over the region through which the Swale passes and are influenced by altitude, relief, solid and drift geology, farming practices and flooding. In upland areas, the Millstone Grit is generally overlain by thick beds of Winter Hill peat (Figure 3.4). Nearly all land above 300 m, except for the steeper slopes, is covered by hill peat formed over the last 7000 years (Carroll, 1974) or soils derived from peat. However, toward the Vale of York peat deposits are rare. Bradley & Allison (1985) found very few deposits, these were locally confined to hollows to the south and west of the Topcliffe to Brafferton ridge.

From the Pennine foothills eastward, the soils are influenced by the Swale and its feeder streams as well as the glacial drift geology. The soils overlying the Triassic rocks consist of fine loamy stagnogleyic rankers, whereas, in the vicinity of the river, the "Swale Series" are typical sandy loam alluvium deposits, rich in calcium carbonate with a high pH (Allison & Hartnup, 1981). Brown rendzinas, in part, overly the Jurassic limestones as well as loams with limestone fragments known as the Wetherby Series (Carroll *et al.*, 1979)

Agricultural land use tends to reflect the soil type. In the upland part of the catchment, undrained peat soils are given over to rough grazing. Drained loam and gley soils in the valley bottom provide improved grazing for both cattle and sheep. The traditional hay meadows are protected by SSSI and Environmentally Sensitive Area (ESA) status (Figure

3.5). Arable farming prospers in the Vale of York on well drained loams and river alluvium, however, wherever this soil is only thin over the underlying clays, grass is more common (Bradley & Hartnup, 1985)

3.24 Water quality

The Environment Agency (formerly the National Rivers Authority) monitors the chemical and biological water quality of rivers in England and Wales. The chemical general quality assessment (GQA) of water is divided into 6 classes (A to F) based on DO, BOD, and ammonia, which primarily to reflect organic inputs. Table 3.1 shows concentration of determinants which defines the chemical class.

Table 3.1 GQA chemical classification used by the Environment Agency based on the determinants: DO, BOD, ammonium-N.

GQA		DO % saturation 10 percentile	BOD mg L^{-1} 90 percentile	ammonium-N mg L^{-1} 90 percentile
A	very good	80	2.5	0.25
B	good	70	4	0.6
C	fairly good	60	6	1.3
D	fair	50	8	2.5
E	poor	20	15	9.0
F	bad	< 20	> 15	> 9.0

Biological assessment of water quality is carried out using a macro-invertebrate methodology developed by the Biological Monitoring Working Party. The method was designed to detect organic pollution, which reduces of dissolved oxygen because of increased BOD. The quality of the water is graded from A (very good) to F (very poor) based on how near the observed fauna is to a predicted fauna with no polluting influences. The prediction is made by the software package RIVPACS (Wright, 1995; Wright *et al.*, 1996) using a range of environmental data collected at each site.

The biological and chemical quality of the Swale, is in general very good, an example of data from 1995 (courtesy of the Environment Agency) showed that from Keld (km 5.1) to Thornton Manor (km 107.9) was classed A. However, some upland tributaries to the Swale; Whitsundale, Birkdale, Arkle and Marske Becks, have impoverished invertebrate fauna attributed to low pH (NRA, 1996), although the influence of metals cannot be ruled out.

The chemical and biological assessment of R. Wiske were class C, caused by diffuse agricultural sources and effluent from Northalleron and Romby sewage treatment works (Environment Agency, 1997). This problem was noted in the National Rivers Authority catchment management plan (NRA, 1996), and measures for improvement are still ongoing. The chemical class of R.Ouse at Acaster Malbis was C, influences of York and Naburn STW contributing to this classification. The biological classification was E, a reduction of invertebrate habitat as well as chemical pollutants being responsible. Further aspects of the biology of the Swale are provided by Whitton & Lucas (1997).

Table 3.2 Biological and chemical general quality assessment (GQA) grade from sites within the Swale - Ouse catchment, 1995. Data provided by the Environment Agency.

Site	Distance from source	biological GQA	chemical GQA
R. Swale at Keld	km 5.0	A	A
R. Swale at Hudswell	km 38.4	A	A
R. Swale at Catterick	km 49.9	A	A
R. Wiske at Kirby Wiske	km -2.5, 86.1	C	C
R. Swale at Thornton Manor	km 107.9	A	A
R. Ouse at Acaster Malbis	km 145.0	E	C

3.25 Nutrients

Nutrients are input by both diffuse agricultural and point source pathways. Agricultural inputs include fertillizer additions and spills of slurry or leaching from silage (Robson & Neal, 1997). STW and faulty CSO are major point sources of effluents rich in nutrients. The Swale is a pristine river and nutrient loads are similar to those other non-urban

Yorkshire rivers, Wharf and Ure (House *et al.*, 1997). However, the influence of inputs from York increase exports from the Swale - Ouse, although nutrient loads (5 t a^{-1} NO_3-N; 189 t a^{-1} PO_4-P) are only moderate when compared to other Humber feeder rivers, such as the Trent, Aire and Don (Robson & Neal, 1997).

The first major STW is located at Colburn, near Catterick, and studies by Jarvie *et al.* (1998) showed that nitrate concentration of individual samples from R. Swale at Catterick, exceeded recommended limits of 11.3 mg L^{-1} N (DOE, 1993). The importance of point sources and agricultural inputs of nutrients to the Swale from tributaries was made clear in the results from the LOIS core chemistry programme. Studies at Crakehill (km 103) found that although the majority (85%) of the water originated from the main river the majority of nitrate (74%) and inorganic P (78%) originates from the tributaries including R. Wiske and Cod Beck (House *et al.*, 1997).

Fractionation of the nutrients allows inferences about sources and processes within rivers to be made. A high concentration of nitrite and ammonium as a percentage of TIN are characteristic of sewage effluent (Royal Society, 1983), typical of urban rivers such as the Aire, Don and Calder. Nitrate formed the majority of TIN in the Swale – Ouse, which is consistent with diffuse agricultural inputs (Jarvie *et al.*, 1998). High winter nitrate concentrations in the main river and R. Wiske measured over an annual period (Pattinson *et al.*, 1998), are consistent with winter storm run-off from agricultural sources.

Flow appears to be a factor determining the concentration of P, not only by diluting autochthonous fractions, but also by introducing allochthonous fractions and influencing river processes controlling the availability of P. P fractions in the Swale measured over an annual period at km 49.9 and km 103.0, suggest that much of the P transported in the main river under high flow was particulate (House *et al.*, 1997). The uptake of soluble P by suspended sediments was also important in storm events (House & Warwick, 1998b). However, under

low to moderate flows FRP was the dominant fraction. Indeed, intensive measurements of tributaries and main river sites showed that under low flows, point sources on the tributaries and the main river were particularly important sources of dissolved P (House & Warwick, 1998a).

3.3 Study sites

Study sites were chosen for comparison with LOIS sampling programme and other studies at Durham (see Leeks *et al.*, 1997; Pattinson *et al.*, 1998). Three main river sites: R. Swale at Catterick Bridge, km 49.9 (Figure 3.10); R. Swale at Thornton Manor, km 107.9 (Figure 3.11); R. Ouse at Naburn Weir, km 145.0 (Figure 3.12), and R. Wiske at Castle Farm, km -1.9, 86.1 (Figure 3.13), overlapped with the LOIS sampling programme. Whitsundale beck at Ravenseat, km -2.5 (Figure 3.6) and R. Swale at Ivelet Bridge, km 10.9 (Figure 3.9), were studied to provide data upstream of the STW at Catterick. These six sites (Table 3.3) formed the basis of the water chemistry sampling programme (chapter 4), and two seasonal studies of phosphatase activity (chapter 6 & 9). Stonesdale Beck, km -3.1, 3.5 (Figure 3.7) and R. Swale at Wainwath km 3.1 (Figure 3.8) were visited as part of studies described in chapters 7 and 8.

Table 3.3 Descriptive characteristics of the study sites in the Swale-Ouse system. Distance from the source shown in km (negative numbers indicate distance up a tributary, from the confluence with the main river).

Site name	distance (km)	grid ref.	river	altitude (m)	width (m)	description
Ravenseat	-2.5	NY863032	Whitsundale Beck	400	5	Boulders, cobbles, some sand, no trees. Bryophyte coverage 1 %.
Ivelet Bridge	10.9	SD933977	Swale	230	10	Bedrock, boulders, cobbles, interstitial sand + banks, marginal shade. Bryophyte coverage 10 %, particularly abundant on bedrock.
Catterick Bridge	49.9	SE227994	Swale	180	20	Boulders, cobbles, sand bar, little marginal shade. Bryophyte coverage < 2 %.
Thornton Manor	107.9	SE433715	Swale	5	15	Boulders, cobbles, sand bank. Bryophyte coverage 5 %, very abundant > 90 % on boulders under bridge. Marginal macrophytes present.
Naburn Weir	145.0	SE594445	Ouse	5	30	Sand banks, > wading depth. Bryophytes on weir but unable to sample. Marginal macrophytes present.
Castle Farm	86.1, -1.7	SE376847	Wiske	5	4	Sand, silt, clay, marginal shade Bryophytes virtually absent, abundant marginal macrophytes

Figure 3.4 Tan hill public house at the head of Stonesdale with the surrounding Winter Hill peat deposits (August 1997)

Figure 3.5 The valley of Swaledale at approximately km 15.0 (June 1996).

Figure 3.6 Whitsundale Beck at Ravenseat, km -2.5 (June 1995)

Figure 3.7 Stonesdale Beck at km -3.0, 3.7 (August 1997)

Figure 3.8 R. Swale at Wainwath falls, km 3.4 (August 1997)

Figure 3.9 R. Swale at Ivelet Bridge, km 10.9 (June 1996)

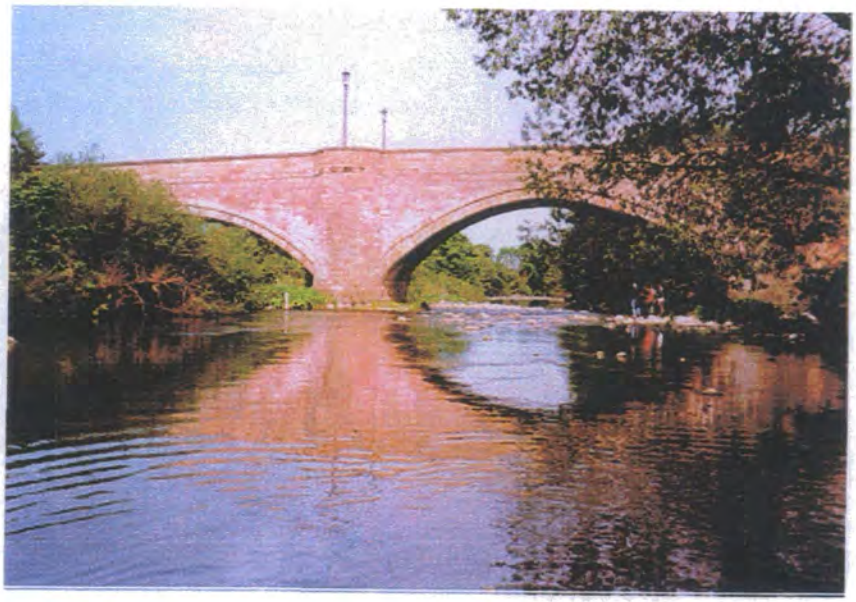

Figure 3.10 R.Swale at Catterick Bridge, km 49.9 (June 1996)

Figure 3.11 R. Swale at Thornton Manor, km 107.9 (July 1997)

Figure 3.12 R.Ouse at Naburn Weir, km 145.0 (June 1996)

Figure 3.13 R. Wiske at Castle Farm, Kirby Wiske, km -1.9, 86.1 (January 1996)

CHAPTER 4

Physical and chemical characteristics of the Swale - Ouse

4.1 Introduction

The LOIS chemistry sampling programme provided background information for the LOIS special topics (1.2), and data to test models of river processes. Sampling at three sites on the Swale - Ouse began in 1994, and continued for approximately three years. However, several of the special topics, this one included, did not begin until 1995. This lag meant that analysis carried out by LOIS would not be available throughout the course of this project. This, and the fact that LOIS did not study sites upstream of the first major STW on the Swale, meant that additional sites further upstream were studied during this project. In the middle and lower reaches of the river, at overlapping sites, a comparison of data was made.

The aim of this Chapter is to provide the environmental characteristics of the river system during two studies of phoshatase activity (Chapters 6, 9). The data presented is that collected during this study, with the exception of flow. LOIS data is included in part for comparison with the results of this work.

4.2 Sites and sampling programme

Water samples were collected and physical measures were taken monthly as part of spatial and seasonal phosphatase studies of the river. Spot samples were taken where possible on intervening days as part of other work. The data cover two studies, moss phosphatase activity from October 1995 to October 1996, and phosphatase activity of water from July 1996 to June 1997. It was unfortunate that commitments assisting other LOIS projects, combined with high flows, meant that a period of 46 days elapsed from the sampling event of 19 January 1996. Therefore, although 12 samples were taken to provide an annual

data set, no sample was taken in February 1996. The implications of the inability to collect samples under the most extreme flow events is discussed later (Chapter 10). There was an overlap period of three months from July to September 1996 when both phosphatase surveys were in operation, therefore, the total sampling period lasted 21 months.

Four sites were surveyed on the main Swale-Ouse river system. Their distances from the source of the river were km 10.9, km 49.9, km 107.9, km 145.0. Another site was located on an upland tributary, 2.5 km upstream from the confluence with the main river, near the source of the Swale. A further site on a lowland tributary, which joined the main river at km 86.1, just over halfway between source and tidal limit, was also studied.

4.3 Variables

4.31 Flow

Flow increased on passing downstream on all sampling occasions, and baseline levels were generally two orders of magnitude greater at the tidal limit of the river km 145.0, compared with the most upstream site, km -2.5. The longest period of low flow was from May to August 1996. Flow increased during storm events, which generally occurred during winter (Figure 4.1). Flows were generally higher at all sites during the water phosphatase survey (July 1996 to June 1997), compared to the moss survey (October 1995 to October 1996). The flood event of greatest magnitude, which was evident at all sites, was a winter storm in February/March 1997. The highest summer flows were recorded in the last week of June 1997, at the conclusion of the water phosphatase study.

4.32 Water temperature

Water temperature showed clear seasonal trends (Figure 4.2) at all sites, with winter minima and summer maxima. In general, sites at higher elevations (> 300 m asl), km -2.5 and km 10.9, had lower temperatures than lowland sites throughout the year, and the winter

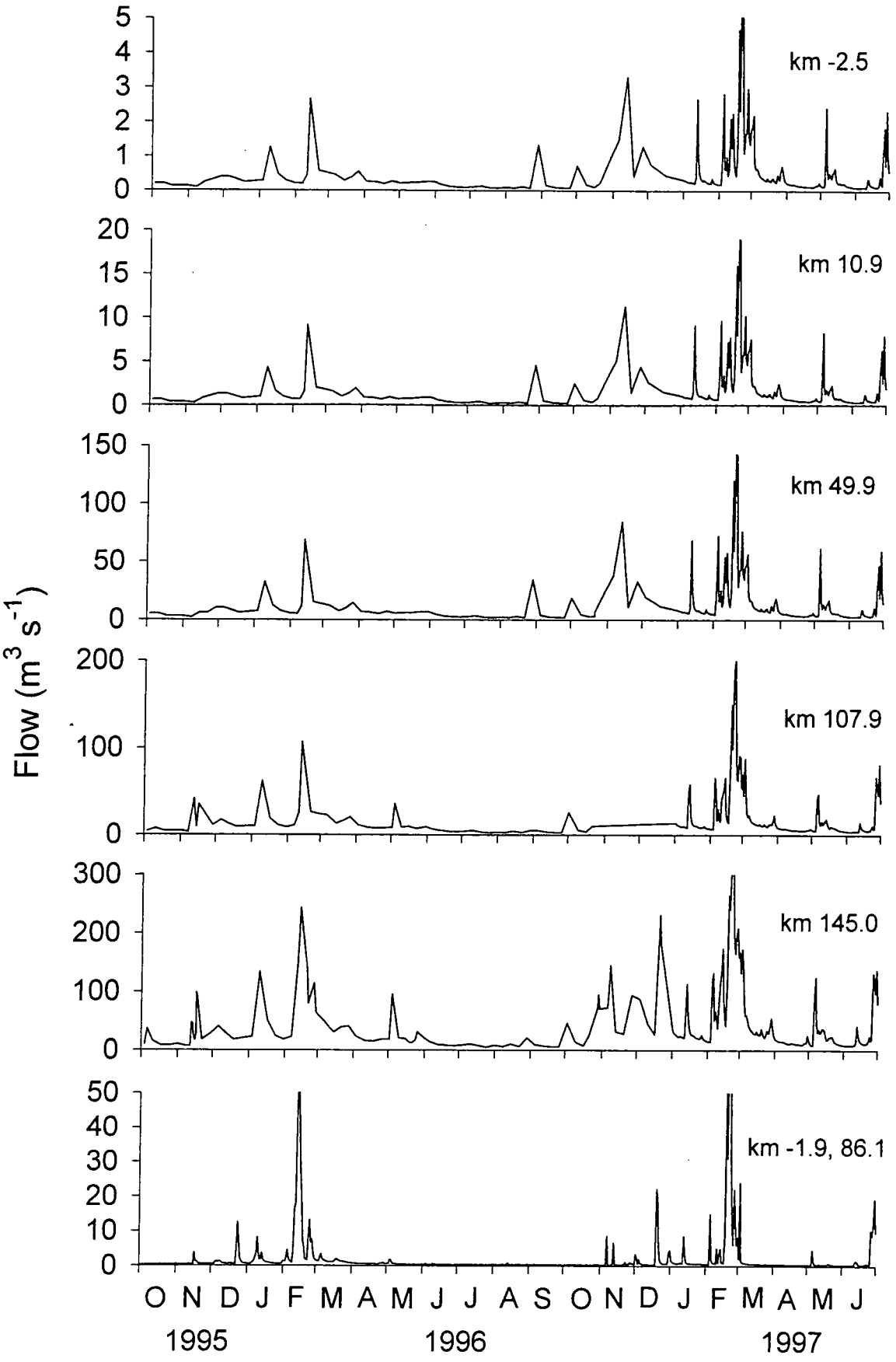

Figure 4.1 Flow at six sites within the Swale-Ouse catchment during two seasonal studies of phosphatase activity from October 1995 to July 1997.

N.B. see 2.2 for details of flow calculation

minima was lower than sites at lower altitudes. Indeed, surface freezing (< 1 cm thick) was evident in the quiescent water at km -2.5 (26 November 1996), when the mid-stream flowing water was 0.2 °C, the lowest temperature recorded. Surface freezing was not seen at any other site, the lowest value recorded was 2.4 °C at km 49.9, 21 January 1997. The highest temperature during the two studies was 24.5 °C at km 107.9, 20 August 1996, after approximately three months of baseline flow (see above). As well as higher maximum values, km 49.9, km 107.9 and km 145.0 had longer periods of warmer water than km -2.5 and km 10.9. Temperature at km 49.9, km 107.9 and km 145.0 was greater than 10 °C on more than half the sampling occasions. In contrast, at km -2.5 temperature was greater than 10 °C on only one third of sampling occasions.

4.33 pH

The pH generally increased with distance downstream. Sites km -2.5 and km10.9 had clear seasonal trends with lower pH in winter compared with summer (Figure 4.3). The remaining sites appeared either not to show this trend (e.g. R. Wiske), or show it less clearly than the upstream sites. However, all the main river sites had the lowest pH recorded in the winter of 1996/7. The lowest recorded pH at km –2.5 site was 4.58, compared to 6.79 at the tidal limits of the river, km 145.0, the only occasion that pH < 7.00 at km 145.0. In general, km 49.9, km 107.9, km 145.0, km -1.9, 86.1 rarely had pH < 7.0. The highest pH was 8.64 at km 49.9, 8 July 1996.

4.34 Oxygen

Dissolved oxygen concentration (DO) decreases below saturation (100 %) due to respiration, often resulting from organic inputs. Dissolved oxygen concentration was always > 100 % at km -2.5, km 10.9 and km 49.9 (Table 4.1 and 4.2), during both surveys. DO was

Table 4.1. Mean conductivity, total alkalinity and dissolved oxygen concentrations from six sites in the Swale-Ouse catchment. Data from October 1995 to October 1996. Min. and max. indicate the range of the data.

Site	conductivity $\mu S\ cm^{-1}$			total alkalinity meq L^{-1}			dissolved oxygen %		
	min.	mean	max.	min.	mean	max.	min.	mean	max.
km -2.5	63	97.2	131	0.14	0.43	0.91	107	117	148
km 10.9	111	198	402	0.65	1.69	5.08	100	112	136
km 49.9	145	330	406	1.37	2.63	8.19	103	112	124
km 107.9	235	602	695	1.93	3.56	13.1	95	105	127
km 145.0	295	618	1320	2.37	4.23	10.8	75	94.2	115
km -1.9, 86.0	1038	1370	1704	2.80	5.49	14.7	35	68.2	113

Table 4.2. Mean conductivity, total alkalinity and dissolved oxygen concentrations from six sites on the Swale-Ouse. Data from July 1996 to June 1997. Min. and max. indicate the range of the data.

Site	conductivity $\mu S\ cm^{-1}$			total alkalinity meq L^{-1}			dissolved oxygen %		
	min.	mean	max.	min.	mean	max.	min.	mean	max.
km -2.5	15.5	76.2	127	0.001	0.18	0.59	104	115	126
km 10.9	62.1	159	276	0.059	1.11	4.58	104	110	118
km 49.9	145	285	406	0.11	1.93	10.1	101	110	139
km 107.9	228	521	686	0.36	1.81	4.57	95	104	120
km 145.0	266	477	660	0.39	1.73	3.90	75	99.1	116
km -1.9, 86.0	746	1320	1547	1.09	2.83	5.57	46	59.7	80

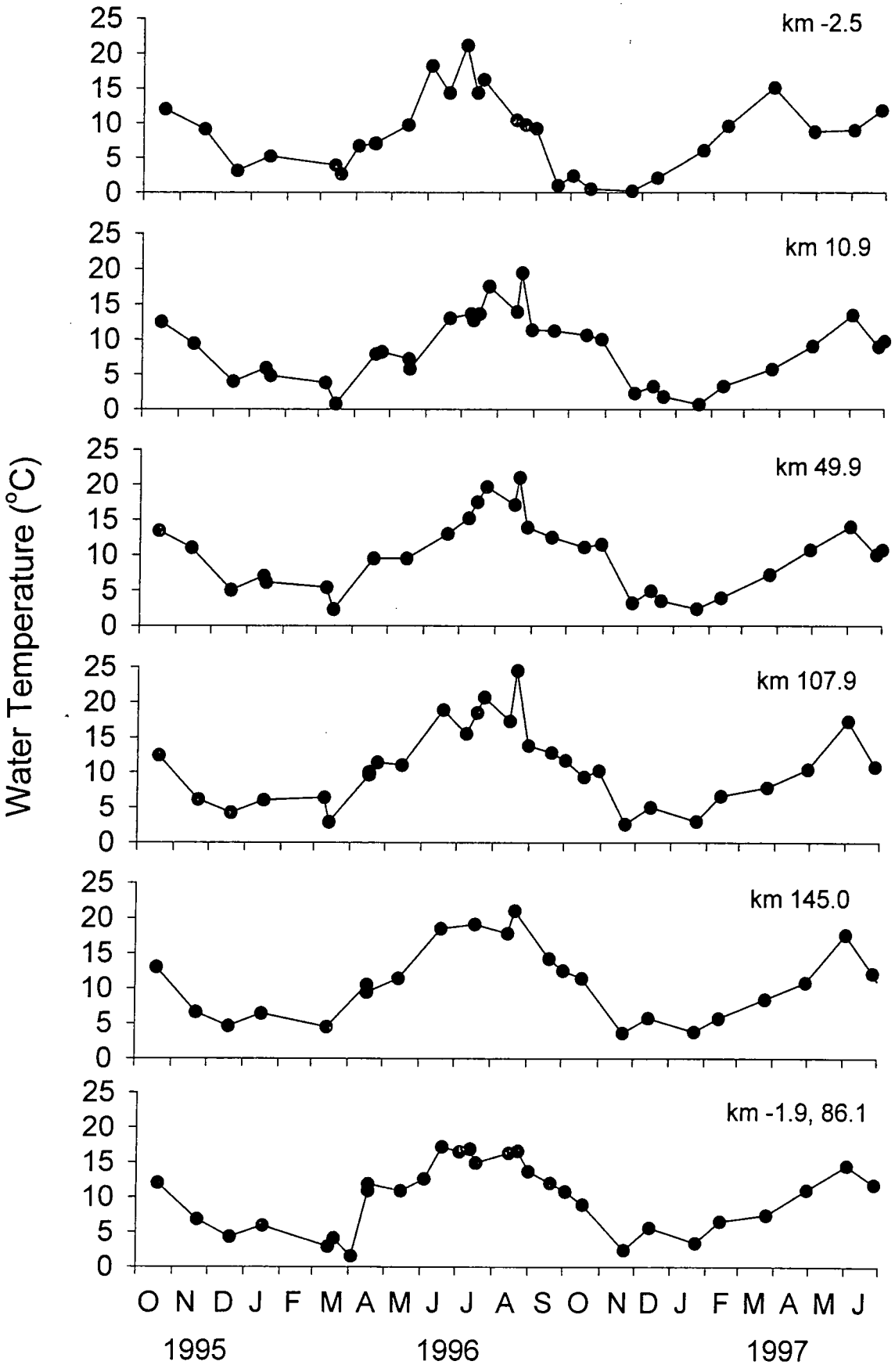

Figure 4.2 Water temperature at six sites within the Swale-Ouse catchment during two surveys of phosphatase activity from October 1995 to June 1997.

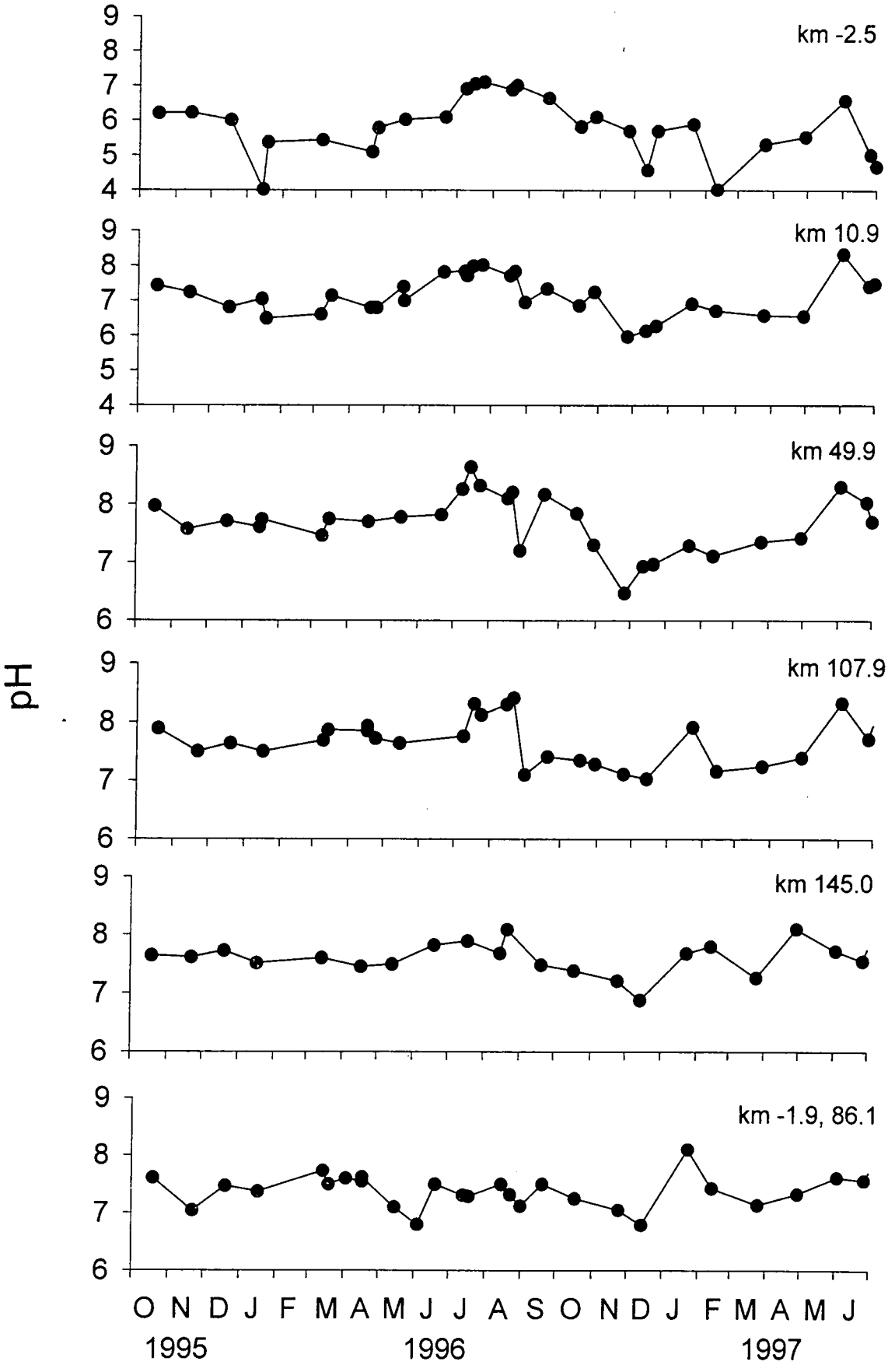

Figure 4.3 pH at six sites within the Swale-Ouse catchment during two studies of phosphatase from October 1995 to June 1997.

< 100 % on 6 of 20 occasions at km 107.9, but was never < 95 %, whereas, at km 145.0 half of the samples were <100 % with the lowest, 75 % on 17 July 1996. Substantial organic load from Northallerton STW affects km -1.9, 86.1, which had lower DO than the main river, and saturation was evident on only 2 of 30 occasions, the lowest was 35 %, 12 July 1996. At all sites where under-saturated values were recorded, the lowest values were recorded in summer. DO was negatively correlated ($p < 0.05$) with temperature at km 107.9 and km 145.0.

4.35 Conductivity and total alkalinity

Conductivity increases with ion concentration in the water and is often correlated with total alkalinity, which is influenced by concentrations of $CaCO_3$. Both conductivity and total alkalinity increased on passing down the river (Table 4.1 and 4.2) during both surveys. At km -2.5, conductivity was typically 100 $\mu S\ cm^{-1}$, whereas at the tidal limit, km 145.0, values were generally six times higher. However, the highest values were found at km -1.9, 86.1, where conductivity was less than 1000 $\mu S\ cm^{-1}$ on only two of 30 occasions. Total alkalinity was generally an order of magnitude greater at the most downstream site km 145.0, compared to the most upstream site. There was no clear seasonal trend to total alkalinity at all sites, but conductivity at sites km -2.5 and km 10.9 showed a summer maxima and winter minima. This was less clear at km 49.9 and not evident at sites km 107.9 and km 145.0 or km -1.9, 86.1.

4.36 Absorbance of water

The absorbance of UV light at 320 nm by water samples (A320) was higher at the upstream sites closest to the peat soils (which leach humic substances), and generally decreased on passing downstream. At km -2.5, A320 was never < 0.100, whereas, the tributary km -1.9, 86.1 not influenced by peat was never > 0.110. In general, all sites had a seasonal trend of winter maxima and summer minima (Figure 4.4), although all had high

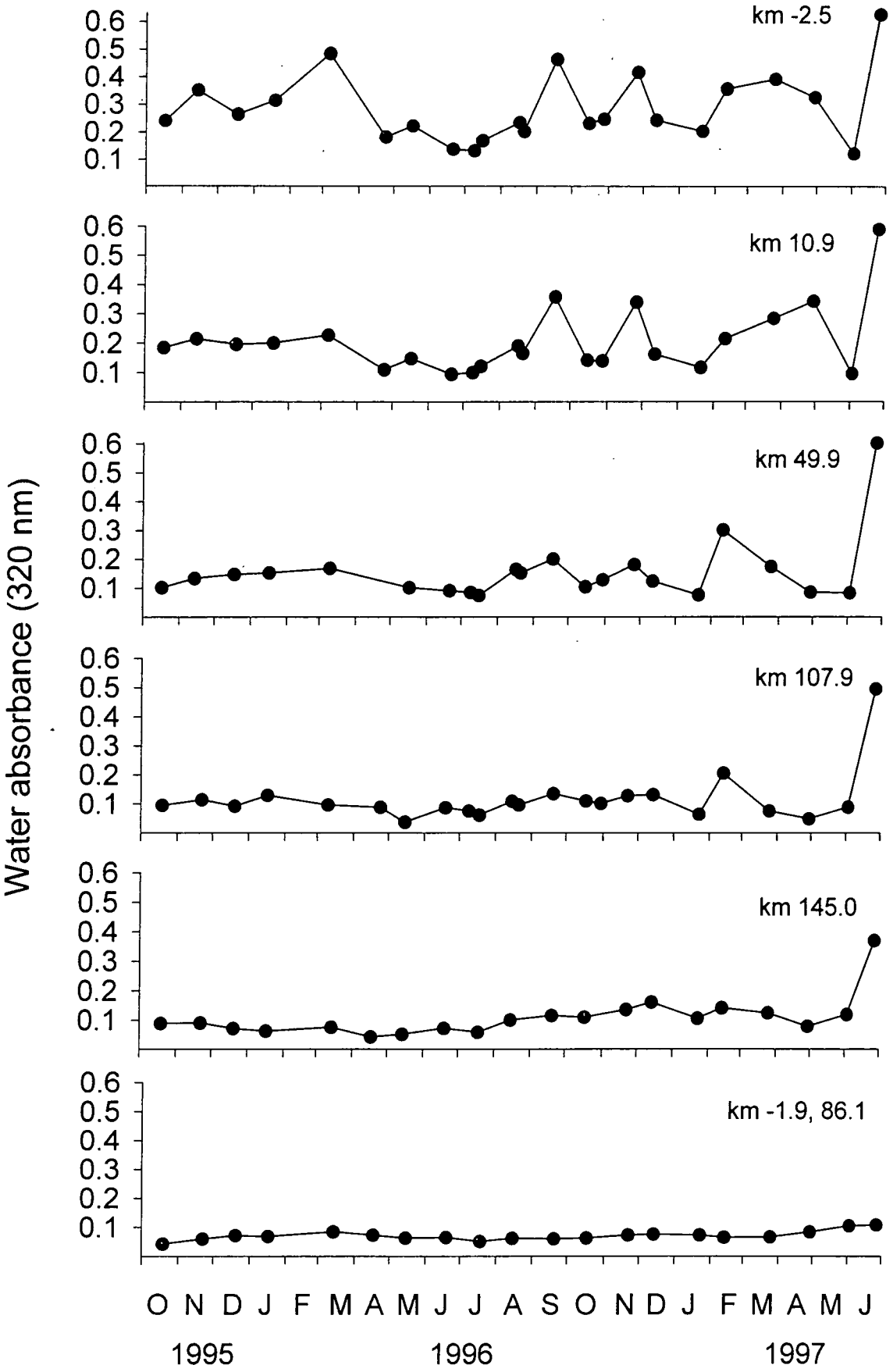

Figure 4.4 Water absorbance at 320 nm, at six sites within the Swale-Ouse catchment during surveys of phosphatase activity, from October 1995 to July 1997.

absorbance on 26 June 1997, during a period of unusually high summer flow. For example, the highest value recorded at km 49.9, 0.602 and was nearly 7 times the value recorded the previous month at this site. All sites had significant positive correlation between flow and A320.

4.4 Nutrient concentration

4.41 Nitrogen

Only inorganic N fractions were determined, both by LOIS and during this study. Organic nitrogen was not recorded and the implications of this are discussed later. Examples of data and general trends from this study are described, and the relationship with other important variables i.e. flow and temperature, mentioned where relevant.

Nitrate concentrations increased on passing down the river. At km -2.5, the highest value recorded was 420 $\mu g\ L^{-1}$ and the lowest 8 $\mu g\ L^{-1}$, compared to 10300 $\mu g\ L^{-1}$ and 2290 $\mu g\ L^{-1}$ at the tidal limit of the river, km 145.0. At km –1.9, 86.1, concentrations were higher than the main river, the highest being 31800 $\mu g\ L^{-1}$ N, 17 January 1996. Winter maxima were observed at all sites, and nitrate was significantly ($p < 0.05$) negatively correlated with temperature at all sites, although the significance of this relationship decreased on passing downstream. Although flows were generally higher in winter, no negative correlation was found between nitrate and flow at any site.

Nitrite formed the least significant contribution to total inorganic nitrogen (TIN) at all sites, and although it increased on passing downstream, the trend was not as marked as for nitrate. At km -2.5 and km 10.9, nitrite was almost always less than 10 $\mu g\ L^{-1}$ N, often close to detection limits (2.35). At km 107.9 and km 145.0, nitrite was always > 10 $\mu g\ L^{-1}$ N, but generally < 100$\mu g\ L^{-1}$ N. Nitrite did not show a seasonal relationship similar to nitrate, and was not significantly correlated with flow.

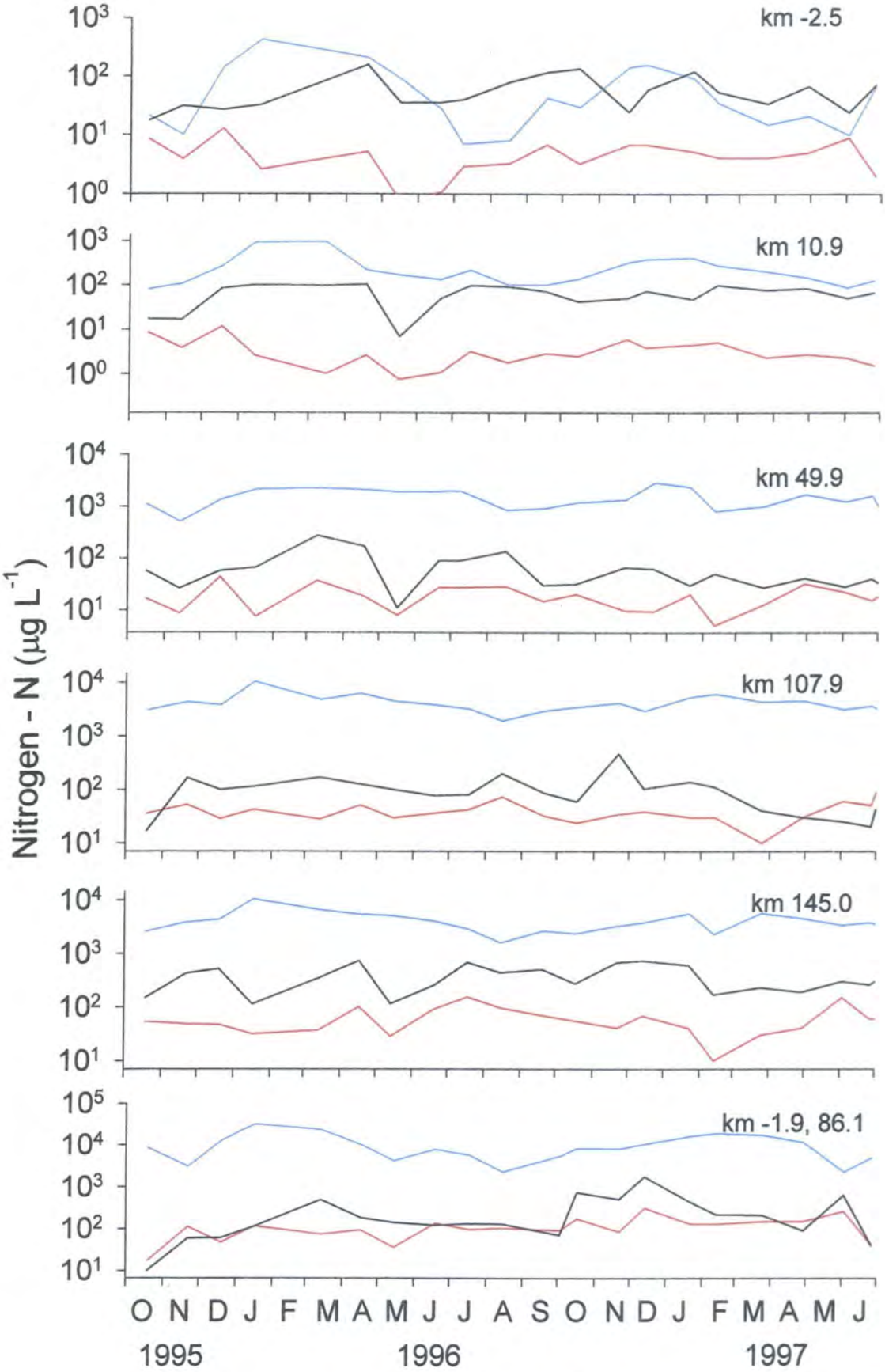

Figure 4.5 Nitrogen concentration at six sites within the Swale-Ouse catchment during two studies of phosphatase, from October 1995 to June 1997.
— NO_3 — NO_2 — NH_4

Ammonium formed an important contribution to TIN at km -2.5, and concentrations were greater than those of nitrate on 10 of 21 occasions, mostly during the summer decline in nitrate concentration. At all other sites, ammonium concentrations never exceeded those of nitrate. Concentrations of ammonium at km -2.5, 10.9 and 49.9 rarely exceeded 100 $\mu g\ L^{-1}$ N, in contrast to km -1.9, 86.1, where concentrations were much greater, and exceeded 1.5 mg L^{-1} N on 14 December 1996. Ammonium had no discernible seasonal trend and was not correlated with flow or temperature at any of the sites surveyed.

4.411 Comparison between datasets

Considering that two data sets (LOIS and this study) existed for three sites, there was the opportunity for comparison. The data for nitrate, nitrite, ammonium overlapped from October 1995 to March 1997, a period of 18 months. LOIS collected 54 weekly samples and these were compared with the 18 monthly samples collected during this study. The data was not normally distributed and was log transformed prior to comparison by Analysis of Variance (ANOVA).

Nitrite and ammonium were significantly different at km 49.9 and km 107.9 but not km 145.0. Nitrate was not significantly different at the three sites.

Table 4.3 ANOVA p- statistics from comparison of nitrogen fractions collected by LOIS sampling programme and this study. Figures in bold represent a significant difference ($p < 0.05$)

Site	nitrate-N	nitrite-N	ammonium-N
km 49.9	0.47	**0.031**	**0.028**
km 107.9	0.19	**0.027**	**0.04**
km 145.0	0.53	0.065	0.81

4.412 Variability of N fractions

A coefficient of variation was calculated to estimate the variability (log transformed data) of N-fractions sampled from October 1995 to June 1997. Variability of nitrate and

nitrite decreased on passing downstream, in contrast, ammonium showed similar variability along the river continuum.

Table 4.3 Mean nitrate-N, nitrite-N and ammonium-N concentration ($\mu g\ L^{-1}$-N) and the coefficient of variation (C.V.), for six sites within the Swale - Ouse catchment, October 1995 to June 1997.

Site	nitrate-N		nitrite-N		ammonium-N	
	mean	C.V. (%)	mean	C.V. (%)	mean	C.V. (%)
km -2.5	46.2	32.7	3.91	49.9	50.9	16.2
km 10.9	204	13.3	2.25	176	56.2	17.9
km 49.9	1435	6.11	16.3	21.2	52.2	18.8
km 107.9	4040	4.25	35.0	11.6	81.8	18.8
km 145.0	3819	5.22	52.3	15.9	326	10.5
km -1.9, 86.1	8370	8.24	96.9	14.8	167	22.5

4.42 Phosphorus

Five P fractions were estimated (2.37), including inorganic and organic dissolved fractions, as well as the contribution from the particulate phase. FRP increased on passing downstream and was approximately two orders of magnitude higher at the tidal limits of the river system, km 145.0, compared with the most upstream site km -2.5. Seasonal trends varied between sites. A clear summer maxima was evident (Figure 4.6) at km 49.9 and 107.9, with values approximately 3 and 4 times the winter minima, respectively. At km -2.5, km 10.9 and km 145.0 no such trend was apparent, although the lowest concentrations at the first two sites were found in winter. High winter flow diluted FRP, and a significant negative correlation with flow was found at km 107.9, km 49.9, km 145.0. These sites had a significant correlation between FRP and TP, demonstrating the important contribution of FRP to total phosphorus at these sites. In contrast, km -2.5 and km 10.9 showed a weak positive correlation between flow and FRP. The highest concentrations of FRP were recorded at km -1.9, 86.1, the concentration was never < 1 mg L^{-1}, and peaked at over 6 mg L^{-1}, 14 May 1996.

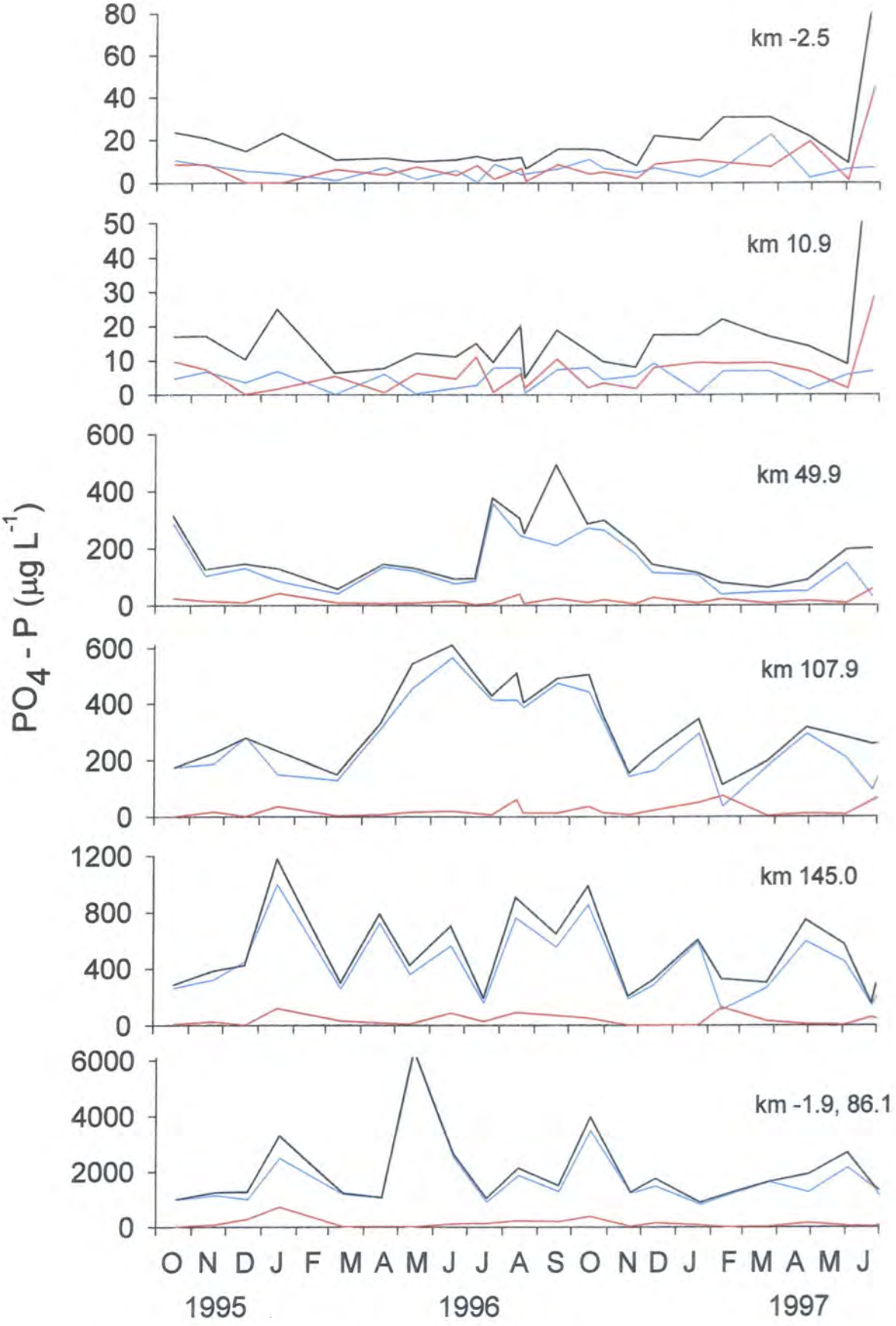

Figure 4.6 Concentration of three phosphorus fractions at six sites within the Swale-Ouse catchment during two seasonal studies of phosphatase, from October 1995 to June 1997.
—FRP —FOP —TP

FOP increased on passing down the river, however, this increase was only 4-fold compared to a more than 60-fold increase in FRP. There were clear differences in the importance of FOP between the sites. At km -2.5 and 10.9, FOP and FRP were similar throughout both sampling periods, whereas at all other sites, FOP concentrations were often two orders of magnitude lower than FRP. The highest concentration of FOP at the most upstream site, 45 $\mu g\ L^{-1}$, 26 June 1997, was 4 times FRP estimated from the same sample. In comparison, the highest concentration of FOP at the most downstream site was 125 $\mu g\ L^{-1}$, 13 February 1997, when FRP was only slightly lower at 108 $\mu g\ L^{-1}$. No seasonal trend of FOP concentration was apparent at any of the main river sites or R.Wiske. However, FOP was very highly significantly correlated with flow, absorbance and TP at sites km -2.5 and km 10.9. Correlation between FOP and flow, absorbance and TP were also evident at other sites, although less significantly.

TP is composed of both filtered and particulate P and tended to follow the trend of the dominant component. TP increased on passing down the river and was approximately 30 times higher at km 145.0, compared to km -2.5. There was marked contrast between km -2.5 and 10.9 and other sites concerning the fractions comprising TP. The two upstream sites had roughly equal proportions of FOP and FRP and a lesser particulate component (PP), which was comparatively more important in winter than summer. At all other sites were TP was influenced by FRP, with much smaller contributions from FOP and PP. The influence of FRP was such, that at the downstream sites, TP had the same seasonal trend as FRP and the two fractions were highly correlated.

4.421 Comparison between data sets

Comparison of P data was carried out using the method described in 4.411. FRP was significantly different at km 49.9 and km 107.9, whereas FOP was significantly different at all three sites. TP was not significantly different at the three sites.

Table 4.5 ANOVA p- statistics from comparison of phosphorus fractions collected by LOIS sampling programme and this study. Figures in bold represent a significant difference ($p < 0.05$)

Site	FRP	FOP	TP
km 49.9	**0.016**	**< 0.001**	0.21
km 107.9	**0.003**	**< 0.001**	0.52
km 145.0	0.17	**< 0.001**	0.12

4.422 Variability of P fractions

The variability of P fractions was carried out using the method described in 4.412, on samples collected during this study from October 1995 to June 1997. The variability of FRP and TP decreased on passing downstream. FOP had high variability at all sites.

Table 4.6 Mean FRP, FOP and TP concentration ($\mu g\ L^{-1}$) and the coefficient of variation (C.V.) for six sites within the Swale - Ouse catchment, October 1995 to June 1997.

Site	FRP		FOP		TP	
	mean	C.V. (%)	mean	C.V. (%)	mean	C.V. (%)
km -2.5	4.57	62.6	6.79	38.8	16.7	19.9
km 10.9	3.49	99.6	5.20	58.8	14.6	21.0
km 49.9	102	14.4	12.0	35.9	145	11.4
km 107.9	211	12.5	11.0	57.3	267	9.17
km 145.0	372	10.6	14.2	66.0	463	8.66
km -1.9, 86.1	1510	6.96	34.6	60.4	1710	6.96

4.5 Relationship between P and flow

A major factor influencing P in lotic systems is flow and this relationship has been addressed by other LOIS studies (3.25). In view of the fact data was collected by this study at sites not covered by LOIS, analysis of the relationship is presented here, and comparison with LOIS data made. Scatter plots of flow versus four P fractions are presented in figure 4.7, and marked differences along the Swale-Ouse river system are apparent.

At km -2.5, the highest FRP concentration was apparent under lower flow conditions and moderate FRP concentrations were still noted under high flow. km 10.9 had similar

relationship, although under high flow FRP was higher than at km -2.5. FRP at moderate and lower flow was variable. Both this study and LOIS had similar results at km 49.9, km 107.9, km 145.0. FRP decreased with flow, and low FRP concentrations were present under high flow conditions. The data fitted an inverse power law regression, r^2 was 0.62, 0.67, 0.74 at km 49.9, km 107.9, km 145.0, respectively.

At the upper two sites, the highest concentrations of FOP were consistent with high flow. The relationship was approximately linear, r^2 was 0.36, 0.34 at km -2.5, km 10.9 respectively. This was not evident at sites further down the river. FOP collected by LOIS at km 49.9 and km 145.0, included several extremely high concentrations under low flows. This was not evident in data from this study, although the high readings were recorded on days when the studies did not overlap.

PP had a positive linear relationship with flow at km -2.5, km 10.9, km 107.9, km 145.0, r^2 was 0.61, 0.44, 0.68, 0.49, respectively. A linear relationship was not found at km 49.9 although both data sets showed the highest flow did coincide with the highest PP concentration.

TP was influenced by its constituent parts. At the two upstream sites TP increased with flow in response FRP, FOP and PP. At km 49.9 and km 145.0, FRP was the dominant fraction and TP reflects the relationship between FRP and flow. At km 107.9 both FRP and PP influence the relationship between TP and flow.

4.6 Nutrient ratios

4.61 FRP:FOP ratio

FRP and FOP are potential sources of phosphorus for biota, although FOP requires transformation by enzymes such as phosphatases prior to utilisation. Considering this, a low FRP:FOP ratio may indicate sites where phosphatase activity may be enhanced. This ratio was calculated for all sites during both phosphatase studies from October 1995 to June 1997.

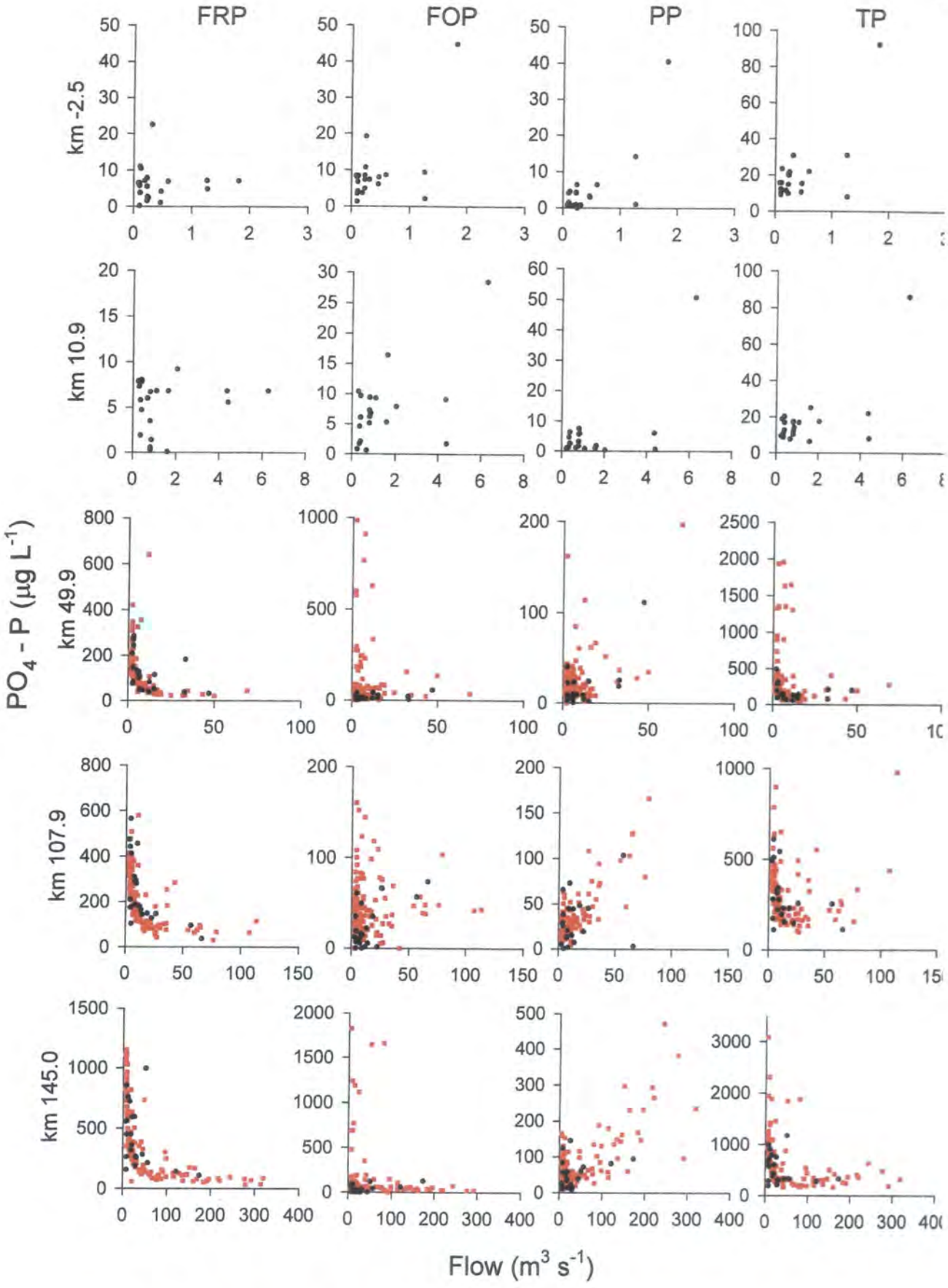

Figure 4.7 Relationship between flow and four phosphorus fractions at five sites within the Swale-Ouse catchment. ▪ LOIS data (weekly), • This study (monthly), from October 1995 to June 1997.

Mean FRP : FOP ratio is presented in table 4.7 and 4.8 and shows marked increases on passing down the river, during both surveys. The most upstream site km -2.5, had ratios of 0.99 and 1.37, compared to 54.3 and 127, at the most downstream site during the two surveys. The highest ratio, 716 was recorded at R. Wiske during the first survey. High ratios of 62.0 at km107.9 during the first survey, and 127 at km 145.0 during the second survey, were caused by FOP being below detection limits. However, the increasing FRP : FOP on passing downstream points to the importance of FOP in the upper part of the catchment. At km -2.5 FRP : FOP was < 1.0 on 7 of 12 occasions during the first survey, and 8 of 12 during the second. The lowest value recorded during both surveys was 0.03 in July 1996. Similar results were found at km 10.9, FRP : FOP was < 1.0 on 8 of 12 and 6 of 12 occasions during both surveys. Greater inputs of FRP increased the ratio approximately 8-fold between km 49.9 and km 10.9, during both surveys. At km 49.9, km 107.9 and km 145.0 FRP : FOP values less than 1.0 were recorded on only one occasion at each site. Only at km -1.9, 86.1 did the ratio always remain > 1.0.

4.62 N:P ratio

A second nutrient ratio, N : P, was also calculated. High N : P is considered indicative of P limitation in freshwaters (1.7); however, it should be compared with total concentrations to maximise its interpretative value. Two ratios; TIN : FRP and TIN : FTP were calculated.. The former is a ratio of dissolved inorganic nutrients, whereas the latter includes both the inorganic and organic P fractions. Data is presented as two surveys as for the previous section (Table 4.3 and 4.4).

TIN : FTP was always lower than TIN : FRP at all sites. The difference between the ratios decreased on passing down the river as the contribution of FOP to FTP reduced. Contrary to expectation, both ratios did not decrease on passing down the river and the

highest values for both ratios, during both surveys, were at km 10.9. During the first survey the TIN:FRP value was 964 at this site, over 10 times higher than the site further upstream, km -2.5. The Ratios during both surveys had the same pattern. From km -2.5 to km 10.9 there was a large increase, followed by a decrease at site km 49.9 due to point sources of FRP. It then increased slightly at km 107.9, the part of the catchment subject to the most intensive agriculture, before decreasing at km 145.0 to values less than km 49.9. The low ratios at the most upstream site may suggest N, as well as P limitation at this site, whereas the high ratio at km 10.9 suggests only P is limiting, at certain times of the year. From km 49.9 downstream, concentrations of both N and P are such that either is unlikely to be limiting, irrespective of high or low ratios.

Table 4.7. Mean nutrient ratios from five sites on the Swale-Ouse and one tributary. Data from October 1995 to October 1996. Min. and max. represent the range of the data.

Site	FRP:FOP			TIN:FRP			TIN:FTP		
	min.	mean	max.	min.	mean	max.	min.	mean	max.
km -2.5	0.03	0.99	2.68	4.49	72.4	325	2.49	16.8	49.7
km 10.9	0.01	2.38	10.0	18.9	964	10400	7.41	39.9	193
km 49.9	2.01	15.8	58.1	4.10	17.6	65.5	3.54	15.2	54.1
km 107.9	4.22	62.0	279	5.29	21.2	70.7	4.61	20.0	57.2
km 145.0	5.87	54.3	448	2.79	11.5	27.0	2.50	10.7	24.2
km -1.9, 86.0	3.49	716	6310	0.68	7.19	20.4	0.70	6.60	20.9

Table 4.8. Mean nutrient ratios from five sites on the Swale-Ouse and one tributary. Data from July 1996 to June 1997. Min. and max. represent the range of the data.

Site	FRP:FOP			TIN:FRP			TIN:FTP		
	min.	mean	max.	min.	mean	max.	min.	mean	max.
km -2.5	0.03	1.37	5.00	2.36	39.9	184	1.78	9.4	25.1
km 10.9	0.06	2.11	9.87	22.8	111	787	5.65	25.0	51.5
km 49.9	0.56	16.5	58.1	4.10	19.6	51.9	3.54	14.6	27.3
km 107.9	0.49	24.2	119	5.29	32.1	170	4.61	20.5	56.3
km 145.0	0.86	24.2	593	2.79	14.4	28.9	2.50	12.2	21.2
km -1.9, 86.0	6.73	129	1110	1.33	7.93	21.3	1.19	7.48	19.7

4.7 Discussion

Seasonal and spatial trends were evident for many of the environmental variables recorded during two seasonal surveys of the Swale-Ouse river system. Much of the spatial variation reflects soils and land use in the catchment. The upper part of the catchment is influenced by Millstone Grit, peat soils and non-intensive farming, giving rise to soft, neutral-acidic waters with low total nutrient concentration. Further downstream, calcareous soils, intensive farming and increased urbanisation, cause chemical enrichment and harder, neutral alkaline water.

TIN increased on passing downstream, and nitrate was generally of greater quantitative importance compared with other nitrogen fractions. However, ammonium formed a significant contribution to TIN at km -2.5. Nitrite was not of quantitative importance at any site, and occasionally was near to, or less than, detection limits. The highest concentrations of all three fractions were found in the tributary R. Wiske, km –1.9, 86.1. Nitrate concentrations varied seasonally at most sites, and summer lows were most likely a function of biotic uptake and denitrification. Nitrate was not significantly correlated with flow, although higher nitrate concentrations were evident under moderate to high flow conditions during winter months. The variability of nitrate and nitrite decreased on passing downstream, whereas, ammonium had similar variability along the river continuum. The reduction in variability on passing downstream is a factor contributing to the difference in results of a comparison between this study and LOIS.

TP increased on passing down the river, and the dissolved and particulate fractions comprising TP varied spatially. In the upper reaches, organic and inorganic phosphorus formed an equal contribution to TDP, this resulted in a FRP : FOP ratio < 1.0 on several occasions. In the middle and lower reaches of the river, FRP was the most dominant fraction, often comprising > 90% of TP, the mean FRP : FOP ratio was > 15. The highest inorganic P concentrations were always found in R. Wiske.

FOP was positively correlated with flow, particularly in the upper reaches. This, and the strong correlation between FOP and A320, suggests that the peat soils form an important diffuse source of FOP in the upper reaches. FRP had an inverse relationship with flow at sites in the middle and lower reaches, and contributions from point sources were more important than diffuse ones. PP was positively correlated with flow and formed a significant contribution to TP during high flows, particularly at upstream sites. The variability of FRP and TP decreased on passing downstream, whereas, FOP remained variable at all sites. Comparison between data collected during this study and LOIS found the FOP data to be significantly different at all sites, the temporal variability was almost certainly a contributing factor.

N : P ratios were calculated for each sampling event within the context of total nutrient concentration to determine sites where N or P may be limiting. Low N : P ratios at the most upstream site during summer are consistent with reduced N concentrations. However, low P meant that both N and P were limiting at this site. The highest N : P ratios were found at km 10.9, whereas, further downstream considerable concentrations of P from point source inputs reduced N : P ratios.

4.8 Summary

i. Flow was correlated with a number of variables and is considered a major factor controlling the concentration of nutrients in rivers.

ii. Water temperature had lower minima at higher elevations and sites at lower altitude had longer periods of warm water.

iii. The upper Swale was generally acid-neutral and pH increased on passing downstream to become neutral-alkaline.

iv. DO was saturated on all occasions in upper Swaledale although increased BOD further downstream meant DO was under-saturated, > 50 % of samples at km 145.0,

most notably those taken in summer. DO in R. Wiske was lower than that of the main river, and was saturated on only two occasions.

v. Nutrients increased on passing downstream with significant contributions to the lower river from R. Wiske.

vi. FRP : FOP increased along the continuum caused by the increasing dominance of FRP in the lower reaches.

vii. N : P ratios (TIN:FRP, TIN:FTP) decreased on passing downstream. The high ratio in the upper reaches is indicative of P limitation.

viii. The variability nitrate, nitrite, FRP and TP decreased on passing downstream, whereas, ammonium and FOP showed little change in variability along the continuum.

ix. Comparison of LOIS nutrient data and this study found nitrate and TP not to be significantly different, whereas, the nutrients with higher variability: nitrite, ammonium, FRP, FOP were all significantly different at the upstream site. The data sets (except FOP) were not significantly different at the most downstream site.

CHAPTER 5

Laboratory studies of moss phosphatase

5.1 Introduction

The water chemistry of the Swale suggests (4.42) that upstream parts of the river system have low concentrations of inorganic P at certain times of the year. Studies of neighbouring catchments by Yelloly (1996), have shown that waters low in inorganic P, which experience intermittent but concentrated pulses of organic P, have biota with enhanced surface phosphatase activity. In view of the close relationship between surface phosphatase activity and phosphorus stress, it was deemed appropriate to assess whether organisms in the Swale were capable of expressing phosphatase activity.

Mosses are present along much of the wadeable reaches of the Swale. In view of the close relationship between aquatic mosses and water chemistry (1.112), it was decided to sample two common mosses, *Fontinalis antipyretica* and *Rhynchostegium riparioides*, and assess their P status. The rapid nature of the phosphatase assay makes it an attractive tool for such studies.

A study of the phosphatase activity of mosses over a twelve-month period was planned, this would reflect the seasonal changes on spatial and temporal scales. However, prior to such a study, a number of experiments were carried out to assess the factors influencing PMEase activity to determine appropriate assay conditions. Many of these preliminary experiments were conducted on mosses collected from km 10.9, due to the abundance of both species, and the ease of access to this site.

As the study developed it became apparent that PDEase as well as PMEase could be of importance to mosses in Swaledale. Therefore, the results include preliminary studies carried out prior to, and further work completed during the annual survey of PMEase.

5.2 Time course

Time course experiments were carried out to test the linearity of moss phosphatase activity. This would determine a suitable incubation period for further studies. Assays were carried out on both mosses sampled from km 10.9 on 17 March 1995. The assay conditions were, substrate concentration 500 μM MUP (final concentration), medium at pH 5.5, 7.5, 10.3, and incubation times of 1, 2, 5, 10, 15, 20, 30, 40, 50, 60 min.

Hydrolysis of MUP was linear at all three pH values, over the period tested (Figure 5.1). Both mosses had higher rates of PMEase at pH 5.5, compared with the other pH values tested. Incubation times of 60 min produced absorbance values in excess of the linear range of the spectrophotometer, and samples required dilution. Therefore, subsequent assays were carried out with incubation times of less than one hour. During the 12-month study, an incubation time of 20 min produced readily detectable activity in samples from all sites.

5.3 Temperature

The water temperature of R. Swale typically ranged between 1 and 25 °C over an annual period (4.32). Therefore, the influence of temperature on phosphatase activity was tested over a similar range. This was carried out on two occasions using three substrates. Both mosses from km 10.9 were assayed for PMEase (100μM MUP, final concentration) at 5 °C intervals, from 5 to 30 °C, and at 1°C, 12 August 1995. A second experiment, again on both mosses, but sampled from both km 10.9 and km 107.9, used PME and PDE substrates (both 100μM final concentration) and temperature intervals of 3 °C from 3 to 30 °C, 14 October 1996.

The response to temperature was linear for both mosses, from both sites, with all substrates on both occasions.

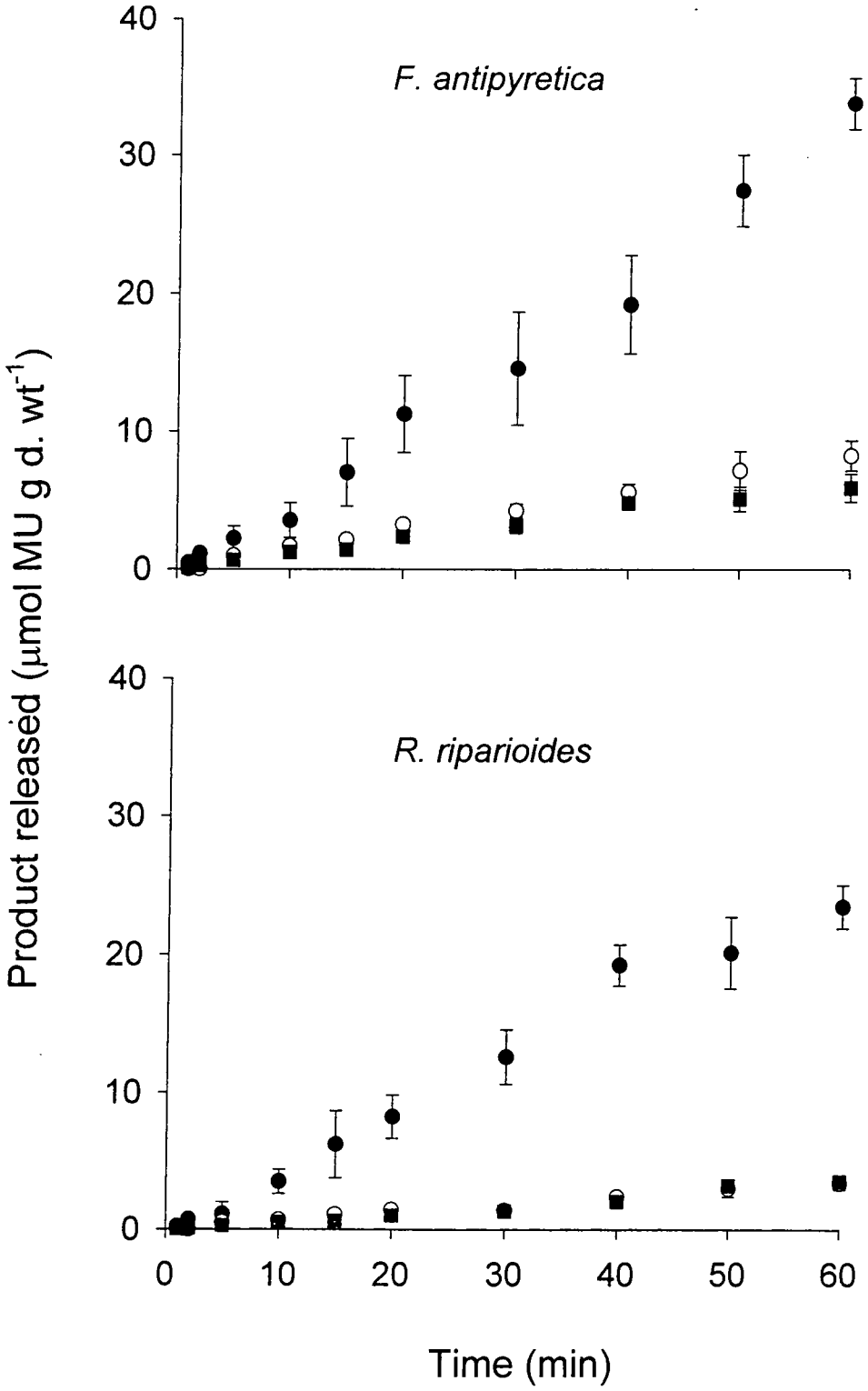

Figure 5.1 Influence of time on hydrolysis of PME substrate (500 µM MUP) at pH ● 5.5, ○ 7.5, ■ 10.3 by 2-cm tips of *F. antipyretica* and *R. riparioides* from km 10.9, 17 March 1995. n = 3 ± S.D.

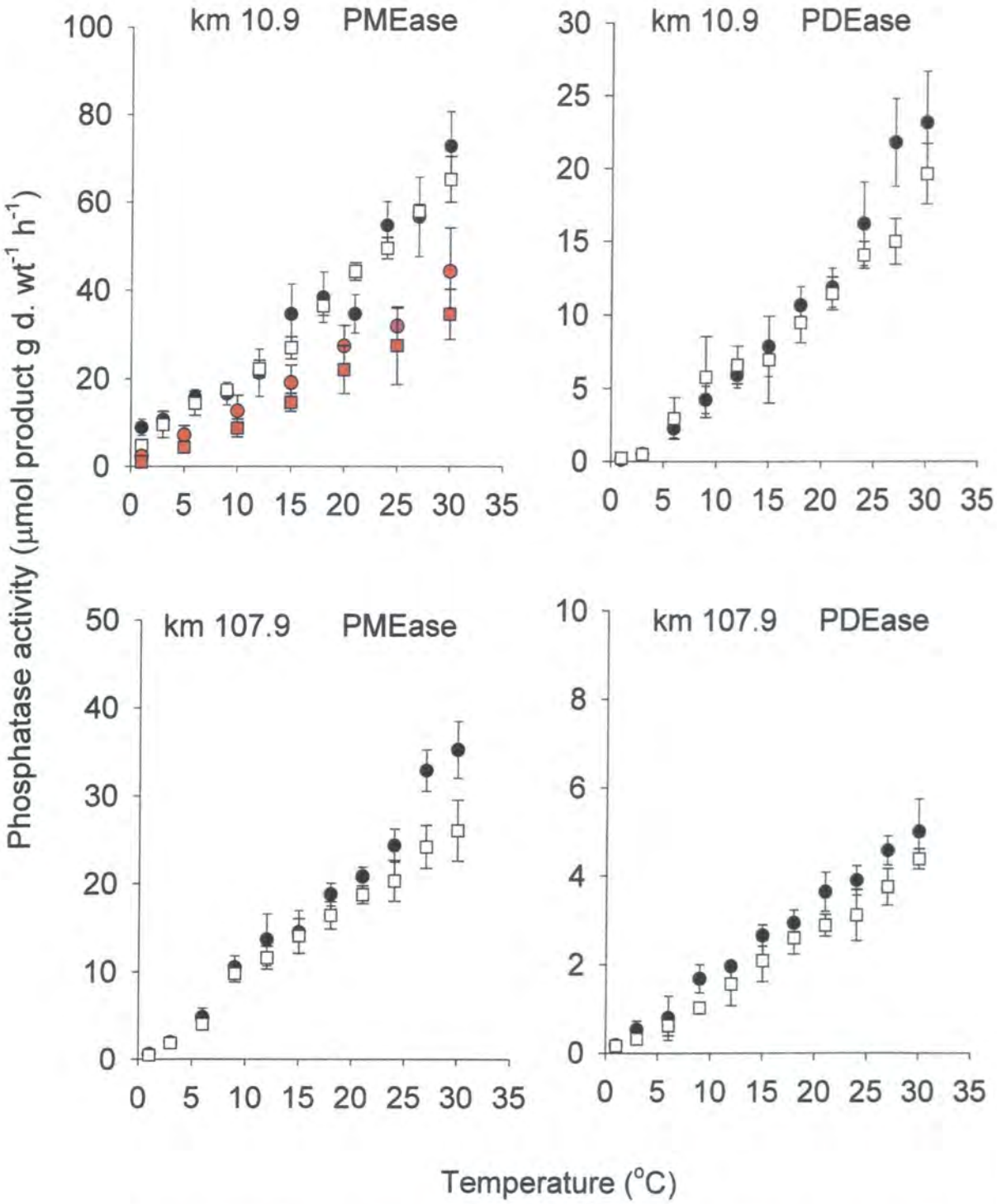

Figure 5.2 Influence of temperature on the PMEase and PDEase activity of 2-cm tips of *F. antipyretica*; ●12 August 1995, ●14 October 1996 and *R. riparioides*; ■ 12 August 1995, □ 14 October 1996, from km 10.9 and km 107.9, R. Swale. n = 4 ± S.D.

5.4 Substrate concentration

The influence of substrate concentration was quantified to assess the apparent V_{max} of the rate of activity. This would determine the substrate concentration suitable for the seasonal study of the two mosses. Plants were taken from km 10.9 on 14 March 1995, and three replicate samples assayed at pH 5.5, 7.5, 10.3, at a substrate concentration of 10, 20, 30, 40, 50, 100, 250, 500, 1000 μM MUP.

Both mosses had similar responses, assays at pH 5.5 produced higher rates of activity than 7.5 and 9.5, at all substrate concentrations tested (Figure 5.3). Saturation was reached using concentrations of approximately 80 μM for both mosses, at all pH values tested.

5.5 Influence of pH

Phosphatase activity, like many other reactions is markedly influenced by pH. Optimum rates of activity have been found at both extremes of the pH scale (1.81), beyond the ambient values of the River Swale (4.33). In view of this, the influence of pH on the phosphatase activity of mosses was tested over a wide range, from 5.0 to 10.0 in half pH unit increments. Mosses from km -2.5, km 10.9, km 49.9, km 107.9 were tested on two occasions, 26 July 1995 and 14 October 1996, using 100 μM MUP as a substrate. The reason for the gap between the sampling events is that *F. antipyretica* was not found at km 49.9 until 9 March 1996, and *R. riparioides* was not sampled at km 107.9 until 23 July 1996. Similar results were found at all four sites, on both sampling occasions (Figure 5.4). The highest rate of activity was at pH 5.5 and the lowest at values > 7.5 for both mosses. In general, *F. antipyretica* had slightly higher activity per unit mass.

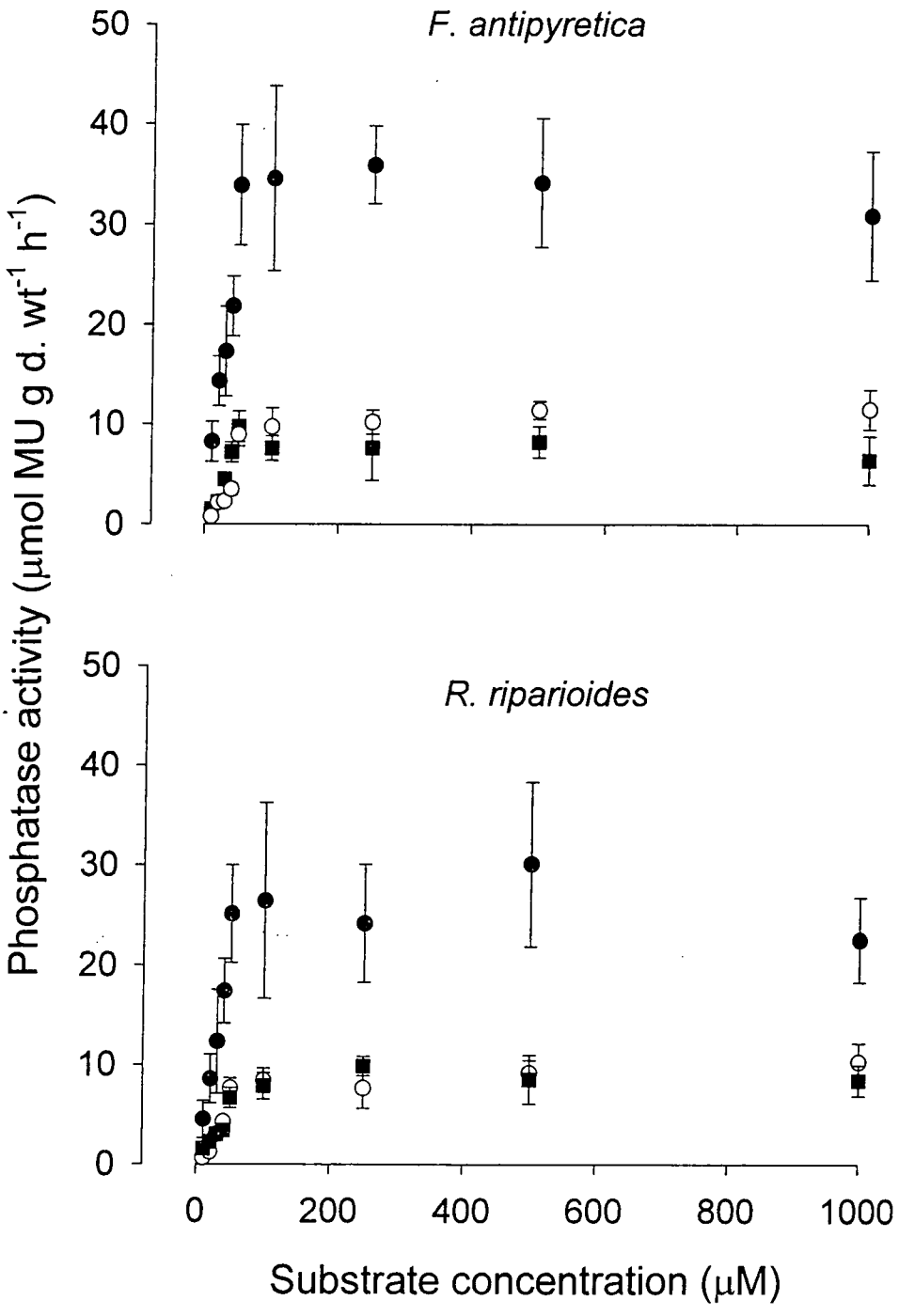

Figure 5.3 Influence of substrate concentration on PMEase activity of 2-cm tips of *F. antipyretica* and *R. riparioides* at ● pH 5.5, ○ 7.5, ■ 9.5. Samples taken from km 10.9, 14 March 1995. n = 3 ± S.D.

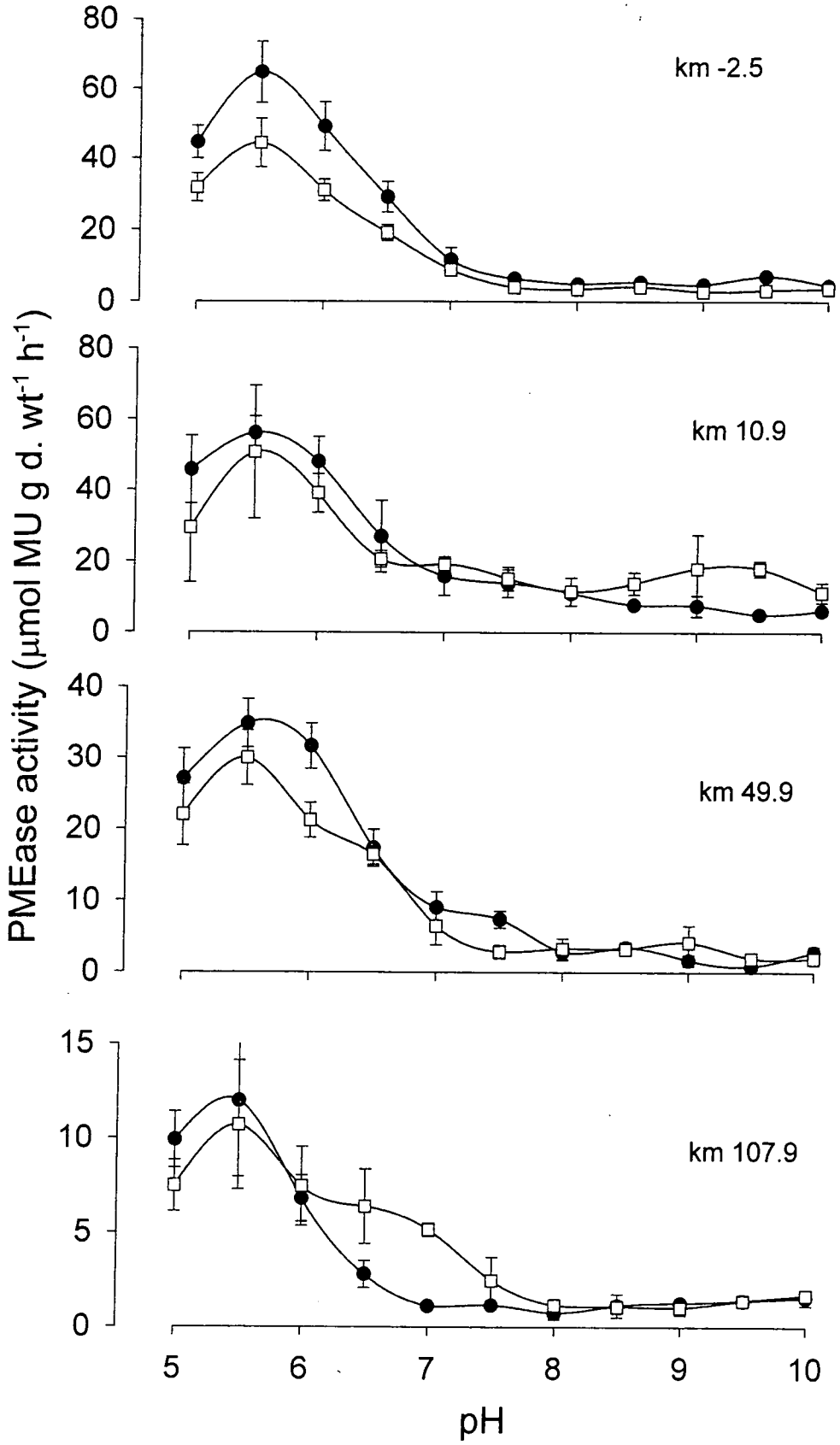

Figure 5.4 Influence of pH on PMEase activity of 2-cm tips of ● *F. antipyretica* and □ *R. riparioides* at four sites within the Swale catchment. km -2.5, km 10.9 on 26 July 1995; km 49.9, km 107.9 on 14 October 1996. n = 4 ± S.D.

The concentration of substrate at which phosphatase activity was saturated (> 80 μM) was generally greater than the potential concentration of naturally occurring substrate. Studies have shown that the pH optimum of phosphatase is often dependent on substrate concentration (1.91). To address this, the experiment was repeated at two substrate concentrations, 100 μM and 1 μM (MUP). This was carried out for both mosses at contrasting sites, km 10.9 (Figure 5.5) low in available P, and km 107.9 (Figure 5.6) sufficient available P, on 26 November 1996. Mosses from both sites showed optimum activity at pH 5.5 at both substrate concentrations.

The influence of pH on the PMEase and PDEase activity was carried out with MUP, pNPP, bis-pNPP, at substrate saturation (100 μM), on mosses from km 10.9 on the 25 March 1997. The PMEase activity of both mosses was similar to previous results (see above), using either MUP and pNPP as substrate. PDEase activity was lower than PMEase at all pH values, but had a similar trend to PMEase, the optimum activity was observed at pH 5.5 (Figure 5.7).

5.6 Influence of element concentration

Concentrations of specific elements inhibit or stimulate acid and not alkaline phosphatase activity (1.92). It was envisaged that a contrasting response to inhibitors or stimulators would indicate whether acid or alkaline phosphatases were hydrolysing analogue PME or PDE substrates. The influence of several elements on acid or alkaline PMEase and PDEase activity of both mosses (Figure 5.8, 5.9, 5.10, 5.11), from km 10.9, was assessed. The elements Ca and K were added as chlorides, Zn as a sulphate and F as NaF to the assay medium. The concentrations increased logarithmically from 0.001 to 10 mM, final element concentration. Assays carried out with Na_2SO_4 and NaCl at the highest concentration (10 mM), showed that Na, chloride or sulphate had no affect on PMEase or PDEase of both mosses, at pH 5.5 or pH 9.5

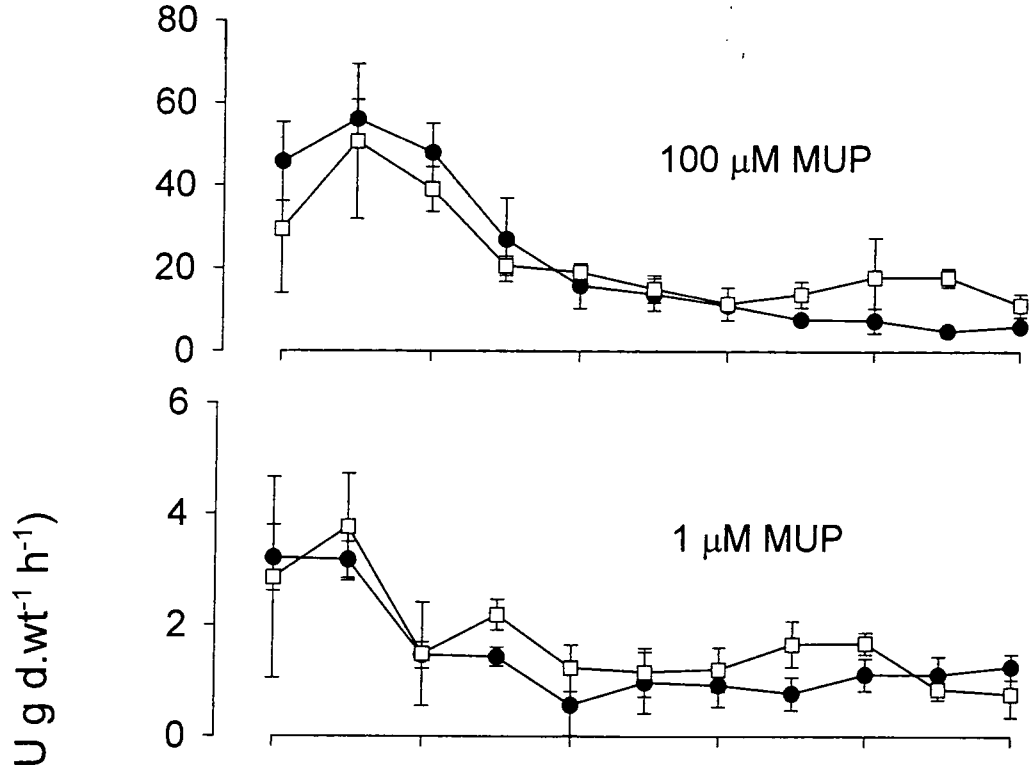

Figure 5.5 Influence of pH on PMEase of 2-cm tips of ● *F. antipyretica* and □ *R. riparioides* at 100 μM and 1 μM final concentration MUP at km 10.9, 26 November 1996. n = 4 ± S.D.

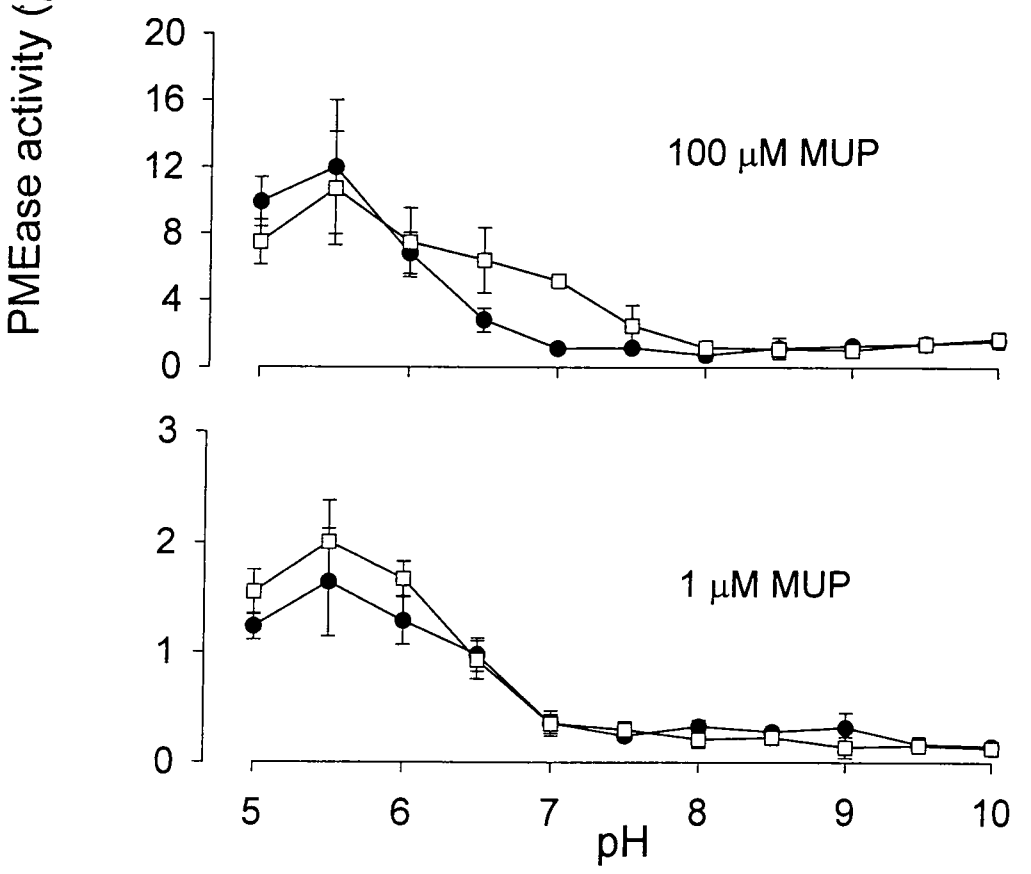

Figure 5.6 Influence of pH on PMEase of 2-cm tips of ● *F. antipyretica* and □ *R. riparioides* at 100 μM and 1 μM final concentration MUP at km 107.9, 22 November 1996. n = 4 ± S.D.

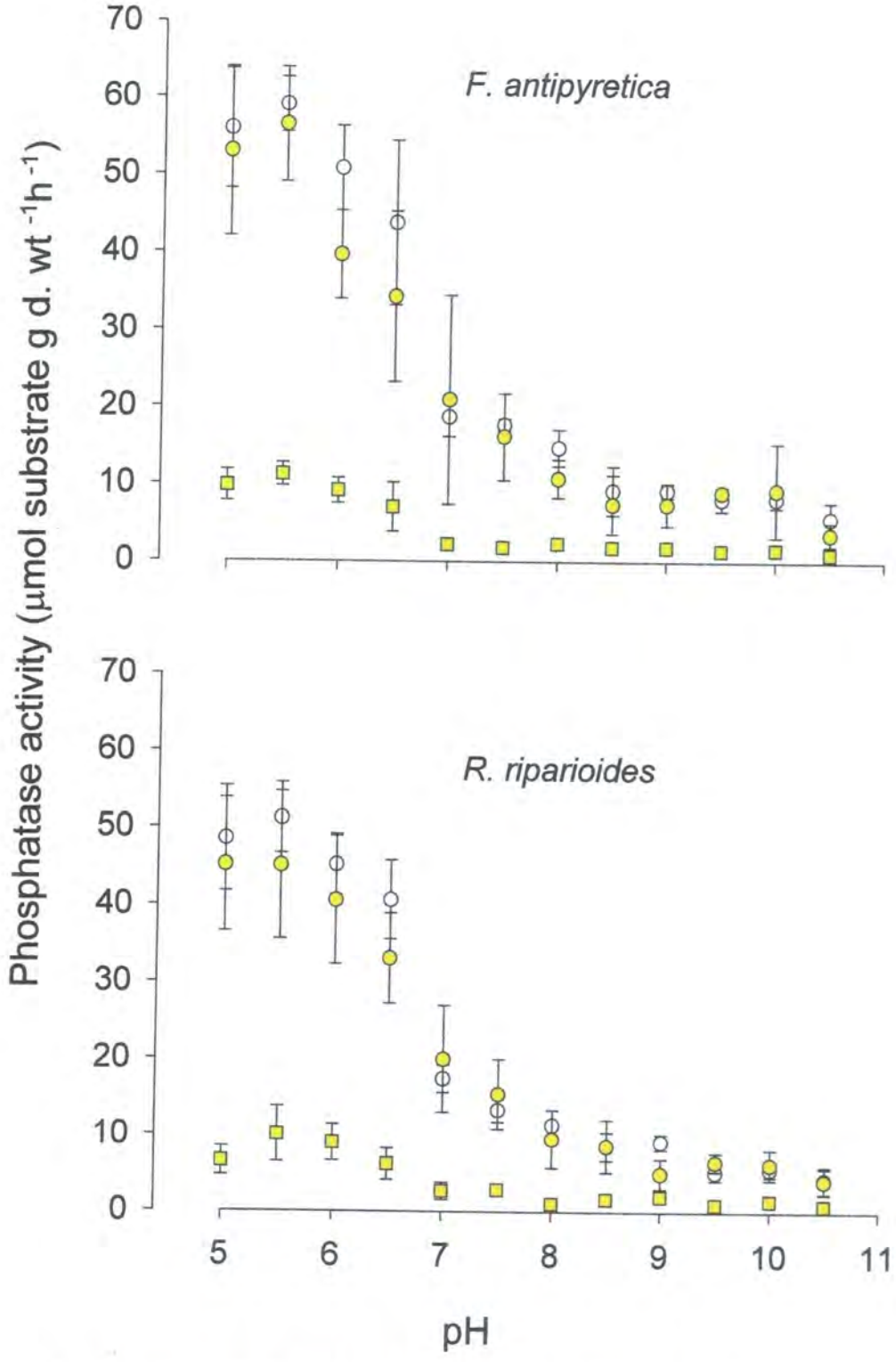

Figure 5.7 Influence of pH on phosphatase activity of 2-cm tips of *F. antipyretica* and *R. riparioides* from km 10.9, 25 March 1997. PME (○MUP, ● pNPP) and PDE substrates (■ bis-pNPP) at 100 µM final concentration. n = 4 ± S.D.

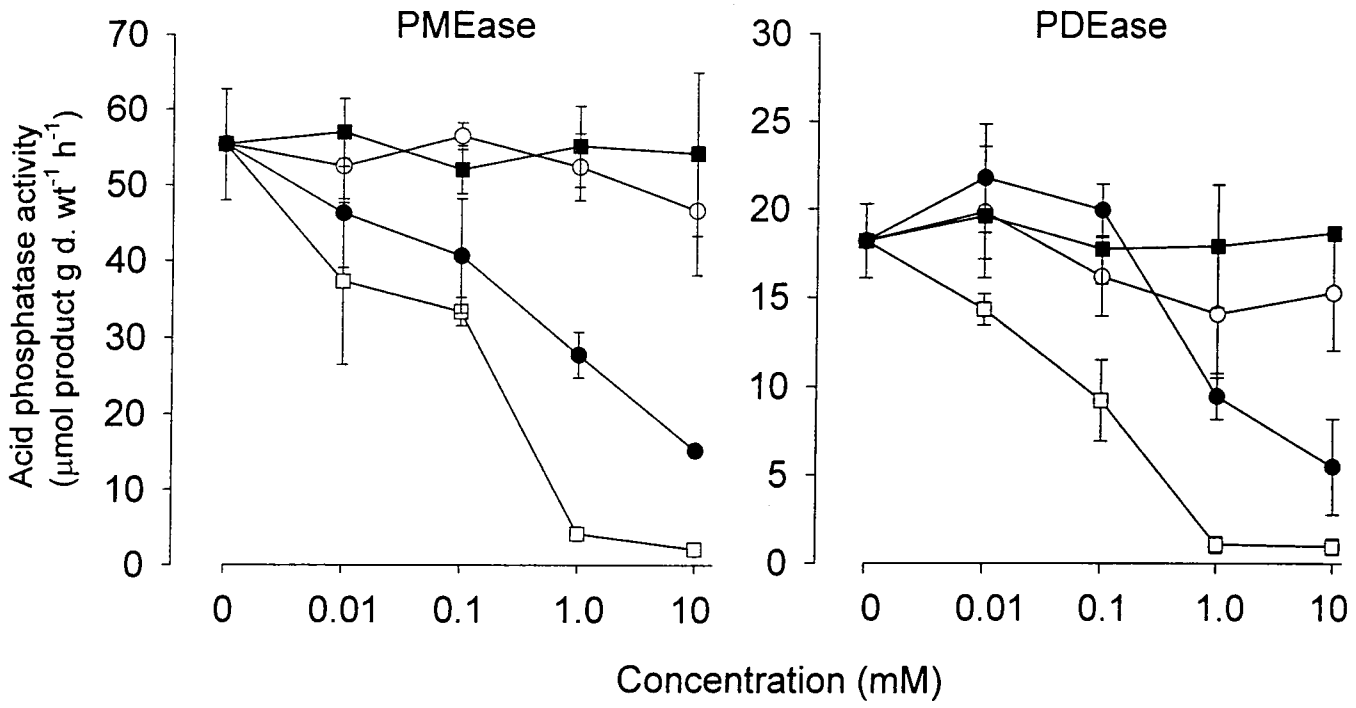

Figure 5.8 The influence of ■Ca, ● F, ○ K, □ Zn on the PMEase and PDEase activity of 2-cm tips of *F. antipyretica* from km 10.9 on 3 May 1997. n = 4 ± S.D.

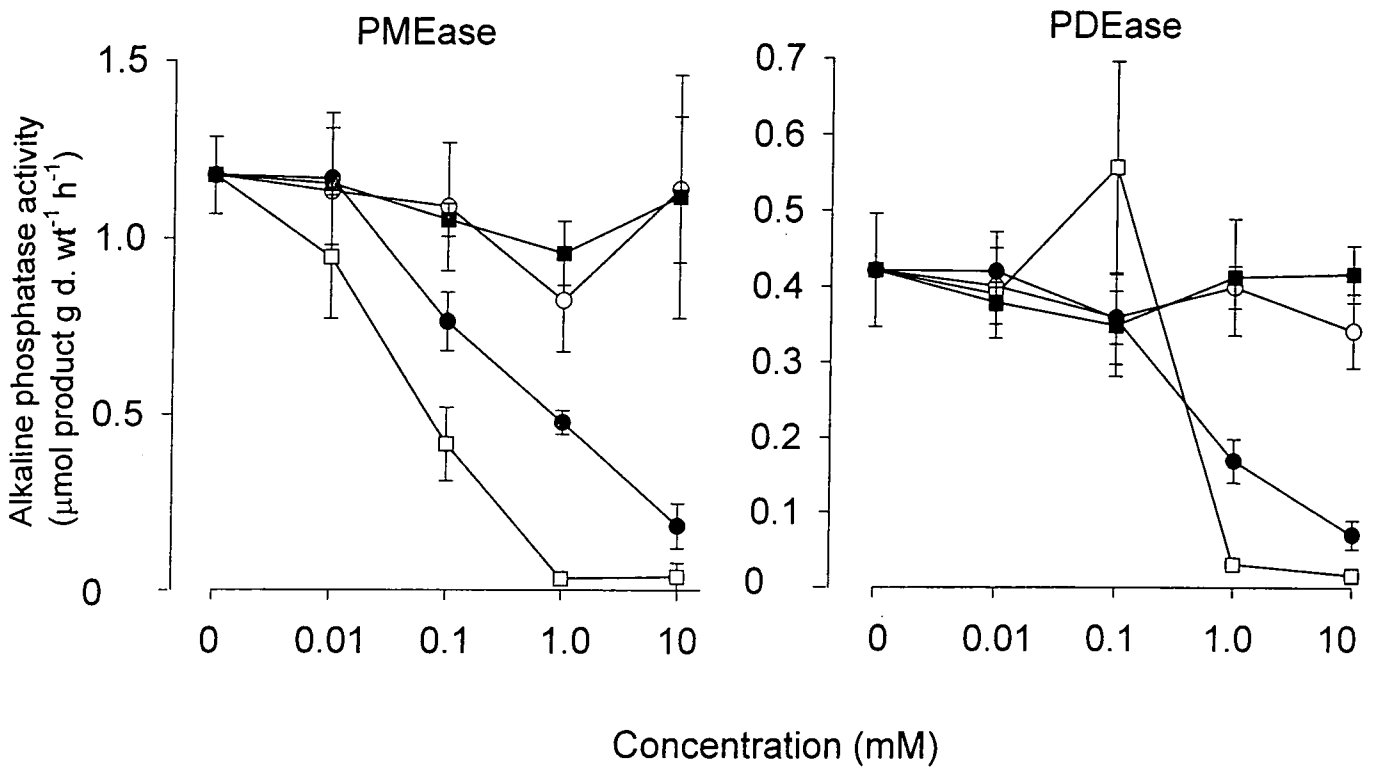

Figure 5.9 The influence of ■Ca, ● F, ○ K, □ Zn on the PMEase and PDEase activity 2-cm tips of *F. antipyretica* from km 10.9 on 3 May 1997. n = 4 ± S.D.

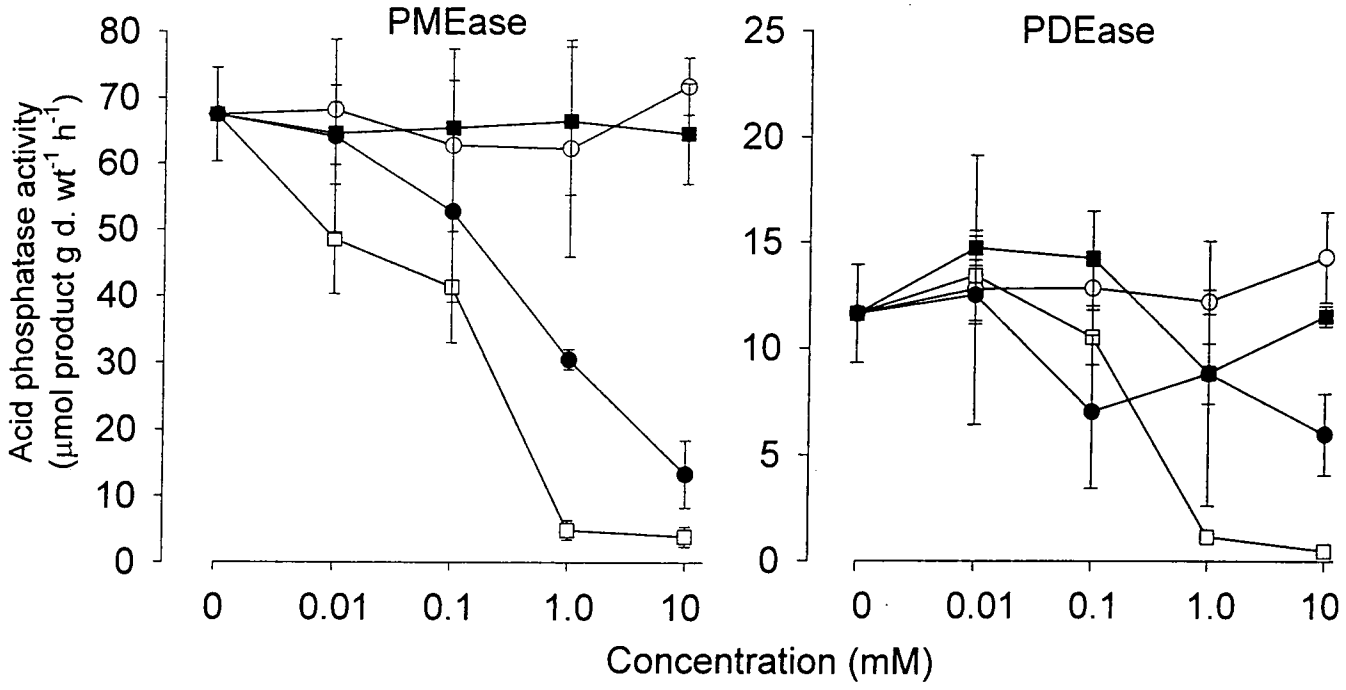

Figure 5.10 The influence of ■ Ca, ●F, ○K, □ Zn on the PMEase and PDEase activity of 2-cm tips of *R. riparioides* from km 10.9 on 3 May 1997. n = 4 ± S.D.

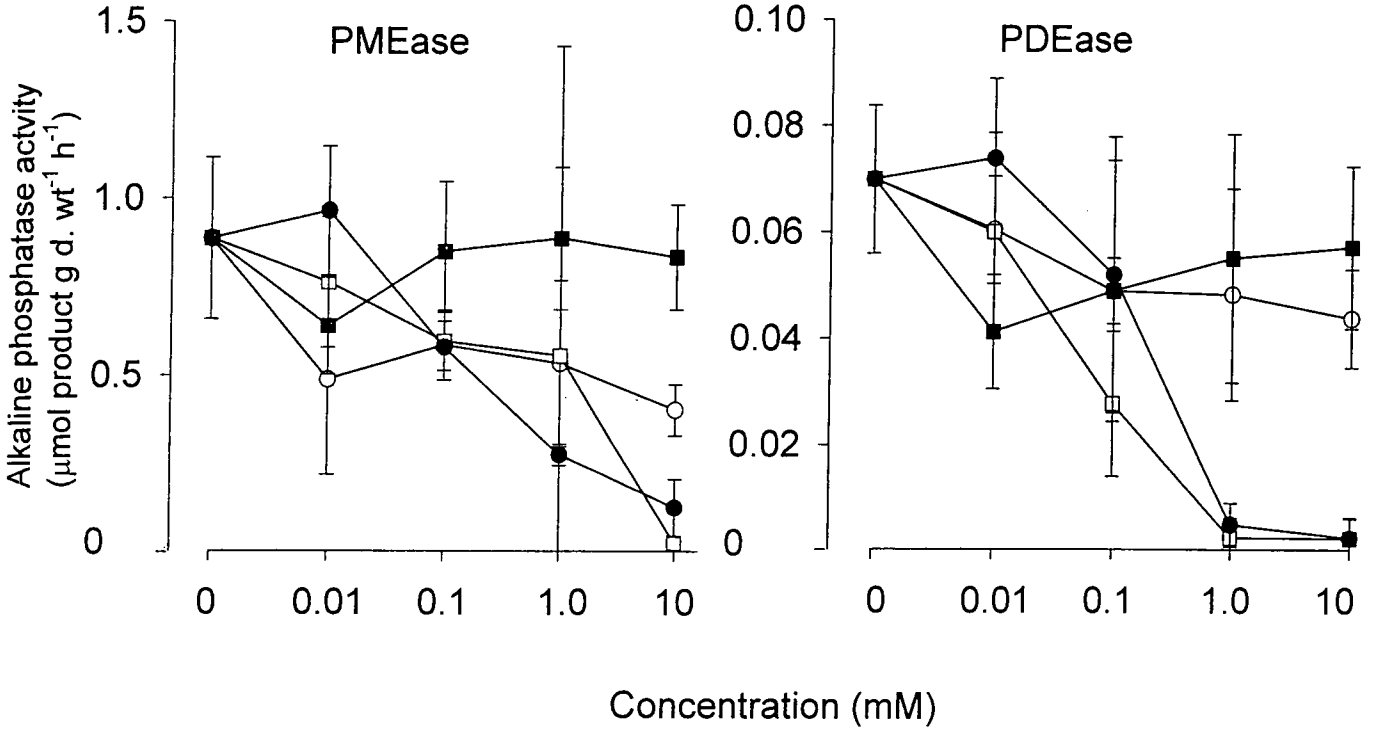

Figure 5.11 The influence of ■ Ca, ● F, ○ K, □ Zn on the PMEase and PDEase activity of 2-cm tips of *R. riparioides* from km 10.9 on 3 May 1997. n = 4 ± S.D.

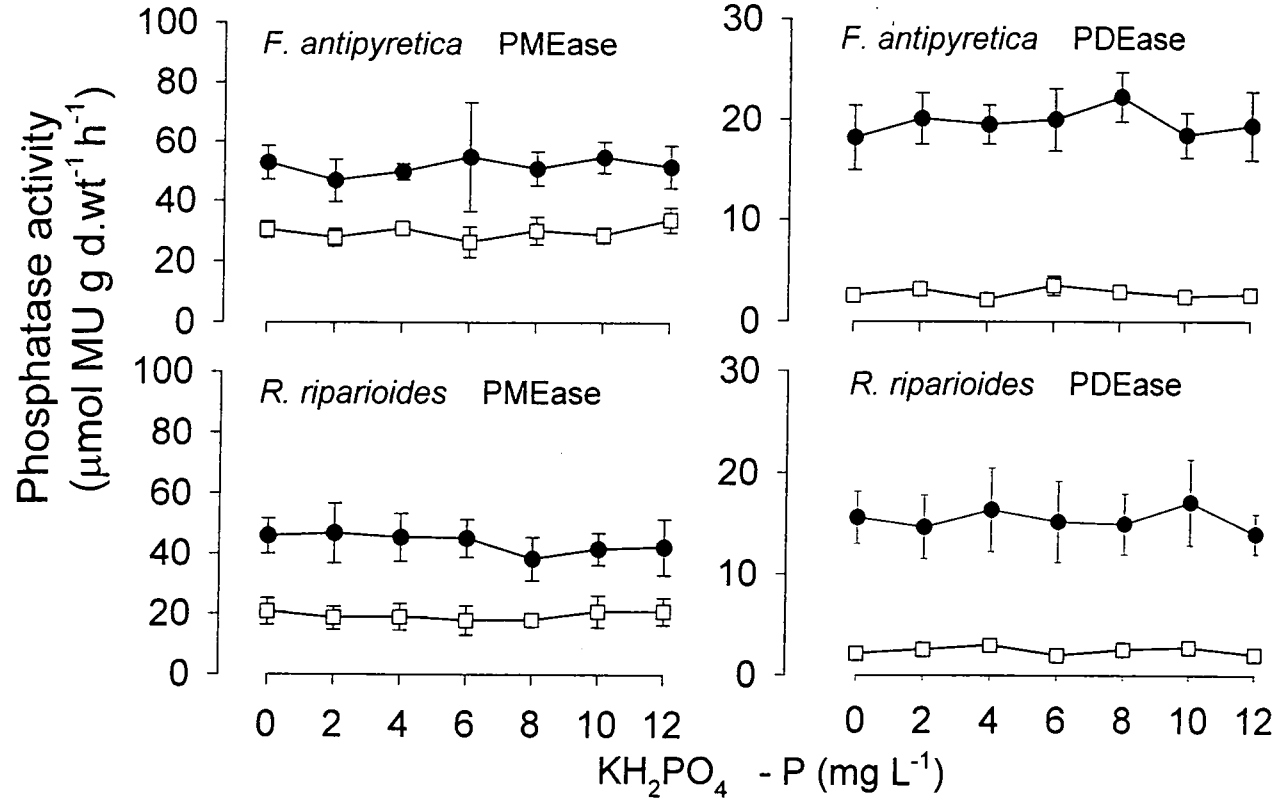

Figure 5.12 Influence of inorganic phosphorus on the PMEase and PDEase of *F. antipyretica* and *R. riparioides* from ● km 10.9 and □ km 107.9, R. Swale, 3 May 1997. n = 4 ± S.D.

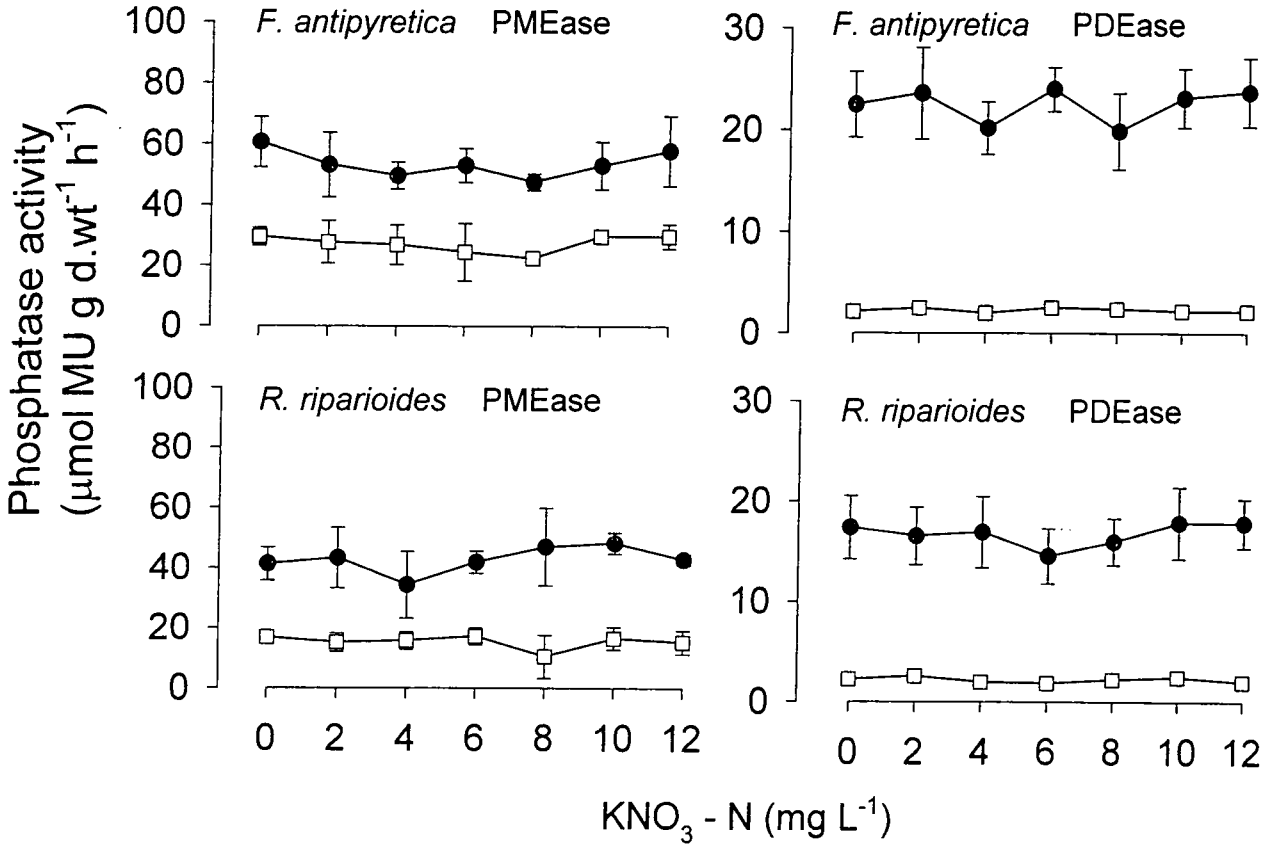

Figure 5.13 Influence of inorganic nitrogen on the PMEase and PDEase of *F. antipyretica* and *R. riparioides* from ● km 10.9 and □ km 107.9, R. Swale, 3 May 1997. n = 4 ± S.D.

Inhibition of acid and alkaline PMEase was detectable at a concentration of 0.01 mM Zn. At 1.0 mM Zn, acid and alkaline PMEase were reduced to "background" levels. F inhibited acid and alkaline PMEase less strongly, not reducing activity to "background" levels, even at the highest concentration, 10 mM F. Acid and alkaline PDEase were also reduced to "background" levels by 1.0 mM Zn, and although inhibition by F was noted, it was not clearly evident until 1.0 mM F. Ca and K had no detectable effect.

Similar results were found for *R. riparioides*, Zn and F inhibiting both acid and alkaline PMEase and PDEase. Ca and K appeared to have no detectable effect, although variable results were found for assays at alkaline pH values. The high variability of alkaline PMEase and PDEase hampered interpretation.

As K did not appear to inhibit PMEase and PDEase activity of either moss, N and P salts of K were used to test the influence of these nutrients on the acid phosphatase of moss, from km 10.9 and 107.9. Concentrations ranged from 0 to 12 mg L^{-1} N or P, values in excess of concentrations detected at the most nutrient enriched site from which mosses were sampled (km 107.9).

The N (Figure 5.13) and P (Figure 5.12) concentrations caused no detectable change on phosphatase of either moss.

5.7 Discussion

The experiments provided background information necessary for long-term studies, and directed research to answer specific questions. PMEase was shown to be saturated at substrate concentrations > 80 μM MUP, therefore, a concentration of 100 μM was chosen to estimate PMEase under saturated conditions during a seasonal study.

The inhibition of alkaline and acid PMEase and PDEase activity of both mosses by Zn, but more importantly F, suggests that the enzyme activity is characteristic of acid rather

than alkaline phosphatase. This suggests that activity measured at high pH values is likely to be acid phosphatase at non-optimal conditions.

The lack of inhibition by high concentrations of P suggests that there is no surface inactivation of phosphatase activity. This implies that the reduced rates of phosphatase activity detected at P sufficient sites are more likely due to intracellular, rather than extra-cellular concentrations of P.

5.8 Summary

i. The hydrolysis of analogue PME substrate (MUP) by *F. antipyretica* and *R. riparioides* was linear over a 60-min period

ii. PMEase activity of both mosses was saturated at substrate concentrations > 80 μM MUP.

iii. Optimum PMEase and PDEase activity of both mosses was evident under acid conditions (pH 5.5). Saturated and non-saturated concentrations of PME substrate did not alter the pH optimum of the PMEase of the two mosses.

iv. PMEase and PDEase was inhibited by high concentrations of F and Zn, whereas, Ca, K, N and P showed no inhibition or stimulation of enzyme activity at the concentrations tested.

CHAPTER 6

Seasonal study of moss phosphomonoesterase

6.1 Introduction

Dissolved N (4.41) and P (4.42) increase on passing down the Swale-Ouse. It is generally assumed that increased availability of nutrients will be reflected in the biota, by way of internal contents and physiological responses such as phosphatase activity.

A preliminary investigation of *F. antipyretica* and *R. riparioides* (Chapter 5), showed both were capable of expressing phosphatase activity. Comparison between sites of contrasting ambient nutrient concentrations, found elevated phosphatase activity in mosses at sites with low concentrations of dissolved inorganic P.

Further studies presented here, were carried out on these mosses with the aim of investigating spatial and temporal changes of P contents and phosphatase activity on passing down a river, over a one year period. This was carried out under standard laboratory conditions, to minimise the influence of natural fluctuations in temperature and pH, shown in chapter 5 to markedly affect the rate of phosphatase activity. As the variation of ambient pH in the Swale spanned nearly four pH units along the study area, the seasonal survey tested changes in moss phosphatase at three pH values, to estimate acid, neutral and alkaline phosphatase. The data collected over a one year period was correlated with a range of environmental variables.

6.2 Sampling strategy

Mosses were sampled approximately monthly, starting in October 1995, however, it proved impossible to collect material during periods of high flow, so it was sometimes necessary to wait a few days to collect a sample. Unfortunately, it proved impossible to sample any moss during February 1996 due to high flow. Furthermore, it was impossible to

find *F. antipyretica* at km 49.9 on three occasions, or *R. riparioides* at km 107.9 before July 1996, presumably due to a combination of low biomass and deep water making observation difficult. It was therefore decided to include a further sample at the end of the one-year period (October 1996). For simplicity, these records are treated as an annual dataset.

It had originally been hoped to obtain moss samples from Naburn Weir (km 145.0), but unfortunately moss growth was restricted to the weir itself, which proved too difficult and dangerous to sample. Nevertheless, water samples were taken from this site and the data are included in the previous chapter.

6.3 N and P content of mosses

The N contents of both *F. antipyretica* and *R. riparioides* increased twofold between the upstream and downstream sites, whereas the P content increased fivefold. The N : P ratio for both plants therefore decreased downstream (Table 6.1).

Table 6.1 Average N:P ratios by mass of dissolved water fractions and mosses from R. Swale October 1995 - October 1996. n = 12 except [a] n = 9 and [b] n = 4. LOIS: data from Land Ocean Interaction Study weekly sampling program.

Ratio		km - 2.5	km 10.9	km 49.9	km 107.9	km 145.0
Total dissolved N : P in water	This study	11.3	41.1	11.4	15.8	9.56
	LOIS			10.3	17.1	13.7
F. antipyretica N : P		14.9	12.9	10.5[a]	6.89	
R. riparioides N : P		12.5	9.76	4.68	5.55[b]	

Seasonal changes in the N and P contents of both mosses varied between the four sites (Figure 6.1). The N contents of both *F. antipyretica* and *R. riparioides* showed little seasonal variation at both km -2.5 and km 10.9. At both sites, *F. antipyretica* had slightly higher N contents than *R. riparioides*. At km 49.9 and km 107.9, seasonal variation was difficult to assess for *F. antipyretica* and *R. riparioides*, respectively, as full annual datasets were not collected. However, *R. riparioides* at km 49.9 had the highest N content on 14 November 1995 and the lowest on 6 March 1996. *F. antipyretica* at km 107.9 had higher N

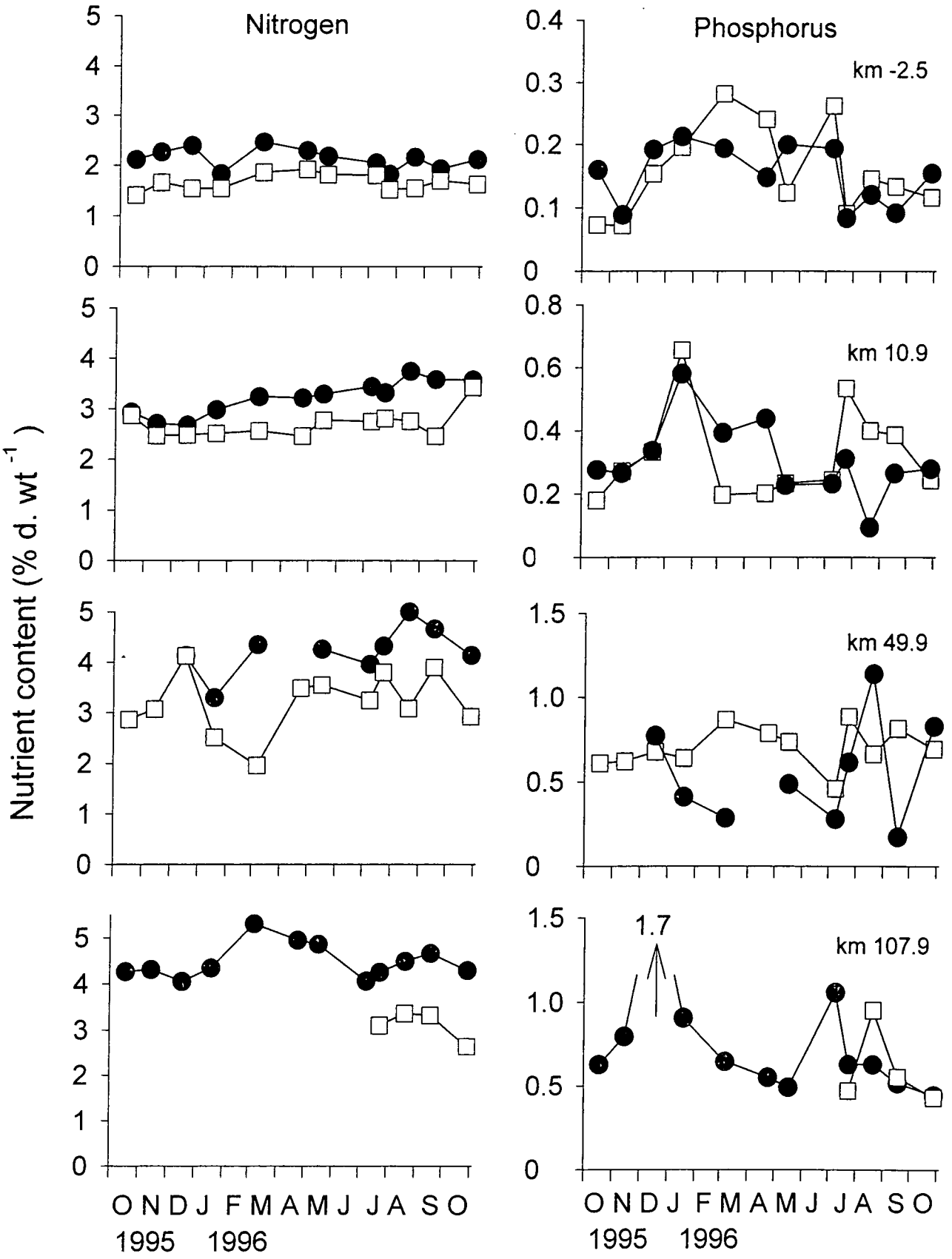

Figure 6.1 Nitrogen and phosphorus contents of 2-cm tips of ●*Fontinalis antipyretica* and □*Rhychostegium riparioides* from four sites within the Swale - Ouse catchment from October 1995 to October 1996.

concentration between January 1996 and May 1996, and lower values during the summer and autumn.

P contents of both mosses were more variable than N at all sites. Clear seasonal patterns were apparent for *F. antipyretica* and *R. riparioides* at km -2.5, higher concentrations were recorded in the winter and early spring, than in summer and early autumn. At km 10.9 a similar pattern was evident for *F. antipyretica*, but *R. riparioides* had high concentrations on 8 July 1996 as well as a winter maxima on 18 December 1995. At km 49.9, *R. riparioides* had high contents in both winter and summer, the data for *F. antipyretica* showed no clear pattern. A winter high was noted for *F. antipyretica* at km 107.9, and although summer values were generally low, a second peak was recorded on 8 July 1996. *R. riparioiodes* had comparable values to *F. antipyretica* on the four occasions it was sampled.

5.3 Phosphomonoesterase activity

F. antipyretica and *R. riparioides* both showed detectable PMEase activity at pH 5.5, 7.5 and 9.5 throughout the year at all the sites sampled (Figure 6.2), with the highest activity at pH 5.5. Both mosses showed similar seasonal and downstream trends. *F. antipyretica* almost always showed higher rates (per unit dry weight) at all three pH values than R. riparioides. In general, the highest activity occurred in late summer and autumn, and the lowest in late winter, early spring. The highest rates of activity were detected at km -2.5. Further downstream, rates for both mosses were an order of magnitude lower and the seasonal variation was less marked. Based on data for all sites, the relationship between acid PMEase activity and P content (Figure 6.3) suggests that phosphatase activity is enhanced in both mosses when tissue P concentration falls below 0.3 %. The relationship between PMEase and N : P of the moss (Figure 6.4) suggests that PMEase activity is enhanced when N : P > 7 : 1.

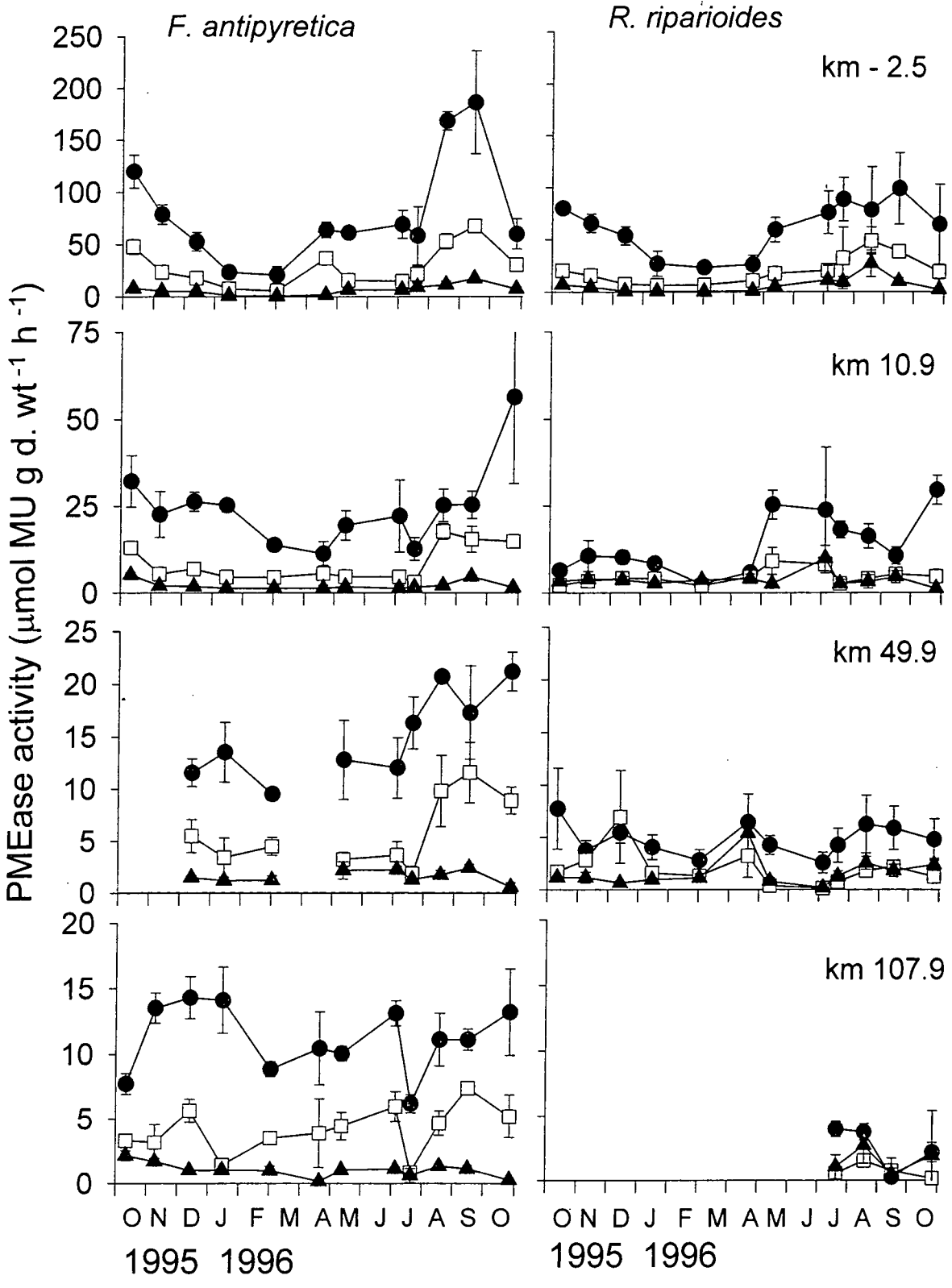

Figure 6.2 Seasonal changes of PMEase of 2-cm tips of *F. antipyretica* and *R. riparioides*. Assays were conducted at pH values; ● 5.5 □ 7.5 ▲ 9.5 and at 100 µM substrate concentration at four sites within the Swale catchment, October 1995 - October 1996

n = 4 ± S.D.

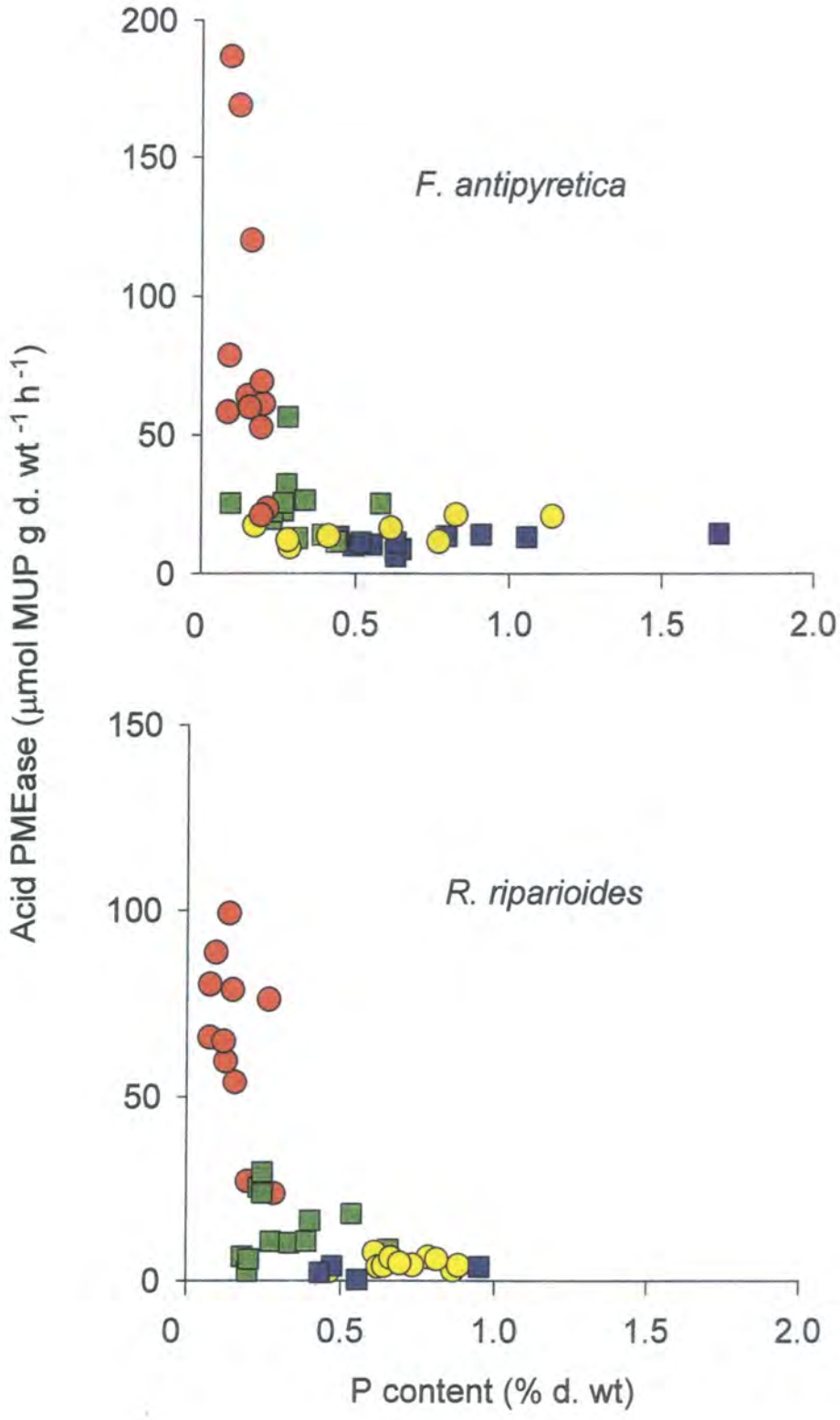

Figure 6.3 Relationship between acid PMEase and phosphorus content of 2-cm tips of *F. antipyretica* and *R. riparioides*. Samples were collected between October 1995 and October 1996 from: ● km -2.5, ■ km 10.9, ○ km 49.9 and ■ km 145.0.

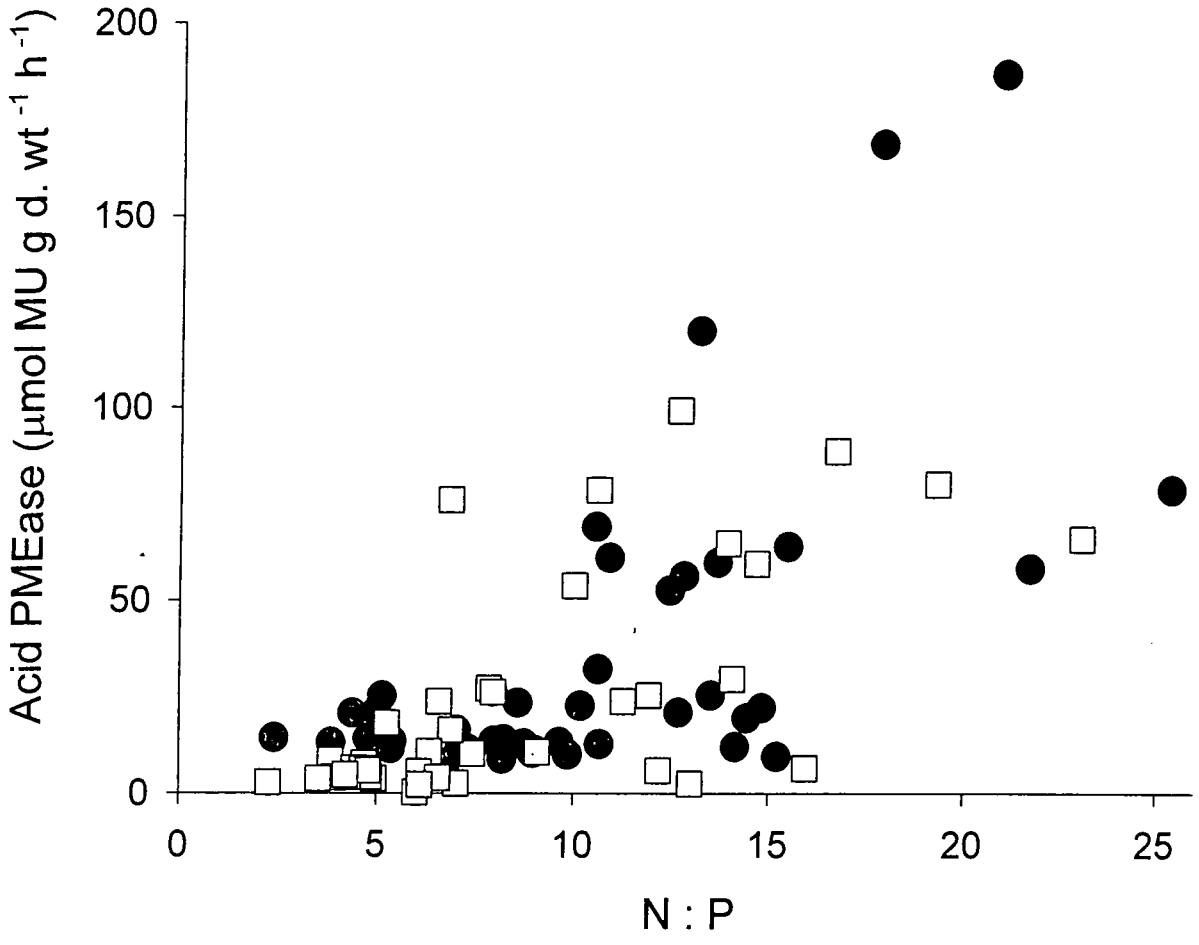

Figure 6.4 Relationship between N : P and PMEase activity of 2-cm tips of ●*F. antipyretica* and □*R. riparioides* from four sites within the Swale catchment from October 1995 to October 1996.

Table 6.2 Correlation between the PMEase activity of *F. antipyretica* and *R. riparioides* measured at three pH values (5.5, 7.5 and 9.5) and environmental variables recorded at the time of sampling, at km -2.5. Only significant ($p < 0.05$) values are shown.

	F. antipyretica			*R. riparioides*		
PMEase	5.5	7.5	9.5	5.5	7.5	9.5
Flow	-0.66	-0.67	-0.75	-0.83	-0.68	-0.53
pH	0.66		0.82	0.90	0.85	0.76
Temp.	0.54		0.65	0.78	0.80	0.68
Cond.	0.67	0.54	0.73	0.70	0.54	
A320						

Table 6.3 Correlation between the PMEase activity of *F. antipyretica* and *R. riparioides* measured at three pH values (5.5, 7.5 and 9.5) and environmental variables recorded at the time of sampling, at km 10.9. Only significant ($p < 0.05$) values are shown.

	F. antipyretica			*R. riparioides*		
PMEase	5.5	7.5	9.5	5.5	7.5	9.5
Flow						
pH						
Temp.						
Cond.				0.79		0.69
A320			0.57			

Table 6.4 Correlation between the PMEase activity of *F. antipyretica* and *R. riparioides* measured at three pH values (5.5, 7.5 and 9.5) and environmental variables recorded at the time of sampling, at km 49.9. Only significant ($p < 0.05$) values are shown.

	F. antipyretica			*R. riparioides*		
PMEase	5.5	7.5	9.5	5.5	7.5	9.5
Flow	-0.61					
pH	0.67					
Temp.	0.69					
Cond.			0.66			
A320		0.65				

The relationship between the PMEase activity of mosses and a range of environmental variables was tested by correlation at each site with a complete annual dataset (Table 6.2, 6.3, 6.4). At km -2.5, a significant positive relationship was found between PMEase and temperature, conductivity and pH, and negative relationship with flow. Correlation at other sites (km 10.9 and km 49.9) showed similar, but less significant results. There was no significant correlation between PMEase and environmental variables at km 107.9. There was no significant relationship at individual sites with any aqueous N or P fraction. There was, however, a positive relationship with aqueous N : P and PMEase, on passing down the river.

6.4 Discussion

F. antipyretica and *R. riparioides* showed spatial and temporal changes in both nutrient content and PMEase during a one-year study period. The N and P contents of both mosses increased on passing downstream, consistent with increases of nutrients in the water. No seasonal pattern was noted for N contents, but a clear pattern of increased P content was evident for both mosses over the winter and early spring at the two most upstream sites. However, this was less clear further downstream. The N : P ratio of both mosses decreased by a factor of 2 on passing downstream.

The PMEase activity of both mosses was highest in plants with low P content. The PMEase activity at pH 5.5 was higher than at pH 7.5 or 9.5 at all sites, and PMEase at pH 5.5 and 7.5 decreased on passing downstream. Correlation between PMEase activity at pH 5.5 and pH of the water was significant at km -2.5, but not at other sites. This relationship will be discussed in chapter 10. Seasonal trends of PMEase at pH 5.5 and 7.5 had winter minima and late summer maxima, again this was not apparent at pH 9.5. Seasonal variation in PMEase was the inverse of P content at km -2.5, although this was not so clear at other sites. It may be that PMEase changes rapidly with internal P fluctuation at sites of maximum P

stress, and less so where plants have greater internal P reserves. The absence of correlation between PMEase and dissolved nutrients suggests there is no instant response of the enzyme to external nutrient concentration, although longer term responses to substrate is discussed in the following chapter.

6.5 Summary

i. Acid, neutral and alkaline PMEase was detectable for both mosses at all four sites. The highest rates were recorded at the most upstream site for all pH values. Acid PMEase was always higher than neutral or alkaline PMEase at all sites.

ii. The relationship between PMEase and internal nutrient contents suggests that PMEase is elevated when P content is less than 0.3 % and N : P was > 7 : 1.

iii. Positive correlation was found between PMEase and temperature, conductivity, and pH and a negative relationship was found with flow. No relationship was found between PMEase and aqueous nutrients.

CHAPTER 7

Studies of moss phosphodiesterase

7.1 Introduction

The majority of studies on phosphatases in the environment focus on PMEase, although a range of organic phosphates may be expected in nature, and nucleic acids for instance, may well be more important than PME in many environments. The literature reviewed in chapter one points to the non-specific nature of PMEase for PME substrates. The relationship between the enzyme and potential substrate, as estimated by the use of analogue substrates, is unclear. What is clear, however, is that few purified PMEases have the ability to hydrolyse PDE substrates (Yoshida *et al.*, 1989). Therefore, investigating just the PMEase activity of an organism may not provide an accurate reflection of the organisms relationship to phosphorus in the environment.

In upstream streams and rivers, it is likely that soil leachate is often important as a source of organic P (1.42), but there may be considerable differences between different types of soil, which in turn may influence the streams. For instance, peat has been shown to contain higher concentrations of PDE than mineral soils. PDE such as DNA often enter soils from the initial breakdown of living matter. That they are less abundant over the range of soil types, suggests that they are highly labile.

The aim of the present study was to compare PDEase and PMEase activities of *F. antipyretica* from tributaries which drain both peat-dominated and agricultural mineral soils. The enzyme activity and the utilisation of phosphorus compounds may provide insight into what processes govern phosphorus cycling in streams of different physical and chemical character.

7.2 Environmental background

The PMEase activity of two mosses from the Swale catchment has been described previously (Chapter 5, 6). This Chapter includes streams within that catchment, and also some draining the same upland area, but forming part of other catchments (Tees to the north, Eden to the west). All the upland streams drain Ravenseat Moor (NGR NY 860 060), an upland gritstone plateau which is radially drained.

Land use in the catchments of the streams changes from undrained heathland and rough grazing ('moorland') in the upper parts of their catchment, to tilled land and mixed grazing and arable agriculture in the lower parts. Nearly all land above 300 m, except for the steeper slopes, was covered by hill peat.

7.3 Sites

Studies to investigate changes down the river continuum used one headwater site and some or all of six sites down the main river. The headwater site was changed to another stream from that used in chapter 6, because much of the *F. antipyretica* population had been removed in winter 1996 floods. The replacement site was on Stonesdale Beck, at a position 3.7 km upstream of its confluence with the river (km 3.5 downstream of the source of R. Swale). As the previous study showed marked differences in PMEase between a headwater site and km 10.9, two intermediate sites were added (km 3.4, km 4.9), and another site at km 30.5. Details of all the sites are listed in Table 7.1. A comparative study was also included on populations sampled at 20 sites on streams and rivers draining Ravenseat Moor (Table 7.2).

7.4 Sampling programme

Moss was sampled at all seven sites down the river continuum on 24 April 1997. Subsequent studies on material sampled on 3 and 7 May 1997, 30 September and 2 October 1997, were limited to five sites, because parts of the *F. antipyretica* populations had

Table 7.1 Descriptive characteristics of the sites used for study of the river continuum. Distance from the source shown in km (negative numbers indicate distance up a tributary from the confluence with the main river).

Site	Distance (km)	Grid ref.	River/Tributary	Elevation (m)
Stonesdale	-3.7 , 3.5	NY 863 032	Stonesdale Beck	400
Wainwath	3.4	NY 884 015	Swale	320
Keld	4.9	NY 898 009	Swale	270
Ivelet Bridge	10.9	SD 933 977	Swale	225
Hartley Park	30.5	SE 103 982	Swale	145
Catterick Bridge	49.9	SE 227 994	Swale	60
Thornton Manor	107.9	SE 433 715	Swale	15

been washed away at the other sites. The comparative study at 20 sites on Ravenseat Moor, used material sampled on 27 and 30 August 1997. Ten sites were sampled on each day, and there was no rainfall between the two days. For practical reasons, all sampling was done at times when the flow was relatively low. The 20 streams drained areas with differing components of three types of land use (Table 7.2). Uplands (> 300 m) were typically moorland, whereas areas below 300 m were good pasture or improved grazing. Streams draining moorland had lower pH values and higher absorbance at 320 nm (Table 7.2).

7.5 Results

7.51 Phosphatase response to distance downstream

A study at all seven sites on 24 April 1997 showed that PMEase activity at any particular site were similar, whether assayed with MUP or pNPP (Figure 7.1). PMEase activity was highest at km 3.4. There was a marked decrease in PMEase activity when tissue

Table 7.2 Environmental characteristics of 20 stream and river spot samples used for comparative study on, 27 and 30 August 1997.

Stream	Catchment	Altitude m	Grid ref.	pH	Cond. $\mu S\ cm^{-1}$	A320	FRP $\mu g\ L^{-1}$-P	FOP $\mu g\ L^{-1}$-P	TP $\mu g\ L^{-1}$-P	Upstream Land Use
River Swale	Swale	145	SE 103 982	8.24	380	0.117	17.6	4.6	27.5	Good pasture
Gill Beck	Swale	155	SE 096 976	8.28	564	0.006	25.5	4.4	40.5	Good pasture
River Eden	Eden	180	NY 782 044	8.50	210	0.088	2.6	2.7	6.6	Improved grazing
Argill Beck	Eden	200	NY 825 128	8.17	462	0.131	3.9	7.7	15.2	Improved grazing
River Belah	Eden	200	NY 823 120	8.13	325	0.134	13.9	9.0	27.2	Improved grazing
River Swale	Swale	225	SD 933 977	8.02	180	0.220	7.9	3.0	14.6	Good pasture
Straw Beck	Swale	240	SD 911 978	8.12	232	0.107	8.9	2.0	13.2	Improved grazing
Oxnop Beck	Swale	240	SD 934 977	8.05	324	0.029	3.6	4.0	10.9	Good pasture
River Greta	Tees	245	NY 995 133	8.12	280	0.385	9.6	5.0	14.6	Improved grazing
Arkle Beck	Swale	275	NY 999 036	8.13	320	0.133	4.3	<2.0	7.6	Improved grazing
Unamed	Eden	280	NY 847 123	8.02	492	0.053	4.9	8.3	17.9	Improved grazing
Thwaite Beck	Swale	290	SD 892 983	8.06	188	0.135	4.6	5.0	10.3	Improved grazing
Unamed	Eden	320	NY 868 131	7.97	427	0.189	16.6	2.0	18.6	Moorland
Birkdale Beck	Swale	350	NY 868 014	7.91	128	0.277	5.3	5.0	10.3	Moorland
SleightholmeBeck	Tees	360	NY 955 105	7.64	188	0.685	11.6	4.6	18.9	Moorland
Stony Gill	Swale	360	SD 929 963	8.15	280	0.014	3.6	6.0	10.6	Improved grazing
Stonesdale Beck	Swale	405	NY 863 032	7.57	240	0.320	16.2	<2.0	25.9	Moorland
Rea Gill	Tees	447	NY 888 079	7.79	157	0.533	5.6	9.6	15.2	Moorland
Birkdale Beck	Swale	455	NY 822 036	6.65	85	1.856	4.6	48.2	81.7	Moorland
Great Wygill	Tees	460	NY 884 081	7.83	229	0.220	4.3	6.3	13.6	Moorland

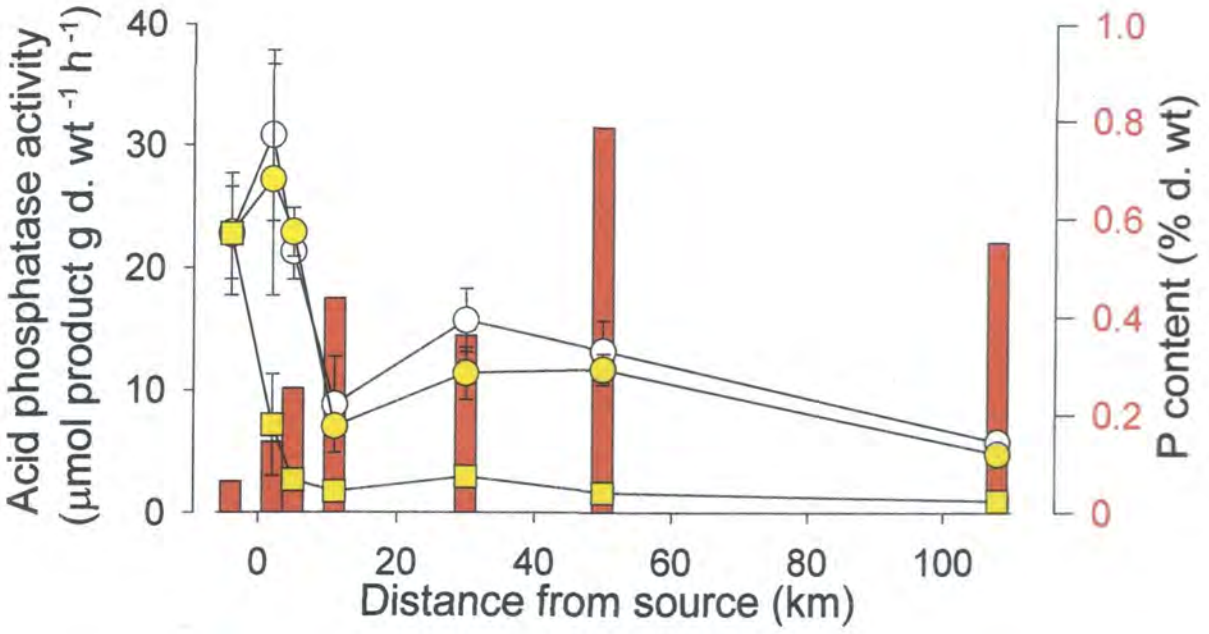

Figure 7.1 ■ Changes in P concentration, PMEase (○ MUP and ● pNPP) and PDEase (■ bis-pNPP) of 2-cm tips of *F. antipyretica* on passing down R. Swale on 24 April 1997.

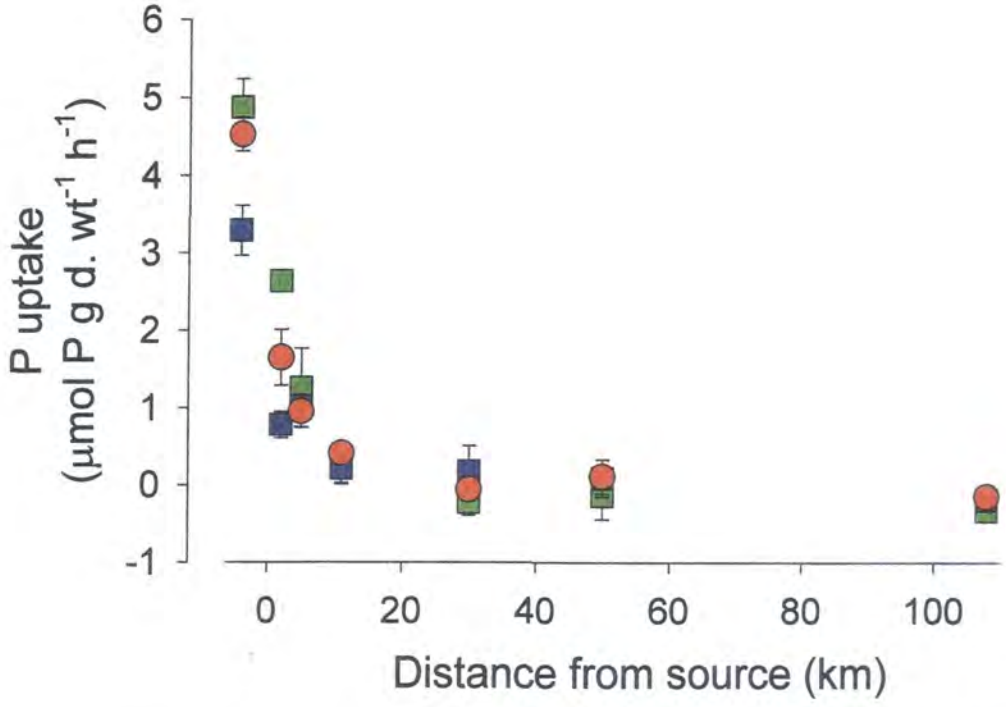

Figure 7.2 Changes in rate of P uptake from ● KH_2PO_4 ■ DNA and ■ phytic acid of 2-cm tips of F. antipyretica on passing down R. Swale on 24 April 1997.

P concentrations rose above 0.3 %. PDEase activity was highest at km - 3.0 (headwater site), but decreased more rapidly than PMEase activity down the river, it was less than PMEase at all except km - 3.0. PDEase activity (1 μmol substrate g d. wt^{-1} h^{-1}) at the most downstream site was much lower than PMEase.

7.52 Phosphorus utilisation

Two experiments were carried out to compare the ability of material from various sites to take up phosphate from the environment. These were conducted in the laboratory using 2-cm tips (2.8). The first experiment was carried out with materials sampled at the same time as those for the first study on phosphatase activities (24 April 1997). The efficacy of uptake from three soluble P sources (DNA, phytic acid and KH_2PO_4) was assessed.

Assays for P uptake over an 18-h period, showed there was a significant removal of KH_2PO_4, DNA or phytic acid (1000 μg l^{-1} P) from the medium by tips from the three more upstream sites. KH_2PO_4, DNA accumulated more rapidly than phytic acid, there was little, if any, P removal at the four downstream sites (Figure 7.2). Uptake by tips from km - 3.0, corresponded to an increase in internal P from 0.08 % to 0.33 %.

The second experiment was carried out with materials sampled at the same time as those for a comparison of phosphatase activities at the 20 upland stream sites (see 7.54). Because of the large number of samples, this was restricted to measurements of P removal from KH_2PO_4

7.53 Influence of substrate concentration

As the previous chapter showed that PMEase was lowest in late spring and highest in autumn, these two periods were chosen for kinetic studies during 1997, however, there was only sufficient biomass for assays at five sites. The substrate concentrations of PME and

PDE substrates were 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 250 μM. The values for V_{max} and Ks of PMEase in May, were similar using MUP or pNPP as substrates (Table 7.3).

Table 7.3 Kinetic parameters, V_{max} (μmol g d. wt^{-1} h^{-1}) and Ks (μM), for 2-cm tips of *Fontinalis antipyretica* using three substrates during May and September 1997.

Site	Substrate	May 1997		September 1997	
		V_{max}	Ks	V_{max}	Ks
km –3.7, 3.5	MUP	43.9	31.9	27.4	24.9
	pNPP	55.7	48.2	54.6	42.6
	bis-pNPP	76.9	78.3	136.3	48.6
km 3.4	MUP	74.4	50.3	162.2	83.9
	pNPP	75.5	60.0	186.0	114.2
	bis-pNPP	9.2	14.7	106.2	97.2
km 10.9	MUP	22.5	49.9	50.0	39.8
	pNPP	52.9	101.6	40.7	29.8
	bis-pNPP	10.7	54.4	7.4	13.3
km 49.9	MUP	26.4	47.9	36.3	42.8
	pNPP	28.7	55.2	53.3	86.1
	bis-pNPP	3.8	10.5	9.6	22.4
km 107.9	MUP	22.3	37.7	20.6	19.4
	pNPP	23.7	59.5	58.3	82.0
	bis-pNPP	6.31	42.6	10.7	33.4

However, they were almost always lower with MUP than pNPP in September. V_{max} was lower on both dates and with both substrates at km - 3.0 than km 3.4. However, V_{max} was higher with both substrates in September (MUP, 186.0; pNPP, 162.2 μmol g d. $wt.^{-1}$ h^{-1}) than in May (MUP, 74.4; pNPP, 75.5 μmol g d. $wt.^{-1}$ h^{-1}). Values for Ks changed relatively little on passing from the headwater down the river.

V_{max} and Ks for PDEase were highest at km -3.7, 3.5. Values for both kinetic parameters at this site were higher for PDEase than PMEase on both dates. V_{max} and Ks

were also high at km 3.4 in September, but not in May. V_{max} was typically an order of magnitude lower at km 10.9 and the downstream sties, than at km - 3.0. Ks was always higher than V_{max} at these sites.

7.54 20 stream survey

TP concentrations were usually lower in streams draining moorland than those draining other types of land. The FRP and FOP concentrations did not always fit the assumption that FRP : FOP ratio would be low at upland sites surrounded by organic soils. However, FRP : FOP was approximately 1.0 or less at four of the seven moorland sites. It should also be noted that the sampling was undertaken during a period of low flow. Results presented in Chapter 4 suggest a positive correlation between flow and FOP concentration, in upland streams in Swaledale.

Mosses from moorland streams had higher PMEase and PDEase activity compared with other sites, and there were marked differences in the ratio of PDEase : PMEase with altitude (Figure 7.3), with a positive ($p < 0.01$) relationship between the two. PMEase and PDEase activity showed a negative relationship with tissue P concentration, but none with any aqueous P fraction. P uptake experiments conducted on materials sampled at the same time, showed that there was also negative relationship ($p < 0.01$) between uptake and tissue P content.

It is unlikely that the PDEase : PMEase ratio had a direct causal relationship with altitude. An intercorrelation matrix for a number of environmental varibles (Table 7.4) showed several variables had a significant positive relationship with altitude. These were P uptake, PMEase, PDEase, A320, those with a significant negative relationship were pH and tissue P content. Although no relationship was detectable with FOP, there was a positive significant relationship between A320 and FOP.

Table 7.4 Intercorrelation matrix of environmental variables from the 20 stream survey, on 27 and 30 August 1997. Only significant ($p < 0.05$) values are shown.

Variable	PMEase	PDEase	PO_4-P Uptake	A320	Altitude	Tissue P	PH	FRP	FOP	TP
PDEase	0.86	1.0								
PO_4-P Uptake	0.77	0.89	1.0							
A320		0.55	0.61	1.0						
Altitude	0.77	0.84	0.76	0.56	1.0					
Tissue P	-0.84	-0.70	-0.74		-0.73	1.0				
pH		-0.67	-0.71	-0.91	-0.73		1.0			
FRP								1.0		
FOP			0.54	0.89			-0.80		1.0	
TP				0.78			-0.73		0.86	1.0
Cond.				-0.54	-0.57					

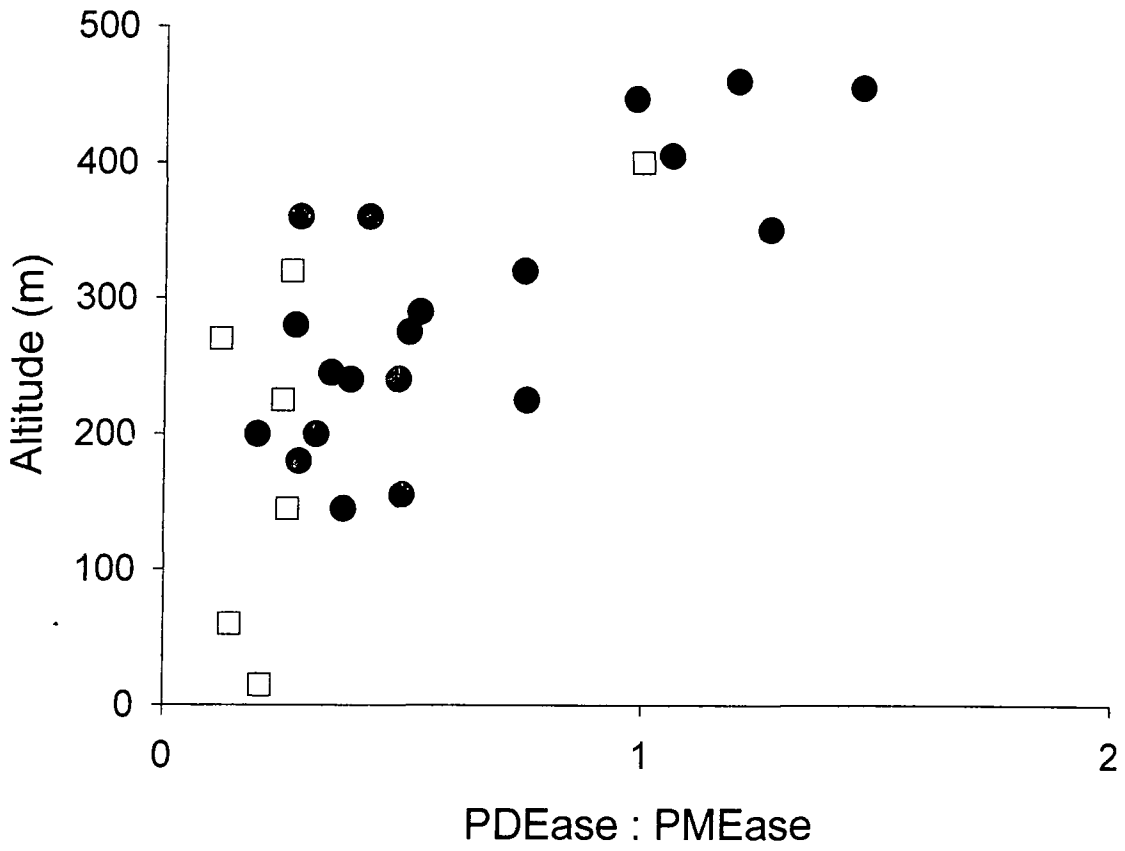

Figure 7.3 Relationship between ratio of PDEase : PMease of 2-cm tips of *F. antipyretica* and altitude of site from which samples were taken. Data are based on two surveys: Swale continuum survey of □24 April 1997; and the 20 stream survey of ●27 and 30 August 1997.

7.6 Discussion

PDEase of 2-cm tips of *F. antipyretica* decreased on passing down a continuum of six sites on the river Swale on 24 April 1997. At the most upstream site, PDEase activity was comparable to that of PMEase, but decreased to background levels further upstream than PMEase. This response may be mosses responding to potential diester substrates supplied by moorland peat. Alternatively, PDEase may be synthesised as part of a suite of enzymes during nutrient limitation. PDE such as DNA are potential sources of C and N as well as P.

The rate of uptake of P from three sources, two organic (DNA and phytic acid) and one inorganic (KH_2PO_4), was greater at the most upstream site of the six sites assessed for phosphatase activity. P from DNA was taken up at a similar rate to that of inorganic P, whilst phytic acid-P was less rapidly utilised. The elevated rate of hydrolysis of analogue diester substrates and the utilisation of P from DNA, add weight to the theory that upland mosses are adapted to sequester nutrients from organic sources.

The kinetic parameters V_{max} and Ks were estimated using three substrates (two PME and one PDE), during two surveys in late spring and autumn 1997. PMEase V_{max} was higher in autumn than spring at all sites, except the most upstream, and both PME substrates had comparable results. PMEase Ks values were variable, and no clear pattern was detected on passing down the river. PDEase V_{max} was higher in autumn than in spring, it was highest at the most upstream site, and decreased on passing down the river. Ks values were higher at the two most upstream sites compared with all others suggesting a low affinity for the analogue substrate.

F. antipyretica collected from 20 sites in three catchments, near to the source of the Swale, had phosphatase activity that increased with altitude. The ratio of PDEase : PMEase also increased with altitude. Although the relationship is presumably not causal, it points to a possible relationship between phosphatase and potential substrate supplied from peat soils

found at higher altitudes. Positive correlation between PDEase and A320 reiterates the relationship between enzyme activity and leachate from peat. However, whether the humic water is related to an organic P source, or if humic substances are binding and immobilising inorganic P is uncertain. PDEase and PMEase were negatively correlated with internal P content, with elevated levels of enzyme activity occurring when P contents were < 0.3 % consistent with studies of plants from the main river.

7.7 Summary

i. PMEase and PDEase decreased on passing down the river Swale, PDEase : PMEase was equivalent at the most upstream location.

ii. Uptake of P from DNA and inorganic P had similar rates at the most upstream sites, whereas uptake from phytic acid was slightly lower.

iii. The influence of substrate concentration (MUP, pNPP, bis-pNPP) tested on two occasions showed higher rates of hydrolysis in autumn compared with late spring for all three substrates. V_{max} of PDEase was higher than PMEase at the most upstream site on both occasions.

iv. PDEase activity was correlated positively with A320, P_i uptake, altitude, and negatively with tissue P, pH. PMEase activity was correlated positively with P_i uptake and altitude, and negatively with tissue P.

CHAPTER 8

Transplantation of mosses

8.1 Introduction

The relationship between inorganc phosphorus concentration and the surface phosphatase activity of mosses appears to be an indirect one. Mosses sampled from Swaledale have been shown to be capable of expressing PMEase and PDEase activity (Chapter 5, 6, 7), particularly in plants with internal P contents less than 0.3 % g d. wt^{-1}. In general, such plants were located in upland streams with low concentrations of dissolved P, although no direct correlation has been found between phosphatase and dissolved P. Laboratory studies (5.6) have shown that neither *F. antipyretica* nor *R. riparioides* show surface inhibition of PMEase or PDEase under high concentrations of P in the medium. However, the occurrence of PDEase in moss from streams influenced by soils potentially rich in PDE, suggests that synthesis of specific enzymes may respond to external organic P substrates, but over what timescale ?

To assess the relationship between dissolved P, the internal P contents and phosphatase activity of *F. antipyretica*, a field study was carried out involving a reciprocal transplant between two sites of contrasting ambient chemistry, an upstream oligotrophic stream and a meso-eutrophic lowland river. The rates of change in phosphatase activity of the transplanted population would be compared to the natural, seasonal, fluctuations of the *in situ* population.

8.2 Sites

Two sites were chosen for reciprocal transplants, both of which had been studied in

Table 8.1 Environmental variables of the upstream site at km 3.4 from 3 May to 22 September 1997 (n = 9), and of the downstream site km 107.9 from 13 August to 22 September 1997 (n = 6) . Cond. = conductivity at 25°C

variable		km 3.4			km 107.9		
		min	mean	max	min	mean	max
Temp.	°C	7.3	11.6	16.2	19.0	20.6	21.2
pH		5.94	6.75	7.61	7.85	8.08	8.24
O_2	%	104	111	126	102	105	108
Cond.	$\mu S\ cm^{-1}$	78	108	134	424	562	626
Tot. alk.	$meq\ L^{-1}$	0.003	0.16	0.34	2.23	2.83	3.21
A320		0.103	0.225	0.334	0.054	0.066	0.081
NO_3-N	$\mu g\ L^{-1}$	75	107	140	4320	4680	5560
NO_2-N	$\mu g\ L^{-1}$	< 2.0	6.0	15	5.0	12	22
NH_4-N	$\mu g\ L^{-1}$	18	34	55	22	36	52
FRP-P	$\mu g\ L^{-1}$	5.8	9.1	14	98	135	160
FOP-P	$\mu g\ L^{-1}$	< 2.0	3.2	5.4	< 2.0	8.8	12
TP-P	$\mu g\ L^{-1}$	6.1	13	20	143	161	184

detail previously (Table 8.1). The upstream site was at Wainwath Falls, 3.4 km downstream from the source of the Swale, the downstream site was Thornton Manor, km 107.9. *F. antipyretica* was abundant and easily accessible at both sites.

8.3 Transplants, sampling and analysis

Transplants from the downstream to upstream site were made on 3 May 1997 and transplants from upstream to downstream on 13 August 1997. Four boulders with attached plants of *F. antipyretica* were selected from each site. Care was taken to select flat-bottomed boulders of about 20 kg, which would not be prone to being moved during high flows, after they had been transplanted. The boulders were placed in the boot of a car, wetted with river water and wrapped in plastic sheeting and frozen gel packs to prevent drying. Transport between the sites took approximately an hour. The boulders were transplanted to the lee of large *in situ* rocks at the new site, away from zones of highest flow.

The experiments were run until marked changes in the phosphatase activity of the transplanted specimens relative to seasonal fluctuations in native plant phosphatase activity were observed. Following the transplant, the upstream site was sampled for water and native and transplanted moss after four days. Then approximately weekly on four occasions, and subsequently monthly on two occasions, until the final samples were taken on 22 September 1997. Following the transplant, the downstream site was sampled approximately every two days on four occasions, and then the final samples on 22 September 1997. The populations were sampled from surfaces which were fully submerged and where the water was flowing. In order to provide sufficient material, typically three whole plants of approximately 30 tips each were harvested. The plants were washed in river water and then transported in individual plastic bags to the laboratory in a cool box. The analysis of dissolved and total P, preparation of shoots and assays for phosphatase were done on the same day, typically within

five hours of sampling. Tips were dried overnight for analysis of internal contents. Filtered water samples for dissolved N fractions were frozen and analysis carried out within two weeks.

8.4 Results

8.41 Water

Differences in the chemistry of the upstream (km 3.4) and downstream (km 107.9) sites are summarized in Table 1, though the experimental period was much longer (142 days) at the upstream than the downstream site (39 days). In comparison with differences between mean values for sites, variability between sample dates for the various determinants was low. The downstream site showed higher values for mean pH, conductivity, total alkalinity, nitrate, nitrite, FRP and TP. Ammonium was about the same at both sites, while A320 was lower at the downstream site. Nitrate and FRP were both about one order of magnitude higher at the downstream site.

8.42 Moss

The first transplant was made from downstream to upstream. C, N and P composition of *F. antipyretica* shoots at both sites on 3 May, and subsequent changes in the *in situ* downstream and transplanted plants are shown in Figure 8.1. N concentration was slightly higher and P concentration much higher at the downstream site prior to the transplant experiment. N concentration in the transplanted moss fell from 3.78 to 3.02 % d. wt during May, and stayed at about this concentration until the final sample in September. P concentration stayed at around 0.5 % d. wt throughout May then declined to a final concentration of 0.32% d. wt, C exhibited no clear pattern.

Variability of PMEase activity was usually quite high, but it is clear that activity showed a slow increase following the transplant from downstream to upstream (Figure 8.2),

with broadly similar results using either of the two PME substrates. PMEase activities of the *in situ* population showed conflicting trends during the first four days according to whether assays were made by pNPP or MUP. PMEase activity (assayed with either substrate) was always greater than that of the transplanted population, though the relative difference between the two populations decreased with time. As a percentage of mean activity of the native population on a particular day, the transplanted population rose from 48 to 73 % (MUP) and 21 to 61 % (pNPP) from the start to the end of the experiment. After the initial sample, PDEase activity was low throughout May, reached a maximum in late June and then dropped again, although it did not return to the low rates recorded in May. It was lower than PMEase activity on all sampling occasions. PDEase activity of the transplanted moss showed no marked change throughout the sampling period, and remained at about 5 μmol pNP g d. wt^{-1} h^{-1}, which is considered to be the "background" level.

In the case of the 32-day study to assess the impact of transplants from upstream to downstream, the *in situ* downstream population showed no obvious changes in the concentrations of C, N and P (Figure 8.3) nor in PMEase or PDEase activities (Figure 8.4).

The transplant showed a slight fall in C content, none in N content, and marked changes in P content (Figure 8.3) and phosphatase activities (Figure 8.4). P increased 12-fold within the first 2 days to concentrations similar to the native population. In contrast, use of pNPP as a substrate revealed an increase in PMEase activity after 2 days, though all subsequent samples showed a decrease in activity. The final value for PMEase activity was about 20 % of the original value. PMEase activity assayed by MUP, and even more so PDEase activity, declined from the start of the transplant; PDEase activity fell to 4 % of the initial activity.

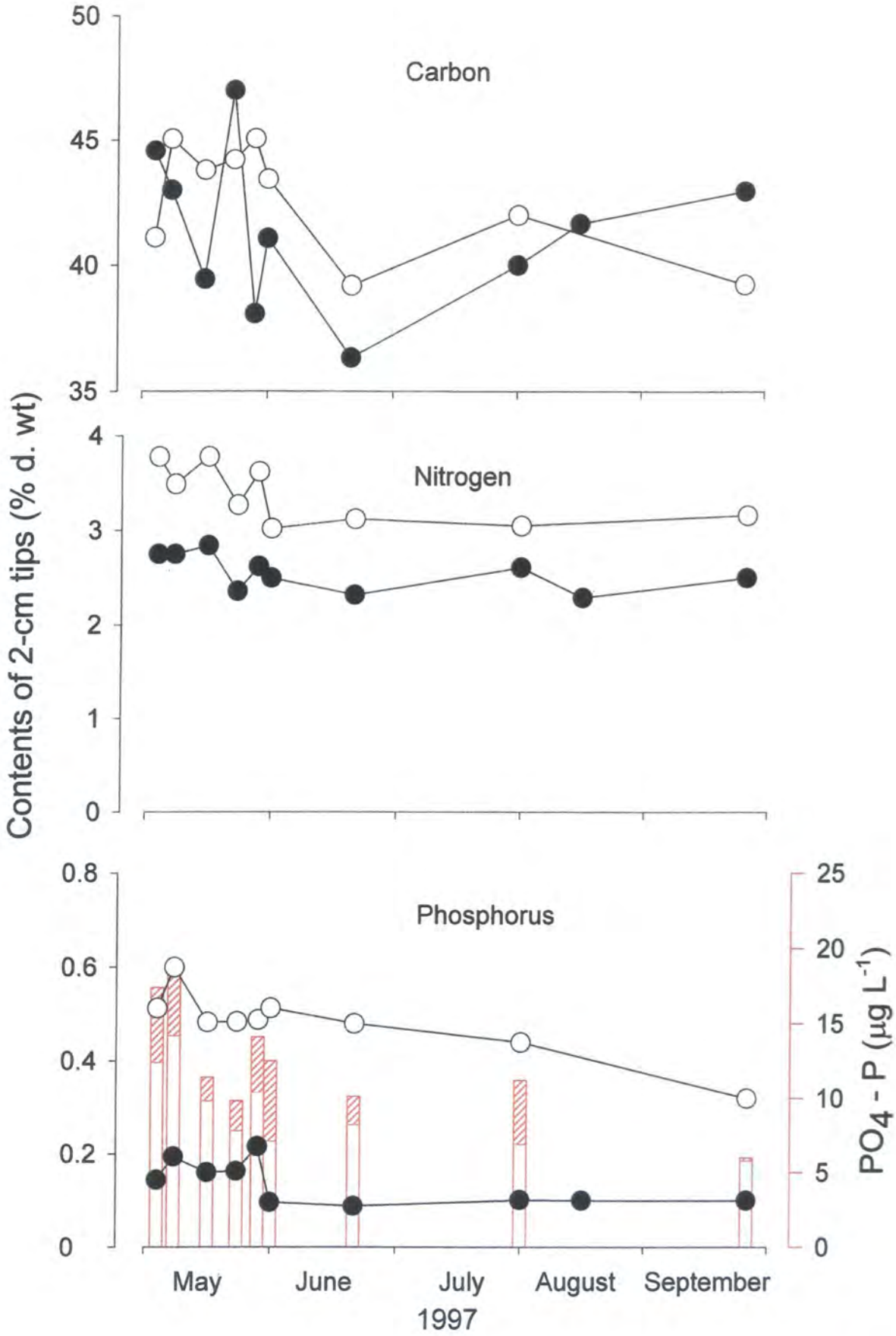

Figure 8.1 Changes in C, N and P contents of 2-cm tips of *F. antipyretica* sampled from *in situ* populations at ● km 3.4 and populations transplanted to km 3.4 from ○ km 107.9. Dissolved P concentrations; ▨ FOP and □ FRP, are also shown.

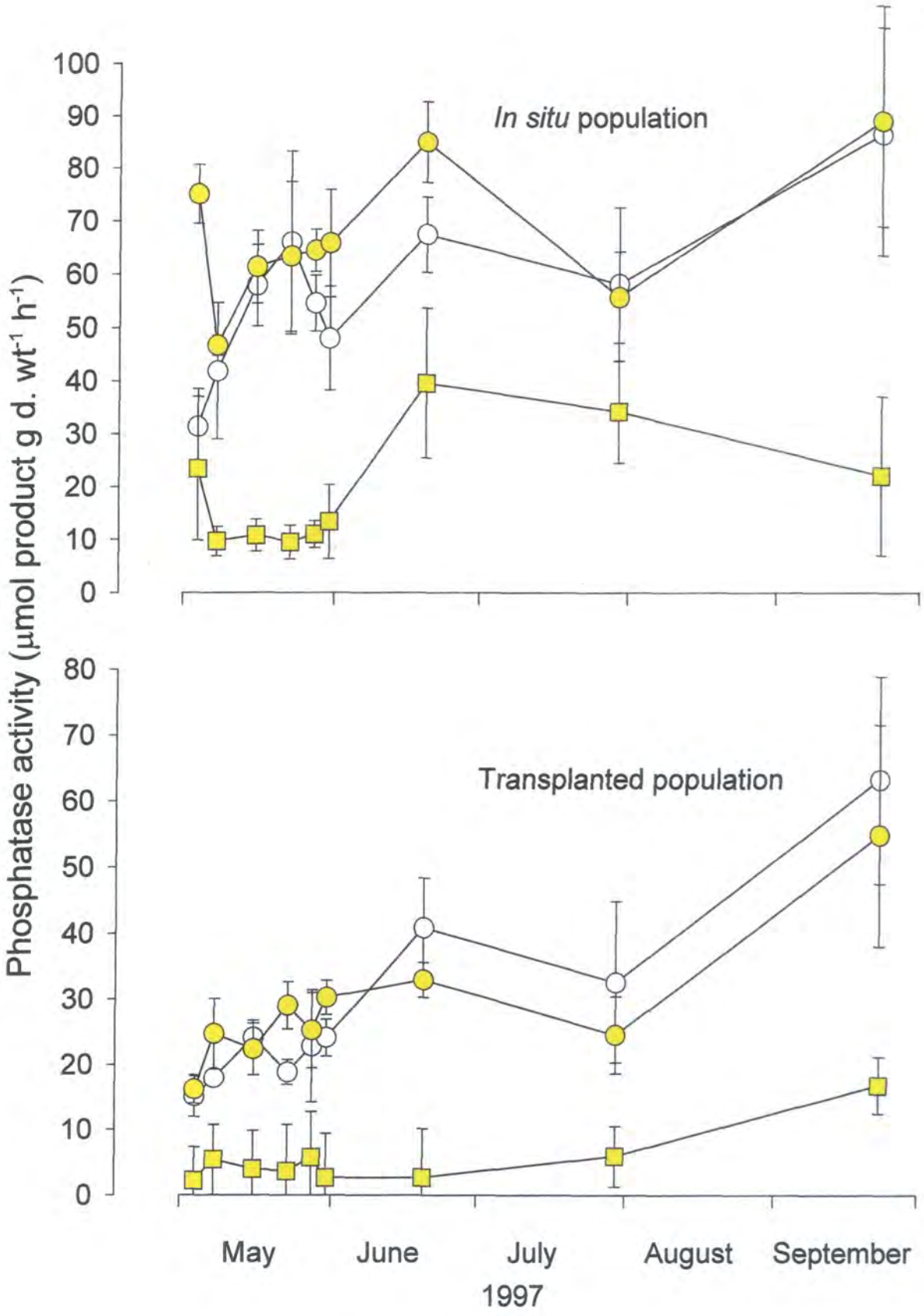

Figure 8.2 Changes in PMEase (○ MUP and ● pNPP) and PDEase (■ bis-pNPP) of 2-cm tips of *F. antipyretica* sampled from *in situ* populations at km 3.4 and populations transplanted to km 3.4 from km 107.9. n = 4 ± S.E.

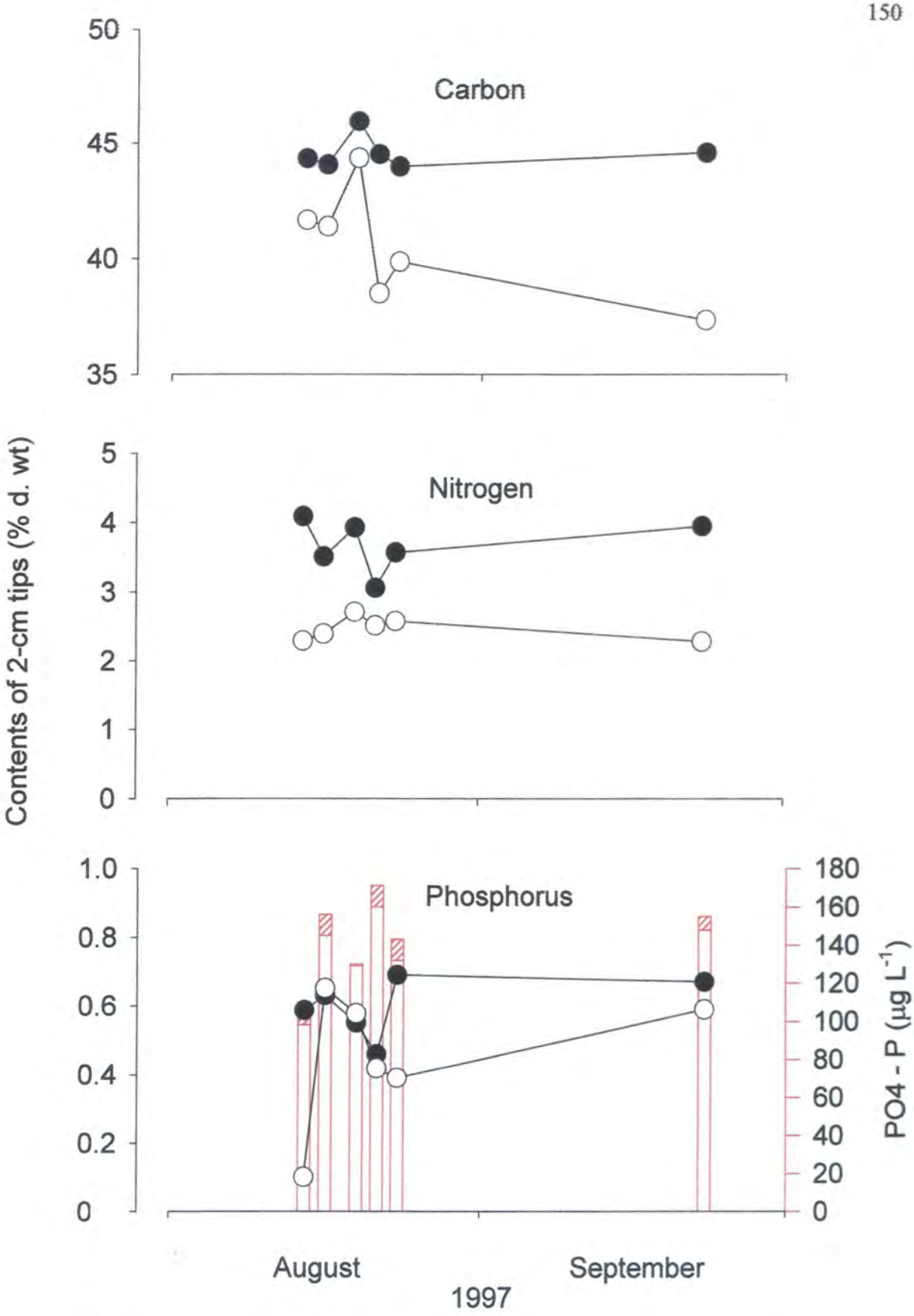

Figure 8.3 Changes in C, N and P contents of 2-cm tips of *F. antipyretica* sampled from *in situ* populations at ● km 107.9 and populations transplanted to km 107.9 from ○ km 3.4 Dissolved P concentrations; ▨ FOP and □ FRP, are also shown.

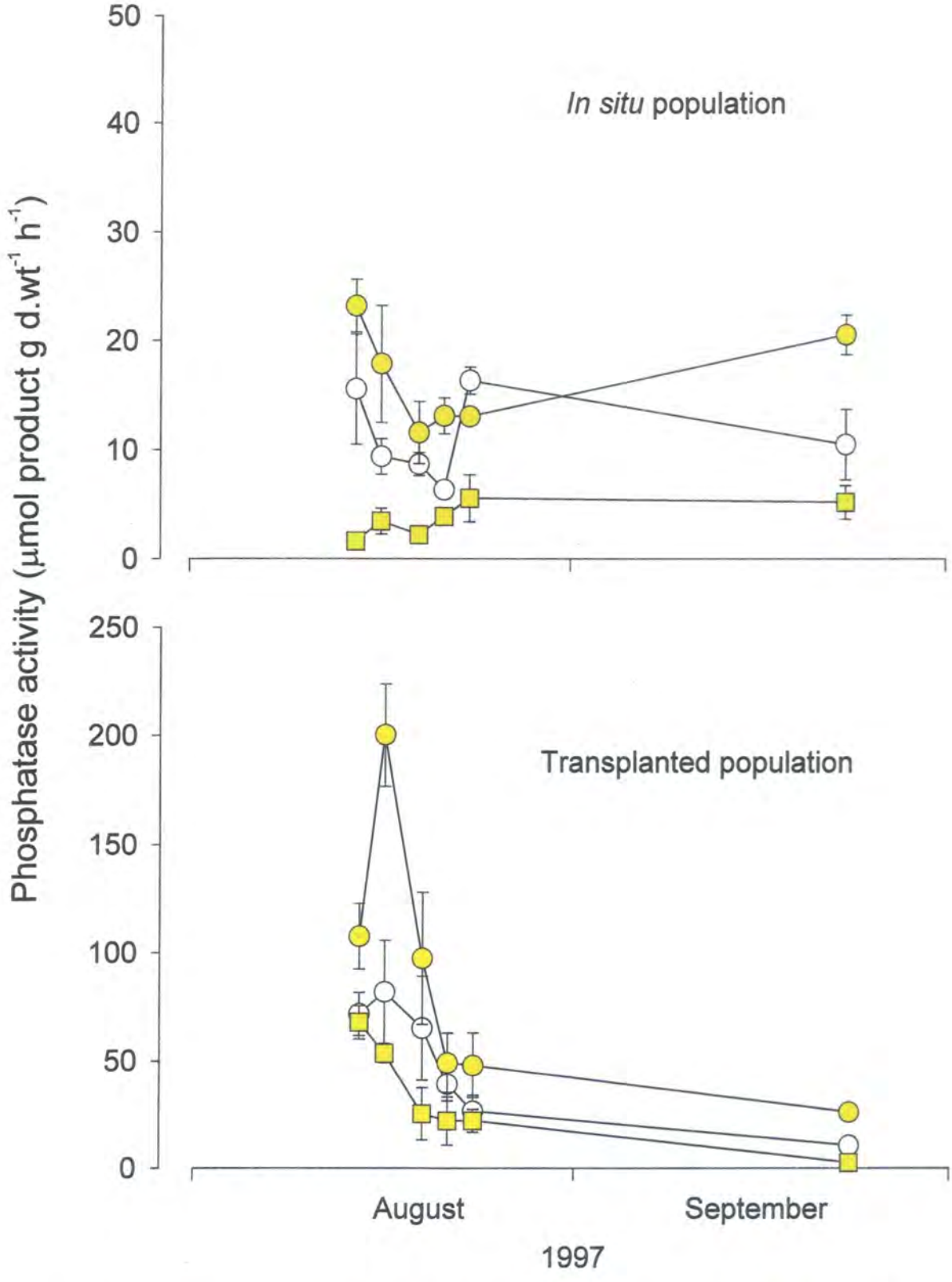

Figure 8.4 Changes in PMEase (○ MUP and ● pNPP) and PDEase (■ bis-pNPP) of 2-cm tips of *F. antipyretica* sample from *in situ* populations at km 107.9 and samples transplanted to km 107.9 from km 3.4 n = 4 ± S.E.

8.5 Discussion

The reciprocal transplants of *F. antipyretica* between sites of different dissolved nutrient concentration, led to marked changes in phosphatase activity of the transplants compared with natural fluctuations observed in the *in situ* populations. *F. antipyretica* transplanted from a P-poor to a P-rich site (FRP > 100 $\mu g\ L^{-1}$ throughout the study period) increased internal P contents twelve-fold in two days. A corresponding reduction in phosphatase was noted after two weeks, however, a decrease in PMEase and PDEase to the values of the *in situ* population took 32 days. These results suggest that a lag of up to 30 days between an increase in P content above the 0.3 % critical value and a corresponding decline in phosphatase activity.

The reciprocal transplant from downstream to upstream decreased the internal P content from 0.60 % to 0.34 %, and phosphatase increased beyond that of natural fluctuations, however, this took approximately four months. The transplanted populations did not achieve rates of PMEase as high as those of the *in situ* population during this study. It is apparent that the phosphatase activity of *F. antipyretica* is more easily repressed than de-repressed in natural conditions. This has important implications for using phosphatase as a tool for monitoring the nutrient status of plants in rivers.

8.6 Summary

i. Transplantation of *F. antipyretica* changed the N and P contents and the phosphatase activity of the transplanted populations.

ii. Transplant from P-poor (mean FRP 9.1 $\mu g\ L^{-1}$-P) to P-sufficient (mean FRP 135 $\mu g\ L^{-1}$-P) conditions, increased P contents 12-fold over two days. PMEase and PDEase

were reduced within two weeks, but took nearly a month to reduce to the levels of the *in situ* population.

iii. Transplant from nutrient sufficient to nutrient poor conditions produced much slower response, both in P content and phosphatase, than in the reciprocal transplant. Notable increases in phosphatase, above the natural fluctuation of the *in situ* population, was noted after two months. P content failed to fall below the critical 0.3% P value, the value at the end of the study, 0.32 % g d. wt^{-1}.

CHAPTER 9

Water phosphatase studies

9.1 Introduction

The use of phosphatase activity of mosses appears to be an effective way of assessing the phosphorus status of the plants, and in turn, provide insight into the long term P availability at a site with greater certainty than a programme of chemical sampling alone. However, there are drawbacks to using *in situ* plants, for example collecting them under high flows may prove problematic. Also, their relative lack of abundance means that role of mosses in the regeneration of Pi from FOP is limited.

The literature points to the importance of phosphatases in the water, both dissolved and particulate fractions as being both a useful indicator of P limitation as well as playing an important role in the regeneration of organic P.

To expand the project, it was decided to investigate the activity of phosphatases within the water, not only to assess the potential use as an indication of P limitation, but to quantify its role in the transformation of organic P. This Chapter presents data on phosphatase activity in the water column of the Swale-Ouse river system at five sites passing down the river and one major lowland tributary. The study investigated the rate of enzyme activity by laboratory assay in twelve samples collected at monthly intervals. This included three fractions separated by filtration and assayed at acid, neutral and alkaline pH conditions. This provided data on rates of enzyme activity, the importance of three fractions separated by filtration, their relationship to assay pH and with environmental variables recorded at the time sampling. Assays were also carried out at ambient field conditions on four occasions to compare the two assay techniques. The null

hypothesis to be tested was that rates of phosphatase activity would not change significantly on passing down the river and there would be no relationship between phosphatase and environmental variables.

9.2 Results

9.21 Time course

Initial experiments were conducted to test the linearity of acid and alkaline PMEase activity over time (Figure 9.1). Measurable fluorescence above that of the background blank was present after 20 min and total PMEase activity was linear over a six hour period at all sites. Therefore, the assumption was made that the phosphoryl, or the organic moiety evolved during the experiment, did not inhibit the rate of activity. The upstream sites had higher rates of activity than the downstream sites, and rates were higher under acid rather than alkaline conditions.

9.22 Influence of pH

The response to a range of pH values was carried out on two occasions under contrasting flow regimes: low flow in June 1996 and high in November 1996 (Figure 9.2). Assays were conducted at every half pH unit from 5.0 to 9.5 (final substrate concentration 100μM) at 25 °C. In general, all sites had the highest rates under acid conditions and showed the lowest activity in the neutral pH range. However, the response to pH was different between sites during the two contrasting sampling occasions.

At km -2.5, 10.9 and 49.9 under high flow conditions, rates of acid (5.0 - 6.0) PMEase activity were much greater than the other pH values tested. During low flow conditions, the difference between acid PMEase and other pH values was not as marked. Indeed, at the most upstream site, km -2.5, there was very little difference between the pH values tested. Further downstream, at two main river sites, km 107.9 and 145.0, and R.

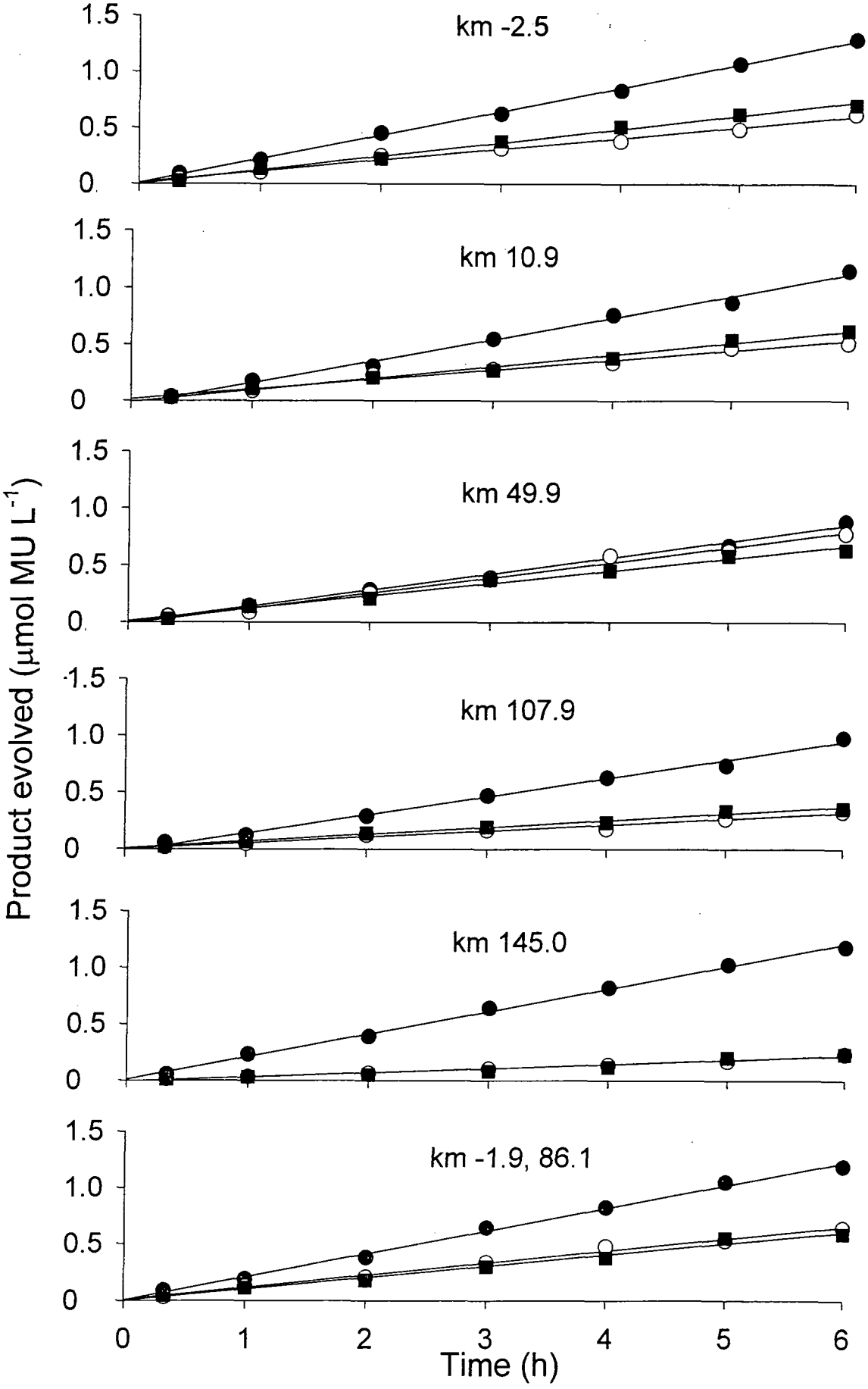

Figure 9.1 Influence of time on the quantity of product evolved by ● acid, ○ neutral and ■ alkaline total PMEase, in water from six sites within the Swale-Ouse catchment, 24 May 1996.

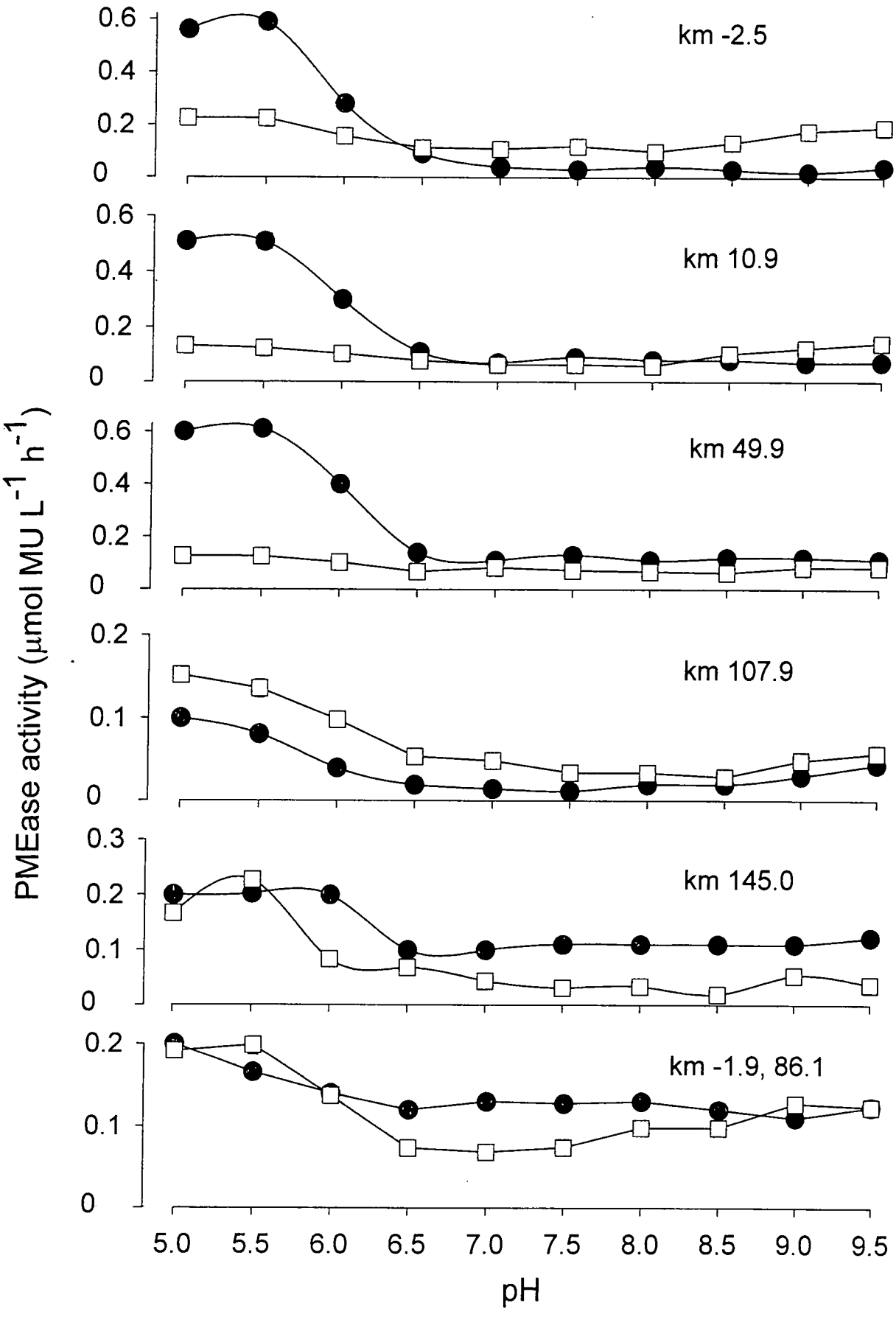

Figure 9.2 Influence of assay pH on total PMEase measured at six sites within the Swale-Ouse catchment, under low flow in □ June 1996 and high flow ● November 1996.

Wiske, PMEase activity had an acid optimum at all three sites, and there was no marked difference in response to pH between the contrasting sampling events.

9.23 Influence of substrate concentration

The influence of substrate concentration on the rate of acid PMEase was carried out twice, under different flow conditions (Figure 9.3). Firstly, June 1996 during low flows and latterly during the high flow event of June 1997. The relationship was fitted to a rectangular hyperbola model. The parameters V_{max} (apparent maximum velocity of the reaction) and Ks (substrate concentration at $V_{max}/2$) were calculated from this model.

Comparison between the two sampling events found the Ks parameter to be broadly similar at each site, although V_{max} was much higher under higher flows. In June 1997, the Ks tended to increase on passing down the river with a particularly high reading at km 107.9. This pattern along the river was less clear in June 1996, although, the highest Ks was again recorded at km 107.9. V_{max} values were generally higher in the upper reaches of the river compared with sites further downstream, during both sampling events.

The results from June 1996 suggested that a substrate concentration of 100μM MUP would provide saturated conditions for a single substrate concentration estimate of V_{max}, during the subsequent seasonal study.

9.24 Influence of temperature

The influence of temperature on the activity of acid PMEase was carried out twice under low flow in June 1996, and high flow in February 1997 (Figure 9.4). At all six sites, under both flow conditions, the response of PMEase to temperature was linear. PMEase recorded during high flows was generally higher than for low flow, at all sites. Under both flow conditions, at all six sites, PMEase had an approximate Q10 response

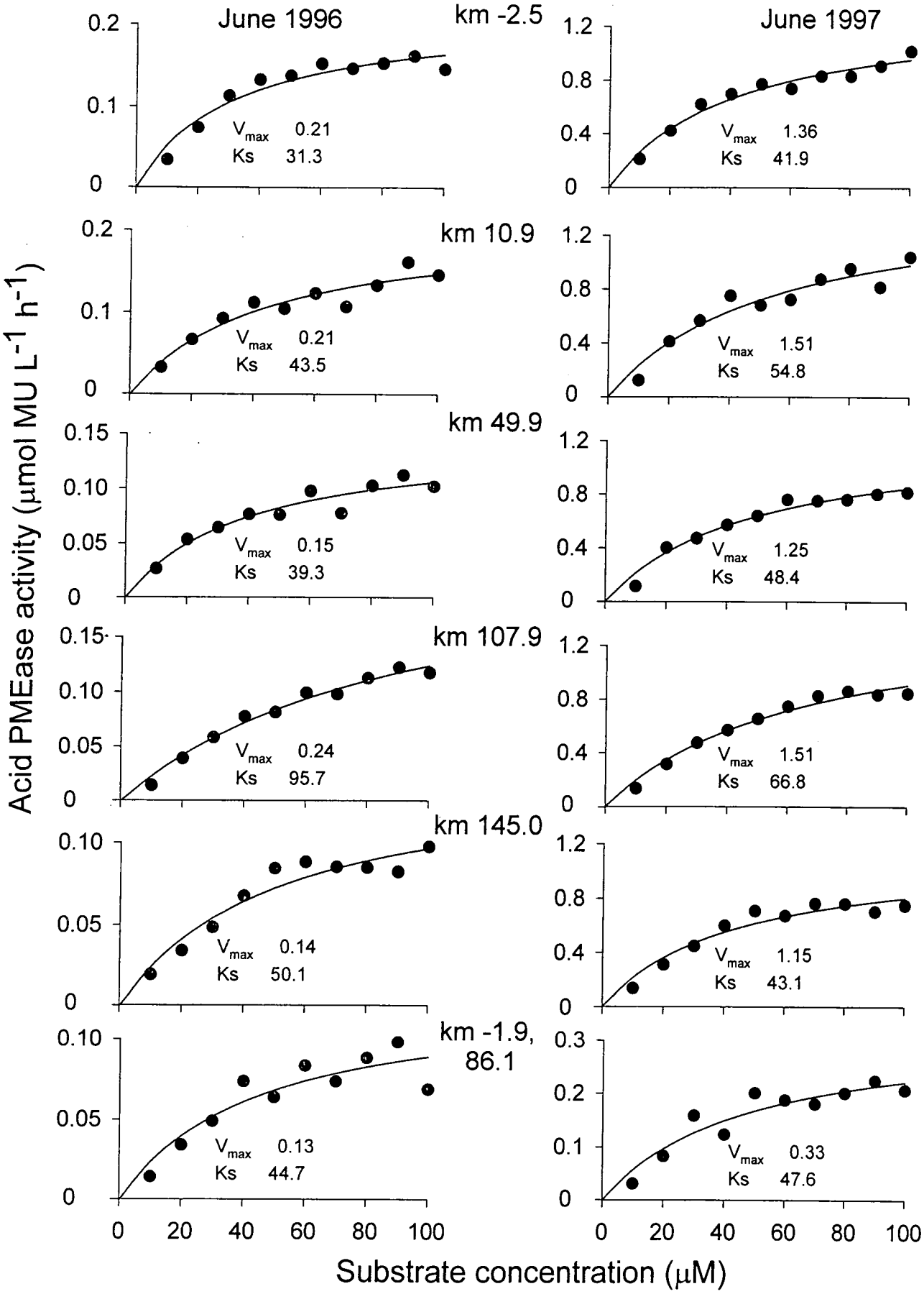

Figure 9.3 Influence of substrate concentration on total acid water PMEase at six sites within the Swale-Ouse catchment, during low flows in June 1996 and high flows in June 1997.

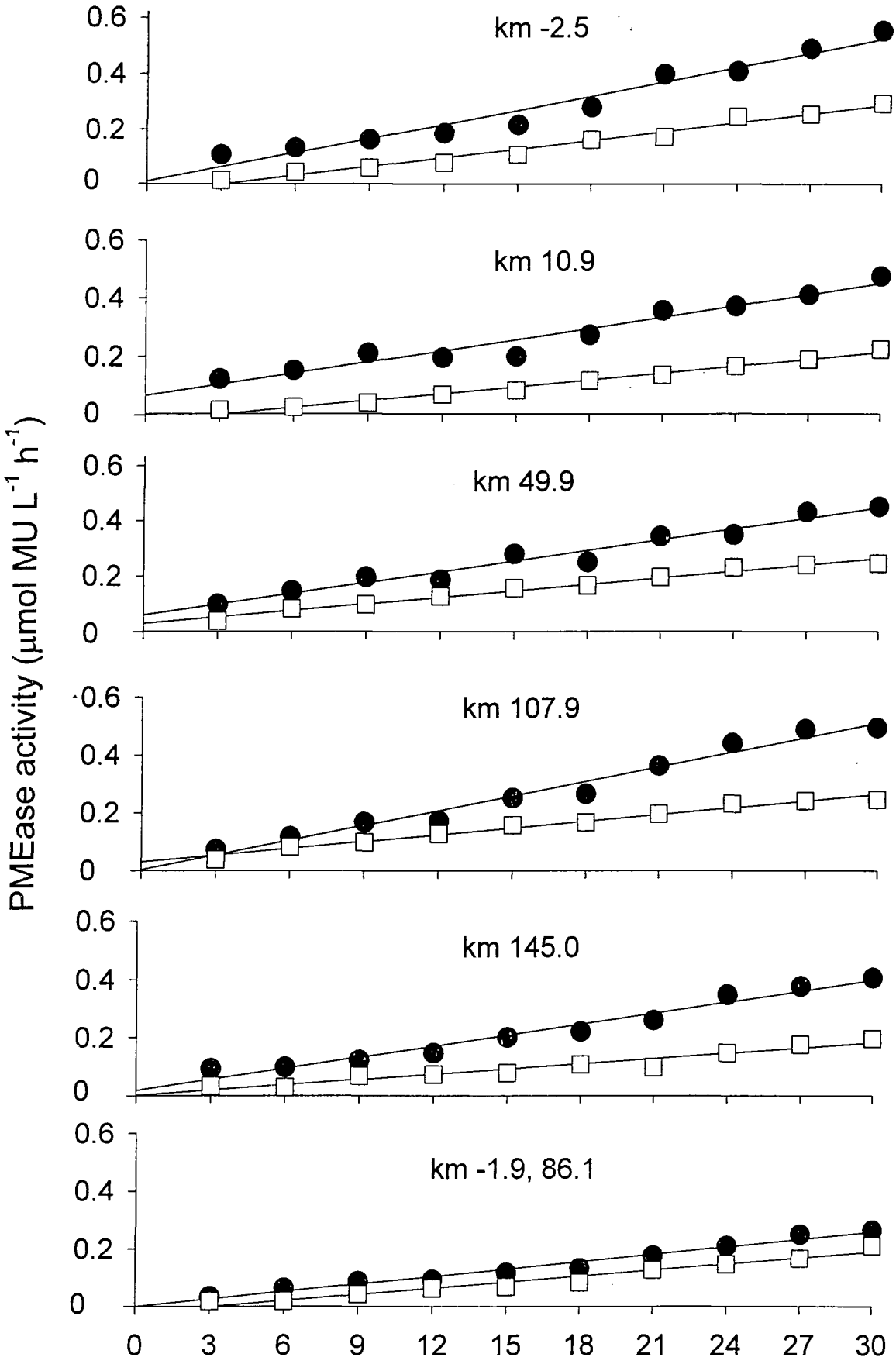

Figure 9.4 Influence of assay temperature on total PMEase in the water at six sites within the Swale-Ouse catchment, under low flow in □ June 1996 and high flow in ● November 1996.

9.25 Variability study

A variability study of PMEase was carried out on water samples at two sites. Four replicate samples were taken at 30-s intervals, at 3/4 total water depth, in the middle of the river, and PMEase calculated for each replicate. The variation of the four replicates was compared to four sub-samples taken from the first replicate sample; i.e. replication of the method. Results (Table 9.1) showed similar mean and standard error for each set of samples and no significant difference (Mann Whitney $p< 0.001$) between the two sampling strategies. However, the replicate of the method had a smaller standard error than the 30 s replicates on seven of eight occasions.

Table 9.1 Mean and standard error of total and filtered water PMEase samples from two sites collected at 30 second intervals compared with replicates of the method from a single sample. (n=4)

Site	Sample	mean	S.E
km -2.5	Filtered 30-s replicate	0.0730	0.0046
	Filtered method replicate	0.0669	0.0023
km -2.5	Total 30-s replicate	0.2019	0.0078
	Total method replicate	0.1919	0.0059
km 49.9	Filtered 30-s replicate	0.0512	0.0025
	Filtered method replicate	0.0543	0.0012
km 49.9	Total 30-s replicate	0.1088	0.0019
	Total method replicate	0.1085	0.0022

Spatial variation was undertaken at three sites to test the influences of intra-site variation on total and filtered acid PMEase (Table 9.2, figure 9.5). The spatial variation survey showed that acid PMEase was not only greater, but also more varied at the upstream tributary sites tested, km -2.5 and km -3.7, 3.5 compared to a main river site, km 49.9. Within the tributary sites, the highest acid PMEase (total and filtered) was found in samples taken from standing water from the stream margins, particularly when iron deposits and inputs of peaty soil pore water were evident. The downstream site, km 49.9,

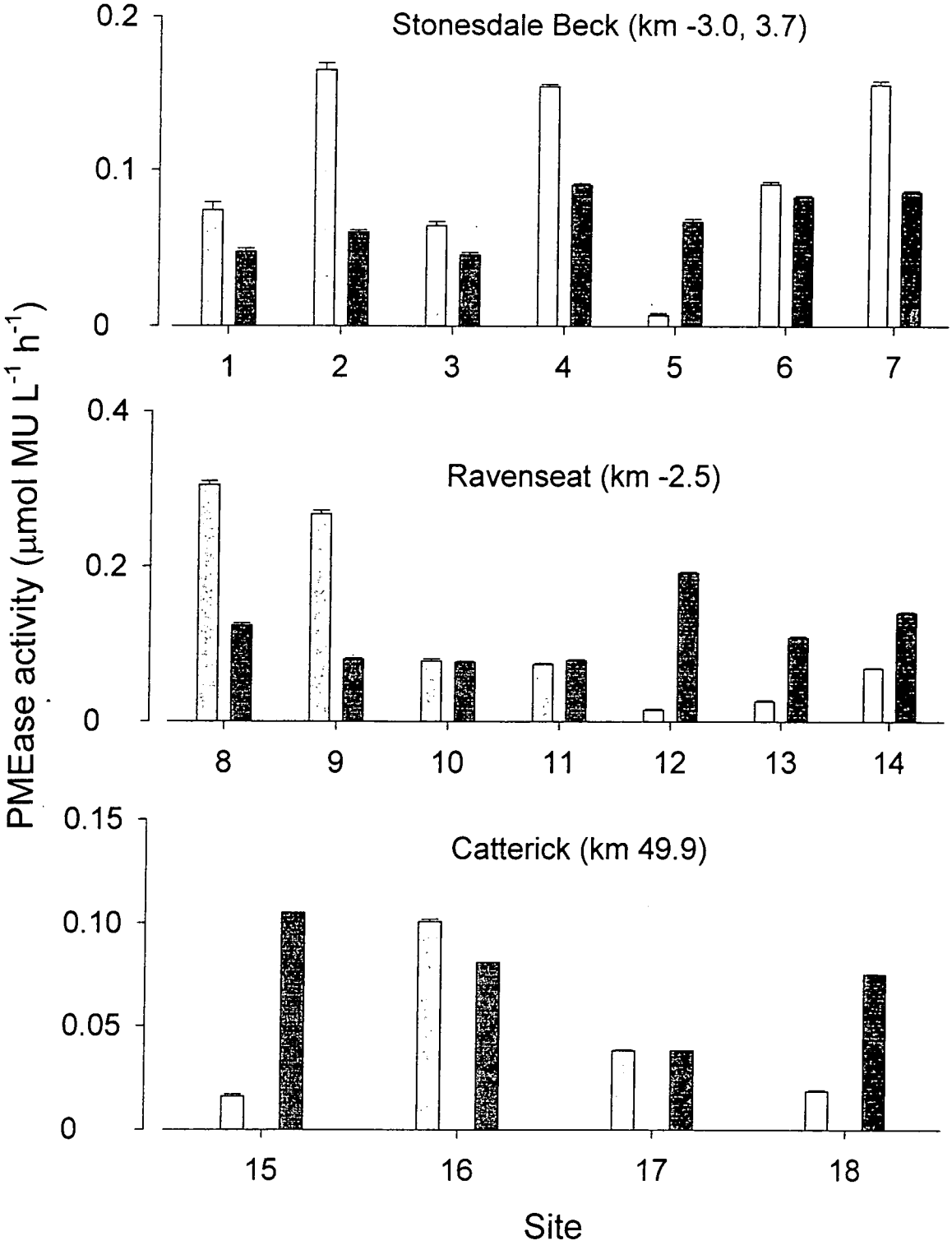

Figure 9.5 Intrasite variability of □ filtered and ■ total acid PMEase in water compared at two upland tributaries (km -2.5) and (km -3.0, 3.7) and one downstream main river site (km 49.9).

had generally lower rates of activity and the difference between samples taken from the margins and midstream locations was less marked than the upland sites.

9.26 PMEase and PDEase activity

Comparison of PMEase and PDEase activity in unfiltered water was carried out using the colourimetric substrates, pNPP and bis-pNPP, on 1 and 2 July 1997 (Figure 9.6). Previous attempts had not detected PDEase activity, and the lower sensitivity of the colourimetric substrates was thought to be responsible. However, during the last week of June 1997 very high PMEase activity had been detected using MUP, so the opportunity to test the other substrates was taken. Comparison between MUP and pNPP was conducted on samples from km -2.5 and similar results were obtained for assays at pH 5.5 and 9.5, but not 7.5. At pH 7.5 PMEase estimated using MUP was markedly lower than with pNPP.

PMEase and PDEase decreased on passing down the river and PMEase was higher than PDEase at all sites at all pH values. At sites in the lower middle reaches PMEase but particularly PDEase was near to detection limits. The highest activity of PMEase and PDEase was at pH 5.5 compared to the other pH values measured. The ratio of PDEase to PMEase decreased from the most upstream sites to the middle reach sites, the ratio increased at the lower sites as both activities neared the detection limits of the method.

Assays at pH 5.5, 7.5 and 9.5 were carried out on unfiltered water from km –2.5 to test the influence of KH_2PO_4 on PMEase and PDEase. There was no significant difference between controls and samples with additions of inorganic P (10 mg L^{-1} final concentration) at all pH values.

9.3 Seasonal study

The seasonal study was undertaken from July 1996 to June 1997 with twelve sampling events occurring at roughly monthly intervals. At all sites, on all sampling

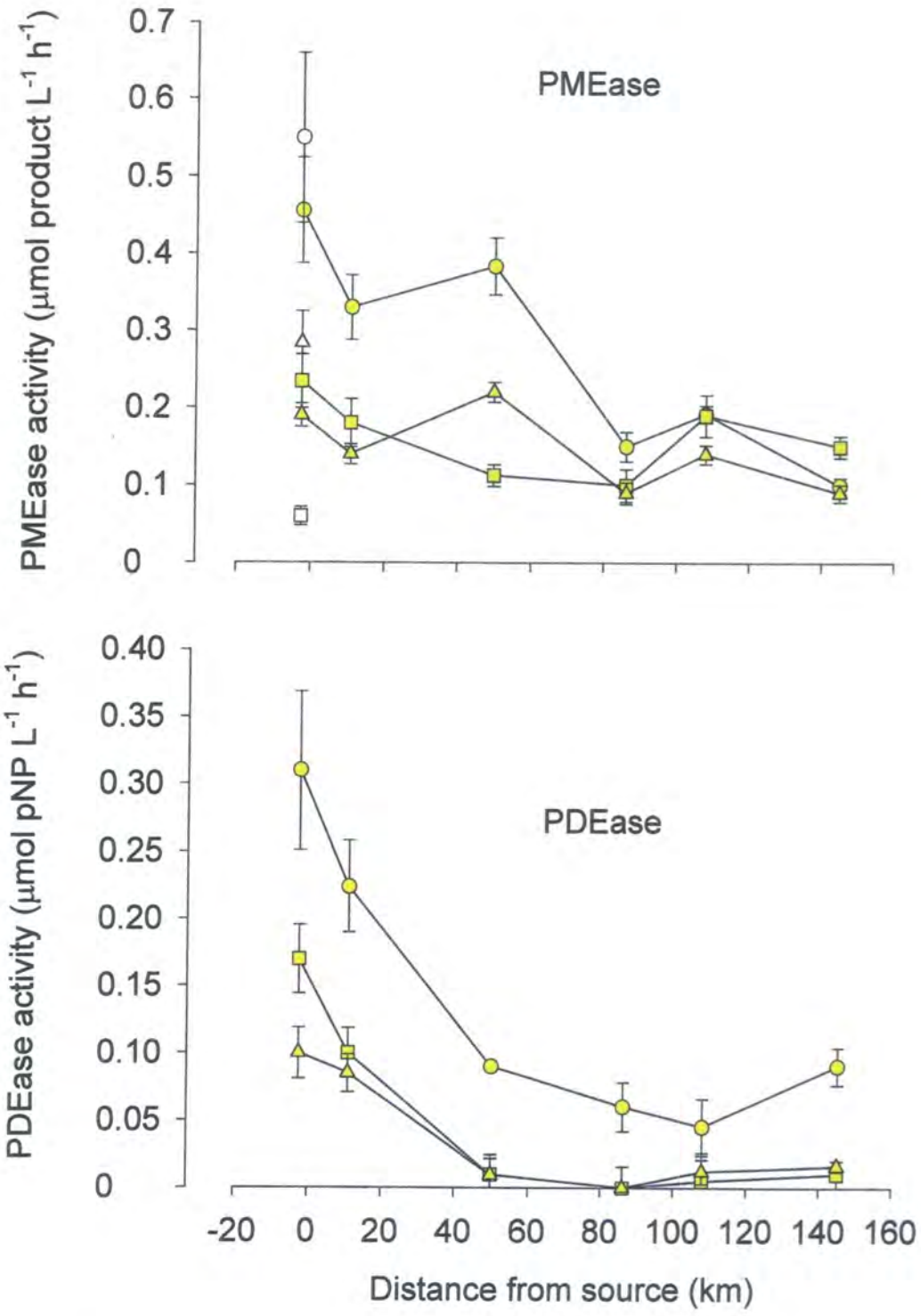

Figure 9.6 Comparison of PMEase and PDEase in unfiltered water from six sites within the Swale - Ouse catchment. Solid symbols represent the colourimetric substrates pNPP (PMEase) and bis-pNPP (PDEase), unfilled symbols represent MUP (PMEase). Assays were conducted at pH ○ 5.5 □ 7.5 △ 9.5, on 2 and 3 July 1997.

occasions, acid (Figure 9.7), neutral (Figure 9.8) and alkaline (Figure 9.9) PMEase was detectable in both unfiltered and filtered water. Assays carried out at standard temperature and pH aimed to provide information on rates of PMEase activity within the water, with minimal interference from environmental variables such as temperature and pH. The highest rate of activity recorded was acid total PMEase, at km 10.9, 26 June 1997, on all other occasions the site further upstream, km -2.5, had higher rates of acid total PMEase. The lowest rate of total PMEase, acid, neutral or alkaline, was recorded at km 107.9 on 17 October 1996, 14 November 1996 and 3 June 1997, respectively. All sites showed seasonal changes in total acid, neutral and alkaline PMEase and in the relative contributions of particulate and filtered water acid, neutral and alkaline PMEase. In general the filtered fraction, an estimate of dissolved and colloidal enzyme activity, formed a larger fraction of the total activity than the particulate fraction. Brief description is given of notable periods of total activity, and mention made of the relative contributions from the filtered and particulate fractions, on a site by site basis

9.4 Site specific trends in water PMEase

9.41 km -2.5

At km -2.5, the rate of acid total PMEase was higher than neutral and alkaline total PMEase on all occasions, except 16 August 1996, when alkaline total PMEase was highest. Filtered PMEase formed a significant proportion of total phosphatase throughout the year at km -2.5, at all three pH values assayed. Briefly, filtered acid PMEase made up > 44 % of total PMEase throughout the study, the highest being 75 %, 15 July 1996. Filtered neutral and alkaline PMEase made up > 35 % and 42 % of total PMEase and peaked at 107 % and 114 %, 26 November 1996 and 12 December 1996, respectively.

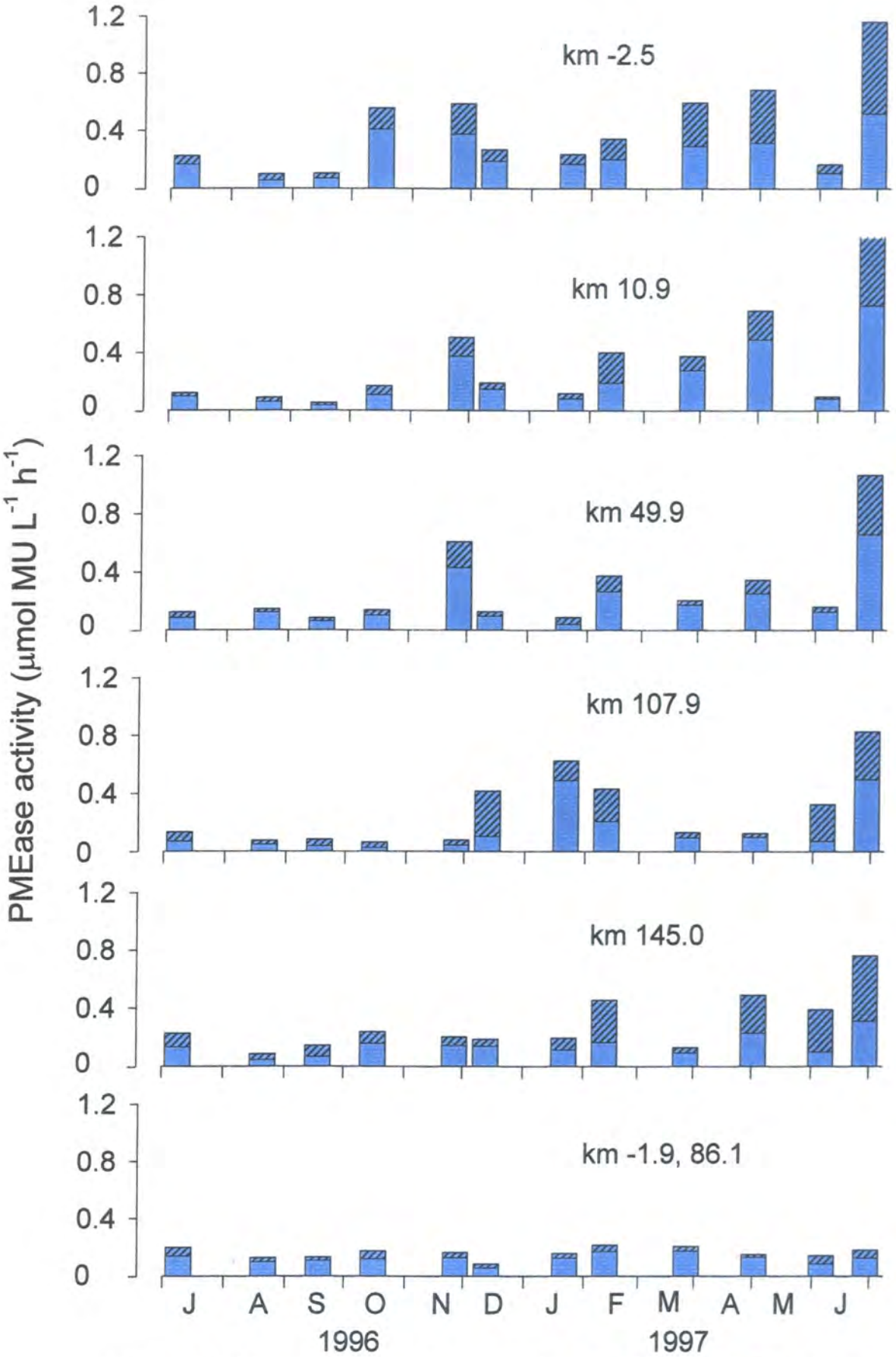

Figure 9.7 Mean total acid PMEase comprising; ■ filtered and ▨ particulate fractions, from six sites within the Swale-Ouse catchment, between July 1996 and June 1997. (n = 4).

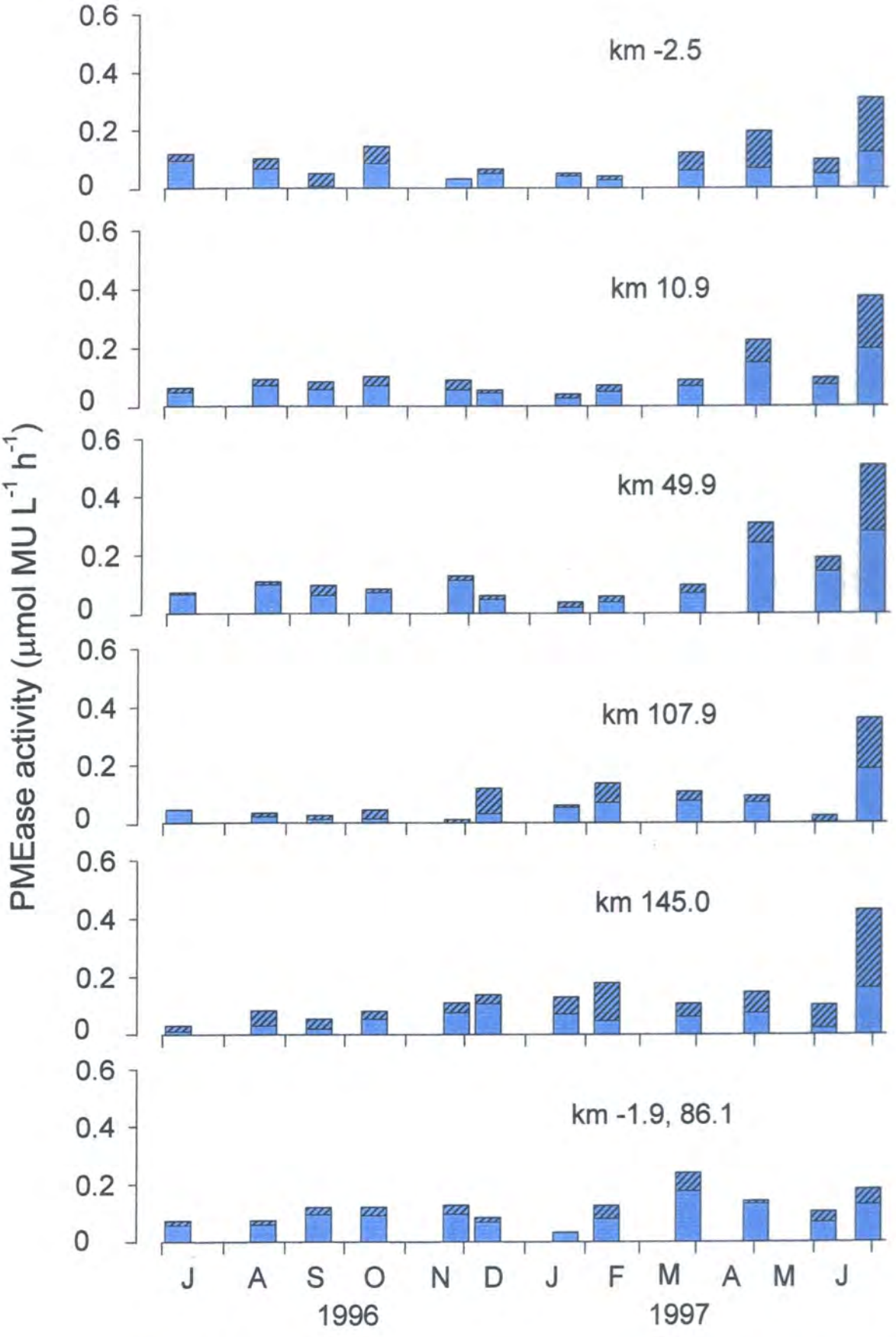

Figure 9.8 Mean total neutral PMEase comprising; ▭ filtered and ▨ particulate fractions, from six sites within the Swale-Ouse catchment, between July 1996 and June 1997. (n = 4).

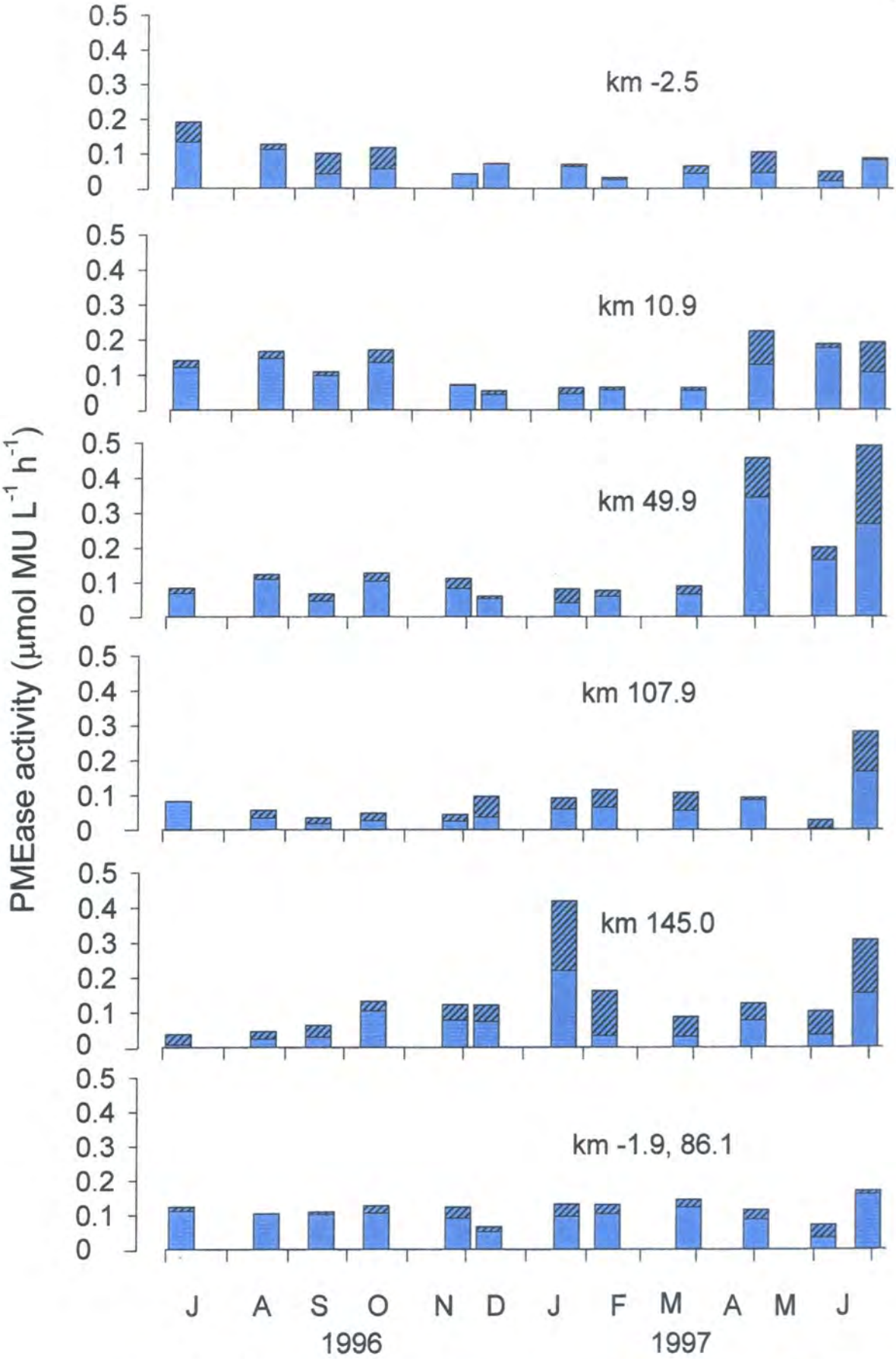

Figure 9.9 Mean total alkaline PMEase comprising; ▭ filtered and ▨ particulate fractions, from six sites within the Swale-Ouse catchment, between July 1996 and June 1997. (n = 4).

Acid total PMEase had three marked peaks: 0.55 μmol MU L^{-1} h^{-1}, 15 October 1996, (filtered 75%); 0.68 μmol MU L^{-1} h^{-1}, 29 April 1997, (filtered 47 %); 1.2 μmol MU L^{-1} h^{-1}, 26 June 1997, (filtered 45%).

Neutral total PMEase ranged from 0.06 to 0.11 μmol MU L^{-1} h^{-1} until November 1996 followed by two peaks: 0.14 μmol MU L^{-1} h^{-1}, 15 October 1996, (filtered 55%); 0.3 μmol MU L^{-1} h^{-1}, 26 June 1997, (filtered 38%). The lowest rates, < 0.06 μmol MU L^{-1} h^{-1}, (filtered > 60%). were during the winter of 1996/7.

The highest rate of alkaline total PMEase, 0.19 μmol MU L^{-1} h^{-1}, was recorded on 15 July 1996 in contrast to all other sites, which had the highest rate recorded on 26 or 27 June 1997. The lowest rates of alkaline total PMEase, < 0.10 μmol MU L^{-1} h^{-1}, (filtered < 20 %) were between November and February 1996. No peak was noted on 26 June 1997, as at all other sites.

9.42 km 10.9

At km 10.9 acid total PMEase was higher than neutral or alkaline total PMEase on all sampling occasions except: 15 July, 16 August, 17 September 1996 when alkaline total PMEase was highest. The filtered fraction made up a significant part of acid, neutral and alkaline total PMEase throughout the year. The proportion of acid filtered PMEase was never less than 48 % and peaked at 87 %, 3 June 1997. Neutral and alkaline filtered PMEase was always greater than 53 % and 56 %, and peaked at 98 and 84 %, 26 November 1996 and 12 December, respectively.

Acid total PMEase had four peaks of activity during the sampling period: 0.509 μmol MU L^{-1} h^{-1}, 26 November 1996, (filtered 75%); 0.404 μmol MU L^{-1} h^{-1}, 11 February 1997, (filtered 48%); 0.694 μmol MU L^{-1} h^{-1}, 29 April, (filtered 75 %); 1.27 μmol MU L^{-1} h^{-1}, 26 June 1997, (filtered 57%).

Neutral total PMEase showed two peaks in activity: 0.227 and 0.374 μmol MU L^{-1} h^{-1}, 29 April and 26 June 1997, of which the filtered fraction contributed 67 and 53 %, respectively.

Alkaline total PMEase had three separate phases of activity: rates ranged from 0.1 to 0.2 μmol MU L^{-1} h^{-1}, July to October 1996, (filtered > 75 %); < 0.1 μmol MU L^{-1} h^{-1}, November 1996 to March 1997, (filtered > 73%); rates of approximately 0.1 μmol MU L^{-1} h^{-1}, April to June 1997, (filtered > 42%).

9.43 km 49.9

Acid total PMEase at km 49.9 was higher than neutral and alkaline PMEase on all occasions, except 17 September 1996, when neutral PMEase was highest, and 29 April and 3 June 1997, when alkaline PMEase was highest.

To summarise, total acid PMEase activity < 0.15 μmol MU L^{-1} h^{-1} from July to October 1996, (filtered > 70%), after which there were three peaks of activity: 0.611 μmol MU L^{-1} h^{-1}, 26 November 1996 (filtered 60%); 0.378 μmol MU L^{-1} h^{-1}, 11 February 1997 (filtered 59%); 1.067 μmol MU L^{-1} h^{-1}, 26 June 1997 (filtered 38%).

Neutral total PMEase was < 0.15 μmol MU L^{-1} h^{-1} from July 1996 to March 1997, a minima of 0.039 μmol MU L^{-1} h^{-1}, 21 January 1997. An increase in activity peaked at 0.31 μmol MU L^{-1} h^{-1}, 29 April 1997 (filtered 71%), the highest rate, 0.509 μmol MU L^{-1} h^{-1}, 26 June 1997 (filtered 18%).

Alkaline total PMEase peaked at 0.457 and 0.492 μmol MU L^{-1} h^{-1}, 29 April and 26 June 1997, (filtered 75 %, 55 %), respectively.

9.44 km 107.9

At km 107.9, acid total PMEase had higher rates than neutral or alkaline total PMEase. There were two periods of high acid total PMEase activity: > 0.40 μmol MU L^{-1}

h^{-1}, December 1996 to February 1997, particulate PMEase formed a significant contribution, > 50% on two occasions. The highest rate, 0.828 μmol MU L^{-1} h^{-1}, 27 June 1997 was composed of 60% filtered PMEase.

Neutral total PMEase < 0.05 μmol MU L^{-1} h^{-1}, July 1996 to April 1997, with the exception of two peaks: 0.11 μmol MU L^{-1} h^{-1}, 14 December 1996 (particulate 81%); 0.14 μmol MU L^{-1} h^{-1}, 13 February 1997 (particulate 50%). The highest rate of neutral total PMEase, 0.361 μmol MU L^{-1} h^{-1}, 27 June 1997 (filtered 52%).

From July to November 1996, alkaline total PMEase < 0.1μmol MU L^{-1} h^{-1}, but from December 1996 to April 1997 > 0.1 μmol MU L^{-1} h^{-1} with significant contributions of particulate PMEase. The highest rate of alkaline total PMEase, 0.281 μmol MU L^{-1} h^{-1}, 27 June 1997.

9.45 km 145.0

Acid, neutral and alkaline total PMEase was markedly influenced on a number of occasions by the particulate fraction. In general, the rate of acid total PMEase activity was higher than neutral or alkaline PMEase. Neutral and alkaline total PMEase had roughly comparable rates of activity throughout the year, apart from 23 January 1997, when alkaline total PMEase activity was significantly greater.

Acid total PMEase was approximately 0.2 μmol MU L^{-1} h^{-1} from July 1996 to January 1997. There were two periods of high activity: 0.418 μmol MU L^{-1} h^{-1}, 13 February 1997 (filtered 32%); > 0.39 μmol MU L^{-1} h^{-1}, between April and June 1997, the highest, 0.764 μmol MU L^{-1} h^{-1}, 27 June 1997. The latter all had significant (55-75%) contributions from the particulate fraction.

Neutral total PMEase activity was < 0.1 μmol MU L^{-1} h^{-1} from July to October 1996 and between 0.1 and 0.15 μmol MU L^{-1} h^{-1}, from November 1996 to January1997

(filtered > 65%). The highest rate of neutral total PMEase recorded throughout the survey was 0.428 μmol MU L^{-1} h^{-1}, 27 June 1997 (particulate 62%).

Alkaline total PMEase activity was approximately 0.1 μmol MU L^{-1} h^{-1} except for two peaks; 23 January and 27 June 1997, the greatest of which, 0.418 μmol MU L^{-1} h^{-1} was in January 1997. Samples from both months had roughly equal contributions from the filtered and particulate fractions.

9.46 km -1.9, 86.1

Acid total PMEase activity was always greater than neutral or alkaline total PMEase, although, it was dissimilar to the seasonal trends noted at other sites. Acid total PMEase was approximately 0.15 μmol MU L^{-1} h^{-1} throughout the study except for a peak, 0.217 μmol MU L^{-1} h^{-1}, 13 February 1997. The contribution of the filtered PMEase fraction was always greater than 62 % and peaked at 85%, 25 March 1997.

The rate of neutral total PMEase had greater seasonal variation than acid total PMEase. In brief, activity was approximately 0.1 μmol MU L^{-1} h^{-1} from July 1996 to December 1996. There was a winter minima; 0.032 μmol MU L^{-1} h^{-1}, 23 January and a spring maxima, 0.238 μmol MU L^{-1} h^{-1}, 25 March 1997. A peak was also noted in summer, 0.181 μmol MU L^{-1} h^{-1}, 26 June 1997. The filtered fraction was > 65% in all samples.

There was little seasonal variation in alkaline total PMEase, rates ranged from 0.05 to 0.15 μmol MU L^{-1} h^{-1} throughout the year.

9.5 Bacterial numbers

Total bacterial numbers, including particulate and free living cells, increased on passing down the river throughout the sampling period (Figure 9.10). Total numbers were almost entirely composed of free living cells on all occasions, numbers of cells associated

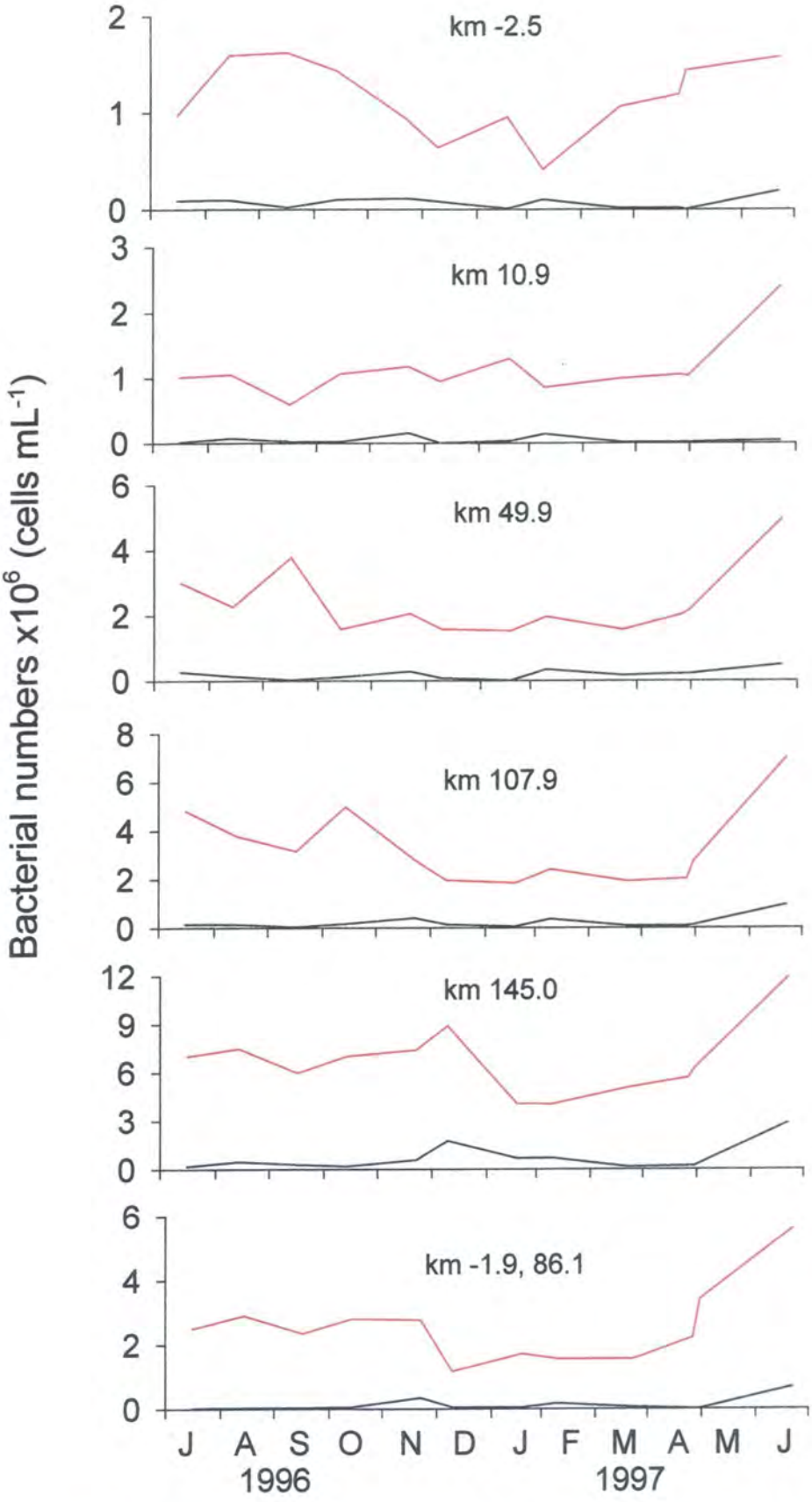

Figure 9.10 Bacterial numbers estimated by direct epifluorescence microscopy counts of —— total bacterial numbers and —— particulate bacterial numbers in the Swale - Ouse catchment, 1996 - 1997

with particulate material often a order of magnitude lower than free living cells. In general, sites had lower total numbers of cells in winter compared to summer, although no clear seasonal pattern was evident at km 10.9 and km 145.0. The highest total numbers at each site were recorded on 26 and 27 June 1997 during high summer flow. Particulate bacterial numbers were high on three occasions: November 1996, February and June 1997, at all sites except km 145.0, consistent with high flow.

9.6 Analysis of PMEase between sites

Correlation matrices were used to test if significant relationships were present between acid, neutral and alkaline PMEase at each site, and seasonal changes in filtered, particulate and total PMEase at a given pH between sites. The relationship between PMEase and environmental variables was correlated. ANOVA tested the annual mean rate of filtered, particulate and total PMEase between sites. Subsequently, a Tukey test was used to group sites with similar mean rates of PMEase.

Correlation of PMEase between sites and between environmental variables and PMEase at each site, was carried out using mean PMEase (n=4) data for each month. Only one measure was taken for the environmental variables on each monthly sampling occasion.

ANOVA was conducted on all filtered (n=48) , particulate (n=48) and total (n=48) PMEase measures made at each site over the year. Normality of data was tested using a Kolmogorov-Smirnoff test ($p > 0.05$). The data was not normally distributed and was transformed using log (n+1) prior to ANOVA.

The combination of ANOVA and correlation allowed sites to be compared for differences in annual mean rate of activity and changes in seasonality. ANOVA tests the average rate of activity over the yearly study but removes the effect of seasonality.

Correlation allows comparison of the periodicity of change between sites but ignores the absolute values.

9.61 Acid PMEase

Between all sites for total acid PMEase, filtered PMEase and particulate PMEase positive significant correlation was found between km -2.5, km 10.9, km 49.9 and km 145.0 (Table 9.3). That is, all sites were correlated with each other and were therefore changing at the same time during the year. Both km -1.9, 86.1 and km 107.9 had no significant correlation with any other site.

Annual means of acid total, filtered and particulate PMEase generally decreased on passing down the river (Table 9.4). ANOVA and a Tukey test of acid total PMEase found that the most upstream site, km -2.5, was significantly different from the sites km 49.9, km 107.9 and km -1.9, 86.1. Site km -2.5 was not significantly different from km 10.9 or km 145.0. The following sites: km 10.9, km 49.9 km107.9 and km -1.9, 86.1 had no significant difference between them but km 145.0 and km -1.9, 86.1 were significantly different.

9.62 Neutral PMEase

The following sites; km -2.5, km 10.9, km 49.9, km 107.9 and km 145.0 had significant positive correlation between sites for both neutral total and particulate PMEase (Table 9.5). R. Wiske, km -1.9, 86.1 was not significantly correlated with any other site for either total or particulate neutral PMEase. Filtered neutral PMEase displayed a more complicated pattern of correlation. Of the main river sites only km 10.9 was significantly positively correlated with R. Wiske km -1.9, 86.1. The following; km -2.5, km 10.9, km 49.9 and km 107.9 were all significantly positively correlated whereas km 145.0 was correlated with all other main rivers sites except km 49.9

The mean annual average rate of neutral total PMEase between sites was tested using ANOVA and a Tukey test (Table 9.6). Sites km 49.9 and km 107.9 were found to be significantly different from the others. Neutral filtered PMEase annual mean rates were highest at km 49.9 and R. Wiske km -1.9, 86.1 and these two sites were significantly different from km -2.5, km 107.9 and km 145.0 but not km 10.9. The highest annual mean particulate neutral PMEase was found at km 145.0 and this site was significantly different from all others except km -2.5

9.63 Alkaline PMEase

Correlation between sites for total, filtered and particulate PMEase was patchy and restricted to sites from the middle and lower reaches (Table 9.7). At the most upstream site km -2.5, none of the fractions analysed were correlated with those of any other site. Total and particulate alkaline PMEase at km 49.9 was correlated with both km 10.9 and km 107.9 but no correlation existed between km 10.9 and km 107.9. There was no correlation between these sites for filtered alkaline PMEase. Further downstream, total alkaline PMEase at km 107.9 was correlated with km 145.0 and R. Wiske whereas filtered alkaline PMEase at km 107.9 was only correlated with R. Wiske. No significant correlation was found between these sites for particulate alkaline PMEase.

Annual mean alkaline particulate PMEase generally increased on passing down the main river, however the lowland tributary, km -1.9, 86.1, had a mean similar to the most upstream site km -2.5 (Table 9.8). ANOVA and a Tukey test showed km 145.0 to be significantly different from all other sites. All other sites were not significantly different except for km 49.9 and km -1.9, 86.1. Mean annual alkaline filtered and total PMEase increased from the most upstream site as far as km 49.9. The subsequent site had mean values similar to the most upstream site.

Table 9.3 Correlation matrix of acid; total, filtered and particulate PMEase at six sites within the Swale-Ouse catchment. Only significant ($p < 0.05$) values are shown.

	km -2.5	km 10.9	km 49.9	km 107.9	km 145.0	km -1.9, 86.1
Total PMEase						
km -2.5	1.00					
km 10.9	0.93	1.00				
km 49.9	0.85	0.94	1.00			
km 107.9				1.00		
km 145.0	0.70	0.82	0.77	0.63	1.00	
km -1.9, 86.1						1.00
Filtered PMEase						
km -2.5	1.00					
km 10.9	0.81	1.00				
km 49.9	0.75	0.91	1.00			
km 107.9				1.00		
km 145.0	0.81	0.87	0.77		1.00	
km -1.9, 86.1						1.00
Particulate						
PMEase						
km -2.5	1.00					
km 10.9	0.92	1.00				
km 49.9	0.85	0.95	1.00			
km 107.9				1.00		
km 145.0	0.65	0.78	0.71	0.60	1.00	
km -1.9, 86.1						1.00

Table 9.4 Annual mean acid; total, filtered and particulate PMEase (μmol MU L^{-1} h^{-1}). Superscript characters denote groups defined by Tukey test ($p<0.05$). n=12, SE = Standard error.

	Total mean	SE	Filtered mean	SE	Particulate mean	SE
km -2.5	0.391^{ab}	0.063	0.234^{a}	0.034	0.167^{a}	0.043
km 10.9	0.307^{ab}	0.071	0.212^{ab}	0.047	0.108^{ab}	0.037
km 49.9	0.266^{ab}	0.059	0.194^{abc}	0.041	0.081^{bc}	0.028
km 107.9	0.257^{bc}	0.056	0.142^{bc}	0.040	0.119^{ab}	0.031
km 145.0	0.024^{abc}	0.042	0.141^{bc}	0.018	0.142^{ab}	0.034
km -1.9, 86.1	0.161^{bc}	0.009	0.123^{c}	0.009	0.038^{c}	0.005

Table 9.5 Correlation matrix of neutral; total, filtered and particulate PMEase at six sites within the Swale-Ouse catchment. Only significant ($p<0.05$) values are shown.

	km -2.5	km 10.9	km 49.9	km 107.9	km 145.0	km -1.9, 86.1
Total PMEase						
km -2.5	1.00					
km 10.9	0.92	1.00				
km 49.9	0.88	0.98	1.00			
km 107.9	0.74	0.80	0.74	1.00		
km 145.0	0.70	0.84	0.81	0.95	1.00	
km -1.9, 86.1						1.00
Filtered PMEase						
km -2.5	1.00					
km 10.9	0.64	1.00				
km 49.9	0.56	0.95	1.00			
km 107.9	0.62	0.74	0.58	1.00		
km 145.0		0.62		0.72	1.00	
km -1.9, 86.1		0.57				1.00
Particulate						
PMEase						
km -2.5	1.00					
km 10.9	0.91	1.00				
km 49.9	0.89	0.97	1.00			
km 107.9	0.63	0.76	0.81	1.00		
km 145.0	0.72	0.87	0.91	0.84	1.00	
km -1.9, 86.1						1.00

Table 9.6 Annual mean neutral; total, filtered and particulate PMEase (μmol MU L^{-1} h^{-1}). Superscript characters denote groups defined by Tukey test ($p<0.05$). n=12, SE = Standard error.

	Total		Filtered		Particulate	
	mean	SE	mean	SE	mean	SE
km -2.5	0.109^{ab}	0.020	0.059^{a}	0.009	0.050^{ab}	0.015
km 10.9	0.113^{ab}	0.023	0.076^{ab}	0.012	0.038^{a}	0.013
km 49.9	0.140^{ab}	0.032	0.103^{b}	0.020	0.040^{a}	0.016
km 107.9	0.085^{a}	0.024	0.049^{a}	0.013	0.038^{a}	0.014
km 145.0	0.128^{ab}	0.024	0.061^{a}	0.011	0.068^{b}	0.018
km -1.9, 86.1	0.118^{ab}	0.014	0.091^{b}	0.010	0.027^{a}	0.006

Table 9.7 Correlation matrix of alkaline; total, filtered and particulate PMEase at six sites within the Swale-Ouse catchment. Only significant ($p<0.05$) values are shown.

	km -2.5	km 10.9	km 49.9	km 107.9	km 145.0	km -1.9, 86.1
Total PMEase						
km -2.5	1.00					
km 10.9		1.00				
km 49.9		0.74	1.00			
km 107.9			0.63	1.00		
km 145.0				0.56	1.00	
km -1.9, 86.1				0.64		1.00
Filtered PMEase						
km -2.5	1.00					
km 10.9		1.00				
km 49.9			1.00			
km 107.9			0.63	1.00		
km 145.0					1.00	
km -1.9, 86.1				0.72		1.00
Particulate						
PMEase						
km -2.5	1.00					
km 10.9		1.00				
km 49.9		0.84	1.00			
km 107.9			0.62	1.00		
km 145.0					1.00	
km -1.9, 86.1						1.00

Table 9.8 Annual mean alkaline; total, filtered and particulate PMEase (μmol MU L^{-1} h^{-1}). Superscript characters denote groups defined by Tukey test ($p<0.05$). n=12, SE = Standard error.

	Total mean	SE	Filtered mean	SE	Particulate mean	SE
km -2.5	0.086^{a}	0.012	0.061^{a}	0.009	0.027^{ab}	0.007
km 10.9	0.124^{ab}	0.016	0.099^{b}	0.011	0.028^{ab}	0.009
km 49.9	0.156^{b}	0.035	0.114^{b}	0.024	0.045^{ab}	0.016
km 107.9	0.086^{a}	0.017	0.055^{a}	0.011	0.034^{ab}	0.009
km 145.0	0.139^{b}	0.027	0.072^{ab}	0.016	0.069^{c}	0.015
km -1.9, 86.1	0.117^{bc}	0.008	0.098^{b}	0.009	0.021^{a}	0.005

9.7 Intrasite relationships

Correlation between assays of a particular fraction carried out at three pH values was undertaken at each site to test if pH could differentiate between groups of enzyme, and if the rates of activity of those groups were changing at similar times of year.

Changes in the relationship between the assays conducted at the three pH values were noted on passing downstream. At the two most upstream sites, km -2.5 and km 10.9, acid and neutral assays were correlated for all three fractions: total, filtered and particulate PMEase (Tables 9.9 and 9.10). No alkaline fractions were correlated with acid fractions at km -2.5, and only alkaline and acid particulate PMEase were correlated at km 10.9. Between neutral and alkaline assays at km -2.5, only filtered PMEase was correlated, whereas, at km10.9 correlation between both total and particulate fractions was noted at these pH values. In general, at these two sites, acid and neutral assays were more closely related than alkaline and neutral assays. Acid and alkaline assays were either not related at all or had a very weak relationship.

The results from further downstream, km 49.9 and km 107.9, (Tables 9.11 and 9.12) were in contrast to the previous two sites. All three fractions at all three pH values were correlated, except for acid and alkaline filtered PMEase at km 49.9. However, further downstream still, km 145.0, the results were similar to the two upstream sites (Table 9.13). Acid and alkaline assays were not correlated for any fraction, whereas, acid and neutral and neutral and alkaline assays were correlated for all three fractions.

At km -1.9, 86.1, acid and alkaline assays were correlated for both the total and filtered fractions but not the particulate fraction. Acid and neutral and neutral and alkaline assays were not correlated for any of the three fractions.

9.8 Relationship with environmental variables

PMEase activity, filtered total and particulate, measured at three pH values was correlated with a range of environmental variables assumed to influence rates of autochthonous enzyme production or allochthonous introduction to the water column. Several variables had significant correlation at all sites whereas others were only notable at single location. Correlation was conducted at each site, the data is presented in tables 9.9 to 9.14 and unless stated otherwise the significance level was 95 %.

9.81 Acid PMEase

Acid total PMEase was positively correlated ($p < 0.01$) with; flow, A320, FOP and TP at both km -2.5 and km10.9. Positive correlation was also found between acid total PMEase and total and free living bacteria at km 10.9. At km 49.9, km 107.9 and km 145.0 acid total PMEase had a positive relationship with; flow, A320, attached bacteria. Acid total PMEase and FOP were positively correlated at km 49.9 and km 107.9 but not km 145.0. Positive correlation with free living bacteria and total bacterial numbers ($p< 0.05$) was found at km 49.9 but not km 107.9 or km 145.0.

Acid filtered PMEase was positively correlated ($p< 0.05$) with flow, A320, and FOP at km -2.5, km 10.9, km 49.9. At km 107.9 positive correlation was found between filtered PMEase and FOP and A320. At km 145.0 positive correlation was found with flow and A320. Significant negative correlation was found between filtered acid PMEase and conductivity at km -2.5, km 10.9, km 49.9 and km 145.0.

Table 9.9 Intercorrelation matrix of PMEase, bacteria and environmental variables at km - 2.5, R. Swale. Only significant ($p < 0.05$) values are shown.

	Total PMEase			Filtered PMEase			Particulate PMEase		
	acid	neutral	alkaline	acid	neutral	alkaline	acid	neutral	alkaline
PMEase									
Acid tot.	1.00								
Neut. tot.	0.77	1.00							
Alk. tot			1.00						
Acid filt.	0.95	0.63		1.00					
Neut. filt.	0.60	0.86		0.56	1.00				
Alk. filt			0.80		0.59	1.00			
Acid part.	0.97	0.83		0.83	0.59		1.00		
Neut. part.	0.77	0.95		0.59	0.66		0.86	1.00	
Alk. part			0.67						1.00
Bacteria									
Total									
Attached				0.56					
Free									
Env. variables									
Flow	0.67			0.62			0.65		
Temp.			0.65						0.62
pH			0.63						
T. Alk.									
Cond.	-0.82			-0.76			-0.80		0.56
O_2 %									
A320	0.69			0.56			0.73	0.55	
FRP									
FOP	0.75	0.83		0.57	0.61		0.84	0.85	
TP	0.78	0.76		0.62	0.57		0.84	0.77	

Table 9.10 Intercorrelation matrix of PMEase, bacteria and environmental variables at km 10.9, R. Swale. Only significant ($p < 0.05$) values are shown.

	Total PMEase			Filtered PMEase			Particulate PMEase		
	acid	neutral	alkaline	acid	neutral	alkaline	acid	neutral	alkaline
PMEase									
Acid tot.	1.00								
Neut. tot.	0.91	1.00							
Alk. tot		0.63	1.00						
Acid filt.	0.98	0.88		1.00					
Neut. filt.	0.87	0.99	0.70	0.86	1.00				
Alk. filt			0.88			1.00			
Acid part.	0.97	0.89		0.91	0.83		1.00		
Neut. part.	0.92	0.98		0.88	0.94		0.93	1.00	
Alk. part	0.72	0.87	0.74	0.73	0.90		0.68	0.81	1.00
Bacteria									
Total	0.80	0.80		0.76	0.72		0.82	0.86	0.58
Attached									
Free	0.78	0.81		0.73	0.73		0.79	0.86	0.61
Env. variables									
Flow	0.79	0.55		0.73			0.82	0.63	
Temp.			0.75			0.89			
pH						0.73			
T. Alk.									
Cond.	-0.75			-0.76		0.58	-0.68		
O2 %			-0.63			-0.70			
A320	0.85	0.81		0.83	0.77		0.82	0.83	0.56
FRP									
FOP	0.69	0.71		0.60	0.62		0.77	0.77	
TP	0.79	0.83		0.69	0.74		0.87	0.89	0.56

Table 9.11 Intercorrelation matrix of PMEase, bacteria and environmental variables at km 49.9, R. Swale. Only significant ($p < 0.05$) values are shown.

	Total PMEase			Filtered PMEase			Particulate PMEase		
	acid	neutral	alkaline	acid	neutral	alkaline	acid	neutral	alkaline
PMEase									
Acid tot.	1.00								
Neut. tot.	0.81	1.00							
Alk. tot	0.69	0.95	1.00						
Acid filt.	0.99	0.79	0.67	1.00					
Neut. filt.	0.73	0.97	0.97	0.73	1.00				
Alk. filt		0.86	0.97		0.94	1.00			
Acid part.	0.98	0.82	0.69	0.96	0.71		1.00		
Neut. part.	0.83	0.94	0.83	0.79	0.82	0.68	0.88	1.00	
Alk. part	0.83	0.96	0.92	0.79	0.88	0.78	0.87	0.97	1.00
Bacteria									
Total	0.59	0.67		0.55	0.55		0.65	0.75	0.66
Attached	0.84	0.67	0.58	0.85	0.63		0.79	0.65	0.63
Free		0.62					0.58	0.71	0.62
Env. variables									
Flow	0.90			0.89			0.87	0.61	0.57
Temp.									
pH									
T. Alk.									
Cond.	-0.77			-0.78					
O2 %									
A320	0.85	0.68		0.82			0.87	0.83	0.73
FRP									
FOP	0.55	0.60					0.59	0.69	0.60
TP									

Table 9.12 Intercorrelation matrix of PMEase, bacteria and environmental variables at km 107.9, R. Swale. Only significant ($p < 0.05$) values are shown.

	Total PMEase			Filtered PMEase			Particulate PMEase		
	acid	neutral	alkaline	acid	neutral	alkaline	acid	neutral	alkaline
PMEase									
Acid tot.	1.00								
Neut. tot.	0.76	1.00							
Alk. tot	0.77	0.99	1.00						
Acid filt.	0.92	0.68	0.75	1.00					
Neut. filt.	0.69	0.94	0.97	0.73	1.00				
Alk. filt	0.62	0.86	0.90	0.69	0.95	1.00			
Acid part.	0.82	0.64	0.57				1.00		
Neut. part.	0.73	0.95	0.90	0.56	0.79	0.69	0.76	1.00	
Alk. part	0.78	0.92	0.89	0.65	0.79	0.63	0.73	0.94	1.00
Bacteria									
Total									
Attached	0.59	0.81	0.80		0.72	0.71		0.81	0.75
Free									
Env. variables									
Flow	0.60	0.72	0.70		0.64	0.57	0.55	0.71	0.70
Temp.									
pH									
T. Alk.	-0.62			-0.56					
Cond.	-0.58	-0.73	-0.68		-0.56		-0.69	-0.81	-0.79
O2 %									
A320	0.676	0.88	0.85	0.58	0.76	0.68	0.61	0.90	0.86
FRP	-0.56	-0.56					-0.63	-0.58	-0.65
FOP	0.71	0.57	0.57	0.70				0.57	0.56
TP									

Table 9.13 Intercorrelation matrix of PMEase, bacteria and environmental variables at km 145.0, R. Ouse. Only significant ($p < 0.05$) values are shown.

	Total PMEase			Filtered PMEase			Particulate PMEase		
	acid	neutral	alkaline	acid	neutral	alkaline	acid	neutral	alkaline
PMEase									
Acid tot.	1.00								
Neut. tot.	0.84	1.00							
Alk. tot		0.59	1.00						
Acid filt.	0.89	0.82		1.00					
Neut. filt.	0.58	0.86	0.59	0.76	1.00				
Alk. filt			0.94		0.62	1.00			
Acid part.	0.97	0.78		0.75			1.00		
Neut. part.	0.88	0.95		0.74	0.66		0.88	1.00	
Alk. part		0.61	0.93			0.75		0.61	1.00
Bacteria									
Total		0.59							
Attached	0.59	0.86		0.64	0.62			0.75	
Free					0.86				
Env. variables									
Flow	0.64	0.68		0.56			0.62	0.72	
Temp.									
pH									
T. Alk.	-0.57						-0.59		-0.63
Cond.		-0.63		-0.57	-0.56			-0.59	
O2 %									
A320	0.68	0.93		0.67	0.82		0.63	0.87	
FRP									
FOP									
TP									

Table 9.14 Intercorrelation matrix of PMEase, bacteria and environmental variables at km -1.9, 86.1, R. Wiske. Only significant ($p < 0.05$) values are shown.

	Total PMEase			Filtered PMEase			Particulate PMEase		
	acid	neutral	alkaline.	acid	neutral	alkaline.	acid	neutral	alkaline.
PMEase									
Acid tot.	1.00								
Neut. tot.		1.00							
Alk. tot	0.75		1.00						
Acid filt.	0.92		0.75	1.00					
Neut. filt.		0.97			1.00				
Alk. filt	0.59		0.93	0.60		1.00			
Acid part.							1.00		
Neut. part.	0.57	0.85			0.68			1.00	
Alk. part									1.00
Bacteria									
Total									
Attached			0.61			0.59			
Free									
Env. variables									
Flow			0.61			0.60			
Temp.									
pH									
T. Alk.									
Cond.			-0.58			-0.62			
O2 %									
A320									
FRP									
FOP									
TP									

9.82 Neutral PMEase

Neutral total PMEase was significantly correlated with FOP and A320 at km 10.9 and km 145.0. At km -2.5 neutral total PMEase was positively correlated with FOP and TP, whereas, at km 107.9 correlation was with FOP and A320. Neutral total PMEase was significantly positively correlated with free living bacteria at km 10.9 and km 49.9, with attached bacteria at km 49.9, km 107.9, and km145.0 and with total bacteria at km 10.9, km 49.9, and km 145.0. Flow was significantly correlated with neutral total PMEase at km 10.9, km 107.9 and km 145.0. Significant negative correlation was found between total neutral PMEase and conductivity at km 107.9 and km 145.0. No significant correlation was found between neutral total PMEase and environmental variables at km -1.9, 86.1.

Neutral filtered PMEase was significantly positively correlated with FOP and TP at km -2.5 and 10.9, with A320 at km 10.9, km 107.9 and km 145.0 and with flow at km 107.9. Significantly negative correlation was found with conductivity at km 107.9 and km 145.0.

9.83 Alkaline PMEase

Alkaline total PMEase was significantly positively correlated with water temperature at km -2.5 and km 10.9, with pH at km 10.9 and with A320 and FOP at km 107.9. Alkaline filtered PMEase was significantly positively correlated water temperature and pH at km 10.9 and with A320 at km 107.9

9.9 Modelling organic phosphorus turnover

Assays conducted under laboratory conditions have been shown to provide data on the rates of filtered and particulate PMEase at specific pH. However, these results are divorced from the ambient conditions within the river at the time of sampling and give

poor insight into the role phosphatases may have on the P dynamics of the river. Ideally, samples would be analysed under both standard laboratory and field conditions for all months of the year. However, time constraints made this impractical and this was done on just four of twelve occasions.

A model based on pH and temperature was developed to assess potential rates of PMEase and consequent breakdown of organic phosphorus along the Swale-Ouse system. Results from assays conducted at standard temperature and pH were recalculated to field pH and temperature at the time of sampling. It was found that log transformation of rates PMEase at pH values between 5.0 and 7.5 and 7.5 and 9.5, resulted in two linear trends either side of pH 7.5. Using this linear equation, rates of PMEase calculated at 25°C were transformed to a predicted rate at ambient field pH. These values were then transformed using the linear equation derived from the temperature curves to adjust the data to field temperature.

9.91 Seasonal trend of model

In general all sites had low rates of PMEase activity in the winter months; December to February, and higher rates in late summer and spring (Figure 9.11). The exception to this was km 107.9 where both December and February had rates similar to those in summer and spring. In general, this suggests that the temperature of the water rather than its pH is a more influential variable controlling the rate of PMEase activity. The maximum rate for all sites was in June 1997.

Predicted values were compared to observed data on four occasions. The observed data were river water samples assayed at field temperatures without buffer. The predicted values of the model were similar to observed values on three out of four occasions, but consistently underestimated PMEase in November 1996.

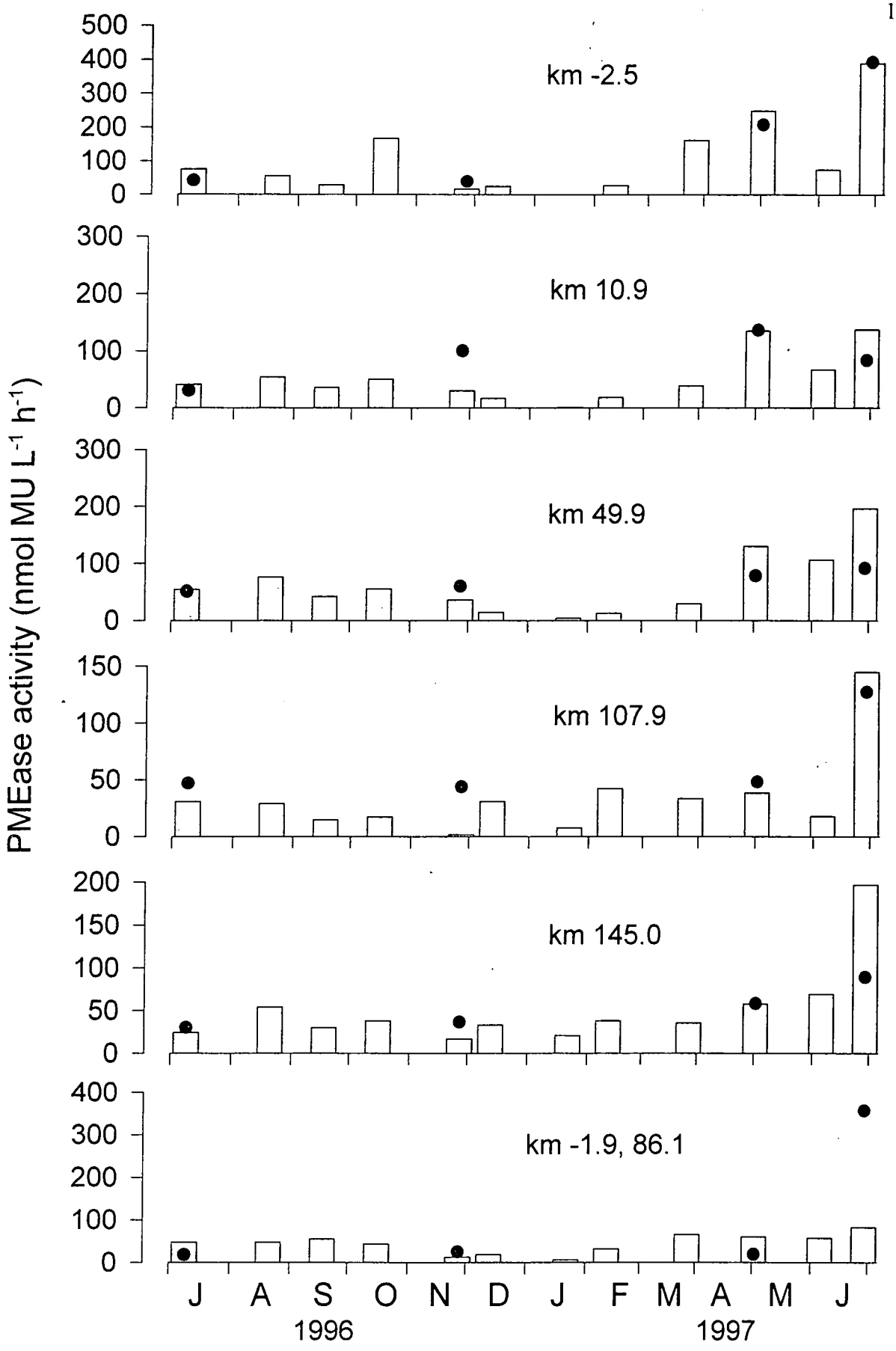

Figure 9.11 Total PMEase recalculated to ambient temperature and pH at the time of sampling. ☐ Predicted data is compared with ● observed data on four of 12 sampling events.

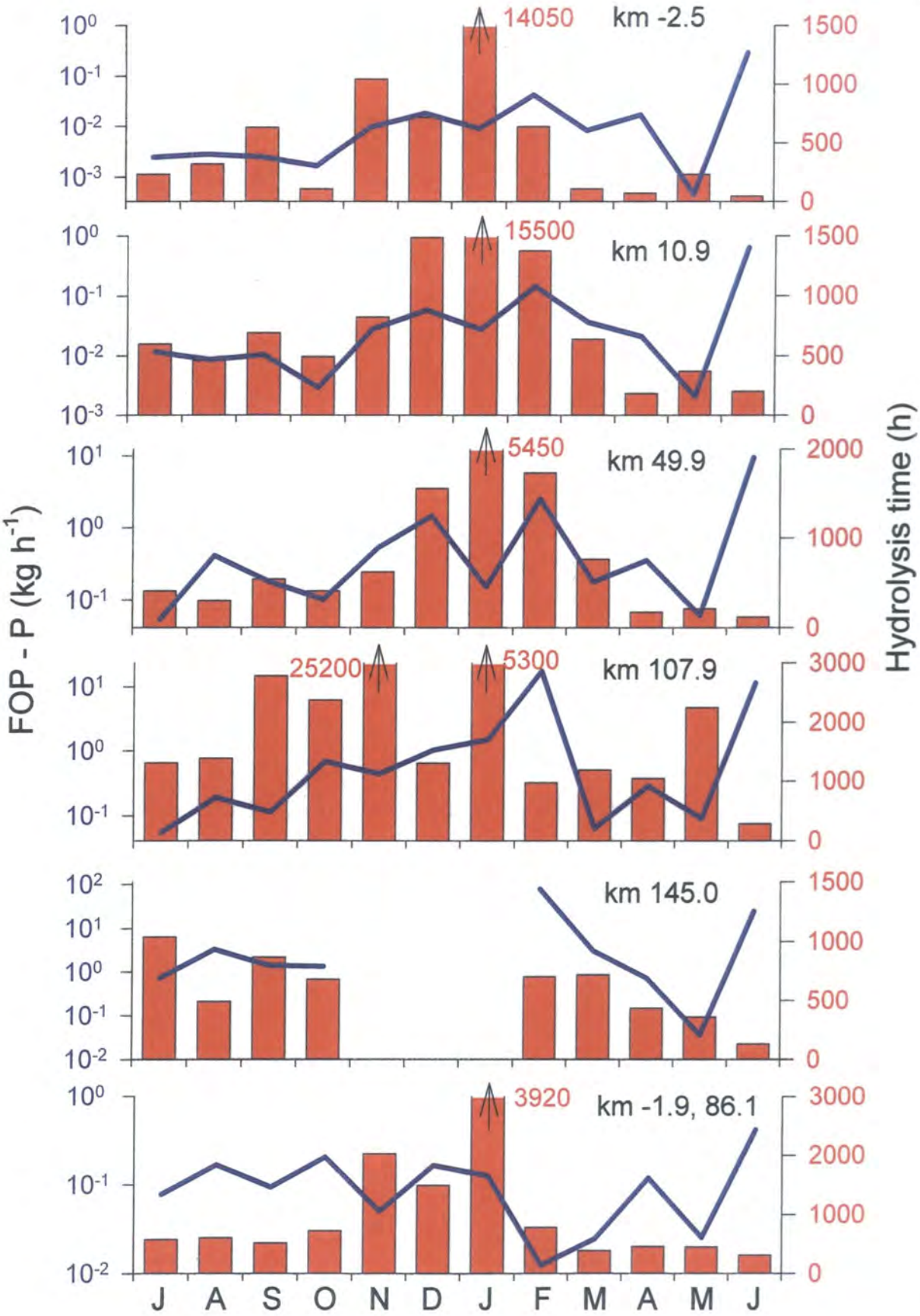

Figure 9.12 Predicted time (h) to hydrolyse 50% of FOP at six sites within the Swale - Ouse catchment between July 1996 and June 1997.
FOP discharge (kg h^{-1}) is also shown.

9.92 Estimated turnover of organic P

The time taken to hydrolyse 50% of the potential substrate was calculated to indicate the importance of water-borne enzyme to the regeneration of inorganic P. A model incorporated the FOP concentration recorded at the time of sampling and the kinetic parameters raised in section 9.23. Under the assumption that all the FOP was potential substrate the concentration of FOP and FOP/2 was used to re-calculate the rate of the reaction using;

PMEase activity = V_{max} x substrate conc. / (Ks + substrate conc.)

The reaction rate was assumed to be fixed at the average of the two FOP concentrations. V_{max} was estimated from the temperature and pH adjusted model and Ks from two kinetic studies under low and high flow conditions. The effect of enzyme inhibition by released inorganic phosphorus and the ambient concentration of inorganic phosphorus was excluded from the calculation.

The results showed that hydrolysis times were greater in the winter than summer (Figure 9.12). The most rapid hydrolysis and was estimated on the 26 and 27 of June 1997 at the most upstream site km -2.5.

9.10 Discussion

Assays of river water have shown there are clear differences in the rate of phosphatase activity on passing down the Swale-Ouse river system. The total activity was comprised of a filtered component, (< 0.2 μm) analogous to "free" or dissolved enzyme, and a particulate fraction. In general, higher rates of PMEase were found in the filtered

component compared with the particulate fraction. Both fractions were assayed at three pH values: 5.5, 7.5, 9.5; for simplicity termed: acid, neutral and alkakline. In general, acid total PMEase was greater than neutral or alkaline PMEase. Mean annual acid total PMEase decreased on passing down the river, whereas, neutral and alkaline total PMEase had higher values in the middle reaches of the river. This suggests that assays over a range of pH values may be one way of separating enzymes with different sources in river systems.

Evidence from the year study and an assessment of variability suggests that the source of acid PMEase is located in the upper reaches of the river, particularly streams influenced by peat leachate. Significant correlation between acid PMEase, FOP and A320 suggests that both the potential substrate and the source of the enzyme are linked in some way. The decrease in acid PMEase on passing down the river may be due to dilution and or an inactivation of the enzyme by increasing quantities of phosphorus input to the river.

9.11 Summary

i. Total PMEase had a linear relationship with assay temperature between 3 and 30 °C under both low and high flow conditions.

ii. The influence of substrate concentration carried out on two occasions had similar results under low and high flow conditions. V_{max} decreased with distance downstream, although the lowest activity was recorded on the eutrophic tributary R. Wiske on both occasions. Ks values were comparable on both occasions at all sites, except km 107.9, where values were almost double that of the other sites on both occasions.

iii. Spatial variability of PMEase was greater at two upstream tributaries than at a site located in the middle reaches of the main river. Peat drainage and iron deposits were notable influences in samples with the high rates of filtered and total acid

PMEase. However, comparison of samples taken mid-stream from a tributary and the main river showed no significant difference in variability.

iv. In a survey carried out during the period of highest PMEase activity, the ratio of PDEase : PMEase decreased on passing downstream

v. Acid PMEase had higher rates than alkaline and neutral PMEase, at all sites, on nearly all occasions.

vi. Mean acid total PMEase decreased on passing downstream whereas neutral and alkaline total PMEase had high values in the middle reaches of the river.

vii. At most sites, acid and neutral PMEase were closely correlated but showed a poor relationship with alkaline PMEase suggesting differences in the origin of acid and alkaline PMEases.

viii. Acid total PMEase was positively correlated with Flow, FOP and A320 and negatively correlated with conductivity at most sites, with the exception of km -1.9, 86.1. Neutral and alkaline total PMEase had variable relationships with environmental variables at the sites surveyed.

ix. A model using recalculated laboratory data to predict rates of PMEase under ambient field conditions closely matched observed rates from experiments carried out at field conditions. This data suggests that temperature rather than pH is the more important factor influencing rates of PMEase acitivity in the Swale-Ouse.

CHAPTER 10

General discussion

10.1 Introduction

Field studies have produced interesting results concerning the relationship between water chemistry and the physiology of mosses along a river continuum. Studies of *in situ* and transplanted mosses have shown that internal nutrient content and surface phosphatase activity reflect the nutrient concentration of the water, along a river continuum. Phosphatase activity in the water itself appears to be an important factor in the P dynamics of the Swale – Ouse river system.

10.2 Water Chemistry

N and P in the Swale increase on passing downstream (Figure 4.6), typical of many rivers feeding the Humber estuary (House *et al.*, 1997; Robson & Neal, 1998). The dominant fractions of both N and P tend to reflect the sources of nutrients. In upper parts of the catchment, TIN is dominated by nitrate, the most likely source being agricultural runoff, whereas downstream, increasing proportions of both nitrite and ammonium are consistent with inputs from STW and CSO.

Organic P formed a larger proportion of total P in upper parts of the river, compared with sites further downstream (Figure 4.6). Although there is no direct evidence, strong correlation between absorbance at 320 nm and FOP suggests the most likely source was peat. In upland reaches, FOP was positively correlated with flow (Figure 4.7), and its is thought that higher rainfall and increased leaching from peat may be the major factor controlling FOP concentrations in many upper Pennine streams. In Teesdale, a neighbouring catchment, Livingstone & Whitton (1984) concluded that a

spring release of FOP was a function of a wide diurnal temperature range, as well as high flow. This study suggests FOP is more strongly related to flow than a particular season. Further downstream, FOP was negatively correlated with flow, dilution being greater than the input of P.

The relationship between flow and FRP was similar to that of FOP. High flows diluted FRP in the lower reaches of the Swale (Figure 4.7), and this is consistent with the LOIS rivers investigated by Jarvie *et al.* (1998). Comparison with LOIS data shows significant difference with the most variable determinants: ammonium, nitrite and FOP. However, the differences are more likely to be the result of the sampling programmes. This study was a time based programme with regular sampling on a monthly basis, whereas LOIS sampled both weekly, and in response to high flow events. A biological study of benthic organisms necessitates sampling in moderate to low flows. With flow being a major factor in the concentration of nutrients, differences between the programmes was almost inevitable.

10. 3 Phosphatase activity of mosses

F. antipyretica and *R. riparioides* are both capable of expressing PMEase and PDEase activity. The optimum activity was at pH 5.5, consistent with previous studies of mosses (Press & Lee, 1982). It remained pH 5.5 under both high and low substrate concentrations. In contrast, studies of neutral-alkaline PMEase activity in mammalian tissues (Fedde & Whyte, 1990) and a freshwater diatom (Hantke & Melzer, 1993), have shown increases in the optimum pH with increased substrate concentration. It is assumed that acid and alkaline 'surface' PMEases respond differently to external concentrations of substrate, the synthesis of one or the other may be dependent on the environment.

Studies using elements known to inhibit or stimulate PMEase and PDEase (1.82), revealed that PMEase and PDEase assayed at pH 5.5 and 9.5 were inhibited by fluoride and zinc (Figure 5.8 – 5.11). Fluoride inhibition is specific to acid phosphatases (Hollander, 1971) and the inhibition observed implies that measurement of PMEase and PDEase at pH 9.5 are not alkaline, but acid phosphatases measured at sub-optimal pH.

During a seasonal study, both mosses showed an increase in N and P contents on passing downstream (Figure 6.1), but relatively more so for P. *F. antipyretica* tended to have a higher N content than *R. riparioides*, though the difference was only slight at km 10.9. *F. antipyretica* showed an obvious increase in N content down to km 49.9, whereas, there was usually little increase for *R. riparioides* below km 10.9. The increase in N content may reflect the fact that the shoots tend to be larger downstream and the 2-cm tips probably include a higher ratio of leaf to stem.

The PMEase activity was highest at km - 2.5, but was variable values at km 10.9 and was low at the two downstream sites (Figure 6.2). In general, *F. antipyretica* showed almost twice the PMEase (per unit mass) to that shown by *R.riparioides* over the whole range of P content. The ratio between the PMEase activities of the two mosses tended to be higher than that between their N contents. Plots of PMEase activity versus P content of the 2-cm tips, showed in both cases that there was a marked increase in PMEase activity when the P content dropped below 0.3 % dry wt (Figure 6.3). Although at the downstream sites PMEase activity was low, rates were always detectable, generally well above the detection limit. It seems probable that both mosses retain low PMEase activity even when the tissues contain a high P content. However, it will require the use of axenic plants before the possibility can be excluded that this residual activity is due to persistent bacteria. The fact that PMEase was not

competitively inhibited by high concentrations of inorganic P suggests the enzyme synthesis is related to internal P and does not respond immediately to external P like de-repressible phosphatases. The presence of non-inducible PMEase would presumably permit the plant to make use of FOP at all times. On the assumption that the 'background' PMEase activity is due to non-inducible activity, the values for *F. antipyretica* and *R. riparioides* are approximately 20 and 10 μmol MUP g d. wt^{-1} h^{-1}, respectively.

The widely quoted study of Redfield *et al.* (1963) suggested that a ratio for N : P in marine plankton above 16:1 (by atomic ratio; by mass = 7 : 1) indicates P limitation in situations where N was available. Figure 6.4 suggests that mosses with a N : P ratio above about 9 : 1 have enhanced PMEase activity. Although the possibility that PMEase is formed in response to some external organic P source, the circumstantial data strongly suggest that levels above the background are de-repressed under conditions of P limitation. However, the study provides insufficient data to establish whether the internal P content or the N : P ratio provides a better predictor of PMEase activity.

Figure 6.4 shows one outlier with much higher PMEase activity than expected for a ratio of 7 : 1, and a few outliers where PMEase actvity is scarcely enhanced for ratios above 9 : 1. A possible explanation for the former is that this was a formerly P-limited population, which had recently experienced a pulse of high ambient phosphate. The latter outliers may reflect populations that had not yet synthesised PMEase in response to P limitation. However, experimental studies will be required to rule out other possibilities, such as clonal differences in response to P limitation.

Positive correlation was found between PMEase activity and temperature, conductivity and pH. The relationship with temperature is explained by an increase in

reaction rate, but higher acid PMEase activity at high environmental pH is more difficult to explain. The relationship between PMEase activity and pH and conductivity is probably not causal, but related to other factors such as internal P concentration and the lag between uptake and repression of phosphatase (10.31). No relationship was found between PMEase activity and aqueous nutrients. It is presumed that the slow growth rate of mosses and the variability of FRP and FOP (4.422) may explain the lack of relationship with P. Comparison between N : P ratio in mosses and N : P ratio in water, shows inconsistencies at the headwater site. Mosses generally had high N : P, whereas, in the water it was low. It is an undoubted flaw of this study that no measure of organic N was made. Considering the importance of organic P in the upper reaches, it could be that organic N is the missing component that would resolve the inconsistency in the comparison of the N : P ratios.

PDEase and PMEase activity of *F. antipyretica* both showed marked decreases on passing down the Swale system, with the highest rates corresponding to where the tissue P concentrations were lowest. PMEase assayed by the two analogue substrates gave similar V_{max} values on passing down the river during April 1997. The rate of PDEase was higher than PMEase at the headwater site, but decreased much more rapidly on passing downstream. The rate of PDEase at the downstream site was so low that it raises the question whether the moss really does show surface PDEase activity under conditions of high ambient phosphate.

The difference in the relative importance of PDEase and PMEase at different positions within river catchments in this region is shown even more clearly in the comparative study of populations in streams draining different catchments (Figure 7.3). Although this figure emphasises the importance of altitude, it seems probable that this is because of the correlation between the area of peat, or peat-derived soil, within the

catchment and altitude. As mentioned above, peat in Swaledale is found mainly at locations above 300 m, and peat is known to contain high levels of diesters (Bedrock et al., 1994). At sites on the main river where PMEase activity is greater than PDEase, yet still well above the background level of activity, the catchment above the sites includes more grazing land where a higher proportion of monoesters may be expected. Further downstream (km 49.9), inputs of inorganic phosphorus from sewage effluents (such as Colburn STW, km 47.0) and non-point sources negate the importance of organic P hydrolysis by phosphatase. Inputs from major tributaries such as the R. Wiske, Cod Beck and Bedale Beck, increase the concentration of inorganic P at the most downstream site km 107.9.

The apparent gradient of PDEase to PMEase to repression of phosphatase activity in response to catchment inputs, although elegant, is difficult to explain fully. Although the phosphatase activity of mosses appears to be independent of external P concentration over short periods (5.6), one possible explanation is that mosses have adapted, over time, to synthesise specific enzymes in response to potential extracellular substrates. Utilisation of organic substrates has the additional benefit of releasing C and N, as well as P. Alternatively, the synthesis of PDEase and PMEase in upland peaty streams may simply be part of a wider stress response. The correlation between sites of P stress and supply of organic P in Pennine streams makes this difficult to resolve.

The phosphatase enzymes of *F. antipyretica* along the river continuum appear to be reflecting the most likely substrates leaching from the soils, with the much higher availability of inorganic P at downstream sites reducing the necessity for phosphatase activity. It would however, require extensive studies to establish to what extent the explanation is as simple as this. Little is known about the forms of P released by

cycling at different positions within the river system, or of their persistence within the system. It is also uncertain to what extent the formation of phosphodiesterases represents a higher energetic cost for the moss than the formation of phosphomonoesterases. Does the moss only form phosphodiesterase when it is under extreme P limitation, or has it evolved to do so because such environments tend to be ones where phosphodiesters are the predominant form of available P? A further complication is that nothing is known about the ability of the moss to utilise the N component of phosphodiesters, nucleic acids for example. This ability might enhance the advantage of hydrolysing them when ambient N concentrations are low.

The Ks values for MUP and pNPP in May 1997 suggest that the affinity for the substrates remains similar along the river continuum, though the values obtained in September were more variable. The affinity for MUP was higher than pNPP at all sites on passing down the river. Yelloly (1996) found similar results for colonies of *Rivularia* in upper Teesdale, an adjacent catchment. It is suggested that PMEase can hydrolyse organic substrates efficiently over a range of concentrations. The Ks of PDEase of *F. antipyretica* is higher at the most upstream compared with the downstream ones. This may imply that surface PDEase of the moss is especially important for pulses of higher concentrations of diesters, rather than continuous low concentrations. This scenario fits with the intermittent, flow related, pulses of FOP in the upper reaches of the Swale (see above). V_{max} for both PMEase and PDEase was higher in September than March suggesting greater production of enzyme at this time of the year.

The experimental studies on P removal from medium add strong support to the importance of PDEase (Figure 7.2). *F. antipyretica* was able to remove P from medium containing acid hydrolysed DNA as fast as from medium with inorganic

phosphate. Furthermore, the rate at which this took place was correlated with the level of PDEase shown by assays using MUP. Therefore, it seems probable that most removal of P from medium is due to uptake and not merely adsorption onto the walls. The addition of P to rivers has shown it is rapidly utilised by mosses in P-limited streams in the Arctic (Finlay & Bowden, 1994), and a 'heterotrophic' headwater stream with low ambient phosphate in N-E. USA (Meyer, 1979).

Comparison of the results on uptake of P from 1 mg l^{-1} DNA-P with those from the kinetic study on ability to hydrolyse bis-pNPP, suggests that uptake was an order of magnitude less efficient than hydrolysis under the conditions used.

10.31 Transplantation of moss

Reciprocal transplants of *F. antipyretica* between upstream and downstream sites differing by about one order of magnitude in ambient phosphate showed marked changes in PMEase activity. PDEase activity increased only slightly on transplant from downstream to upstream, though activity had fallen to the baseline level by day 4 on transplant from upstream to downstream. This is probably due to the fact that PDEase activity is de-repressed at lower tissue P concentrations than PMEase.

Interpretation of the data is complicated by the fact that the upstream *in situ* population also showed changes in all three phosphatase activities during the experimental period. However, the changes in PMEase activity shown by transplanted materials exceeded changes shown by *in situ* populations at both upstream and downstream sites. The most likely explanation for the lack of any enhancement of PDEase activity in moss transplanted to the upstream site is that the plants had still failed to deplete P reserves sufficiently after 142 days for activity to be de-repressed. Another possibility is that the downstream population may differ genetically from the upstream population.

Although most measurements of phosphatase activity above background levels showed relatively high variability, several features merit comment. During the first four days of the experimental period, the *in situ* moss at the upstream site showed a decrease in PDEase and PMEase activity assayed by pNPP, but not by MUP. This suggests that PMEase assayed by MUP may respond more slowly to a rise in ambient phosphate than the other phosphatase activities. In contrast, the transplant from upstream to downstream showed a transient, but significant ($P < 0.01$) increase in PMEase activity assayed by pNPP. This appears to conflict with results from the seasonal study. The critical 0.3 % P concentration was surpassed within two days, but PMEase activity took nine days to reach steady state conditions. Therefore, the lag between uptake of P and the decrease in PMEase activity is an important consideration.

Transplanted mosses from the eutrophic downstream site to the nutrient poor upstream location took longer to show a change in phosphatase activity than the reciprocal transplant. With luxury concentrations of P within the cells, growth can be maintained under low external P concentrations. However, growth will dilute phosphorus within the cells creating the need to obtain phosphorus from external sources. The slow growth rate of mosses, 2 mm $week^{-1}$ (Kelly & Whitton, 1987), means that a reduction phosphorus levels within the cells may take considerable time. Therefore, the need to obtain external P from organic sources by the synthesis of phosphatases would not need to be a rapid phenomenon. This study makes this apparent, it took up to 142 days of spring/summer growth to approach the critical level of 0.3% P g d. wt^{-1} that Christmas and Whitton (1998a) found to be associated with PMEase induction.

As with metals, mosses are excellent accumulators of external chemicals, but require much longer periods to purge them from their cells. This is compounded by the

fact that although some metals are essential for growth, they are by no means as essential as phosphorus in cell biology. Therefore, mosses may adopt phosphorus saving strategies such as the slowing of growth rate during P limitation in order to avoid the energy investment of synthesising phosphatases.

Using transplanted mosses as nutrient biomonitors appears useful when trying to define a site of luxury ambient phosphate conditions. The rapid repression of the enzyme activity implies that mosses are capable of utilising freely available inorganic P from the water. The data shown in this study to assess repression involve transplantation to a site of abundant inorganic P. Transplantation to a site with variable concentrations of Pi and FOP, or only slightly higher levels of Pi, may prove more difficult to interpret. Interpretation of data from transplants from nutrient rich to nutrient poor proves even more problematic. High cellular P concentration would mean even infrequent pulses of low concentrations of inorganic P could sustain growth for long periods without the moss becoming P stressed. This implies that only mosses already expressing high activity should be used in transplantation experiments.

10.4 Water phosphatase

As well as being predictor of P limitation, phosphatase activity can be an important factor in the regeneration of phosphate from organic phosphorus molecules. This study has assessed both aspects with assays under laboratory and field conditions. PMEase activity in the water was detected at pH 5.5, 7.5, 9.5 (Termed: acid, neutral and alkaline) at all sites studied within the Swale – Ouse catchment. 'Free' enzyme (0.2 μm filtered) formed a significant contribution to total PMEase activity. PDEase in the water was detected on one occasion and it decreased on passing down the river. It would be interesting to investigate PDEase using a more sensitive method, for example a fluorometric substrate, so that comprehensive studies could be undertaken. However,

the fact that PDEase was only readily detected under extreme conditions suggests PDEase is not of quantitative importance in the river system.

PMEase activity was generally highest when assays were carried out at pH 5.5. Studies of neutral-alkaline European lakes and rivers have in general shown that PMEase has an alkaline pH optimum. In contrast, in acidified lakes (Jansson *et al.*, 1988) PMEase has an acid optimum close to the pH of the water. Acid phosphatase was high in comparisons of biofilms from acid and calcareous streams (Chappell & Goulder, 1992; Freeman *et al.*, 1990).

Poor correlation between PMEase activity measured at three pH values at a single site suggests enzymes with different pH optima are present at each site. Poor seasonal correlation between sites along a river continuum at each pH value suggests that there are a number of different sources of enzymes along the river continuum. For example, the phosphatase activity of the receiving waters can increase by up to an order of magnitude immediately downstream of STW and fish farms (Chappell & Goulder, 1994; Carr & Goulder, 1990a) and influence activity for some distance downstream.

Mean neutral and alkaline PMEase activity showed variable results on passing down the river but were highest at km 49.9, downstream of the first major STW, a known source of APA (Chappell & Goulder, 1994b). Mean acid PMEase activity decreased on passing down the river (Table 9.4) and two explanations are feasible. Firstly, the source of the enzyme is in the upper reaches and dilution on passing downstream reduces activity. Alternatively, enzyme is inhibited by increasing concentrations of inorganic P. Although acid phosphatases are generally thought to be constitutive, Jansson *et al.* (1988) have shown acid phosphatase to be competitively inhibited by phosphate. In a limited study at km –2.5, high concentrations of phosphate (10 mg L^{-1}) did not inhibit PMEase or PDEase. Therefore, although inhibition cannot

be totally ruled out it is more likely that dilution or sequestering of free enzyme into biofilms is reducing acid phosphatase activity on passing downstream.

Acid total PMEase activity was correlated with flow at all sites which implies that runoff may be an important source of enzyme both from within the river, i.e. pore waters, biofilms and from soils. Particulate phosphatase activity was correlated with bacteria at km 49.9 downstream of a STW. Poor correlation with microbial numbers at other sites suggests bacteria are not an important factor influencing PMEase at sites not directly downstream of sewage inputs.

Although criticism has been raised concerning the strict use of kinetic parameters for mixed populations, it is a useful way of characterising a group of enzymes for comparison (Jansson *et al.*, 1988). Kinetic studies showed that substrate saturation at 100 μM was similar to other studies of lotic environments (Admiraal & Tubbing, 1991; Chappell & Goulder, 1992). As for studies of bryophytes, there is a notable discrepancy between the substrate concentration required for saturation and the potential concentration of natural substrate. Average Ks for was 50 μM, higher than other studies of rivers, 15 μM - 33 μM (Admiraal & Tubbing, 1991; Boon, 1993). However, comparison between assays at standard temperature and pH and those conducted under field conditions is difficult to interpret.

To compare results with field studies a phosphatase activity model was calculated using results from temperature and pH studies (9.9). The data predicted by the model was consistent with assays carried out under field conditions on four occasions. The site furthest downstream, km 145.0, was approximately 8 km downstream of a site used for phosphatase studies by Chappell & Goulder (1995), where the average PMEase activity was 38.7 nmol L^{-1} h^{-1} over 14 months. This study showed slightly higher results; 53.7 nmol L^{-1} h^{-1} (mean of four direct readings), 51.3

nmol L^{-1} h^{-1} (mean of 12 readings predicted by the model). However, the site from this study was downstream of a major STW.

A second model including potential substrate limitation was used to calculate the time to hydrolyse 50 % of FOP. Hydrolysis took longer in winter at all sites, particularly in the upper part of the catchment. The shortest times were found in summer during an unusually high flow. This suggests that temperature is a major controlling factor in the hydrolysis of FOP. Taking an example from the high rates of activity in summer at km –2.5 under high flows, shows that 50 % of FOP is hydrolysed in 46 h. This calculation may be criticised as the actual concentration of enzyme available P is unknown, although it has been 75 % of FOP (1.72). However, it demonstrates the importance of PMEase in the regeneration of inorganic P in lotic ecosystems.

10.5 Concluding remarks

Although the project set out to assess a wide range of factors influencing P dynamics in the Swale – Ouse, it became focussed on aspects of phosphatase and its use in monitoring. The project found that PMEase and PDEase activity of mosses was related to internal P contents rather than short-term changes in external P concentration. However, PDEase was synthesised under extreme P stress where there were potential inputs of diesters. Therefore, mosses may respond to external P over a longer period. Transplantation of mosses showed that PMEase and PDEase could be repressed more quickly than de-repressed. In spite of the lag before nutrient content and PMEase or PDEase respond to ambient nutrient conditions, and probably also a lag before PMEase or PDEase reflects the internal nutrient status, it is suggested that such assays provide a valuable aid to monitoring the nutrient status of streams and rivers. Particularly in

rivers and streams of generally low diffuse inputs of P. Its application to higher nutrient regimes appears doubtful.

The variability of phosphatase activity of water, due principally to the heterogeneity in the source of the enzyme, makes it an unsuitable parameter for the interpretation of P limitation. However, PMEase in the water column appears to play an important role in the P cycle of rivers by regenerating inorganic P from organic molecules.

Summary

i. A study was carried out on aspects of water chemistry, the phosphorus status of two common mosses, and 'surface' phosphatase activities of bryophytes and water, in the Swale – Ouse catchment. This included two seasonal studies over a period of 21 months along the river continuum.

ii. On passing downstream the general trends of environmental variables included increases in pH, conductivity, alkalinity, and nutrients and a decrease in A320

iii. FRP : FOP increased downstream reflecting the enhanced contribution of FRP, particularly from point sources at km 49.9 and R. Wiske. The N:P ratios (TIN:FRP, TIN:FTP) decreased on passing downstream and indicate potential periods of P limitation in the upper reaches.

iv. The variability of nitrate, nitrite, FRP and TP decreased on passing downstream, whereas, ammonium and FOP showed little change in variability along the continuum. Comparison between LOIS nutrient data and this study at km 49.9, km107.9 and km145.0, found no significant difference for nitrate and TP, whereas, the nutrients with higher variability: nitrite, ammonium, FRP, FOP were all significantly different at km 49.9. The data sets (except FOP) were not significantly different at km 145.0.

v. The 'surface' PMEase activity *F. antipyretica* and *R. riparioides* was saturated at substrate concentrations > 80 μM MUP, and was linear over a one hour period. Optimum PMEase activity of both mosses was evident under acid conditions (pH 5.5) at both saturated (100 μM) and substrate limited (1 μM) conditions. The relationship between PMEase and internal nutrient contents suggests that PMEase is elevated when P content is less than 0.3 % and N : P was greater than 7 : 1.

vi. PMEase and PDEase activity of both mosses was inhibited by high concentrations of F and Zn, whereas, Ca, K, N and P showed no effect.

vii. River continuum studies of PMEase and PDEase of *F. antipyretica* showed that the influence of substrate concentration (MUP, pNPP, bis-pNPP) tested on two occasions, showed higher rates of hydrolysis in autumn compared with late spring, for all three substrates. V_{max} of PDEase was higher than PMEase at the most upstream site on both occasions. Uptake of P from DNA and inorganic P had similar rates at the most upstream sites, whereas uptake from phytic acid was slightly lower.

viii. Intensive studies of *F. antipyretica* was carried out on samples from 20 twenty streams draining a peat moorland. PDEase was correlated positively with: A320, Pi uptake, altitude; negatively with: tissue P, pH. PMEase was correlated positively with Pi uptake and altitude and negatively with tissue P.

ix. Transplant of *F. antipyretica* from nutrient poor to nutrient sufficient conditions, increased P contents 12-fold over two days. PMEase and PDEase were reduced within two weeks, but took nearly a month to reduce to the levels of the *in situ* population. Transplant from nutrient sufficient to nutrient poor conditions produced much slower response, both in P content and phosphatase, than in the reciprocal transplant. Notable increases in phosphatase, above the natural fluctuation of the *in situ* population, was noted after two months. P content failed to fall below the critical 0.3% P value, the value at the end of the study, 0.32 % g d. wt^{-1}.

x. Characterisation of total PMEase of water was carried out prior to a seasonal study. The response to temperature was linear between 3 and 30 °C under both low and high flow conditions. Optimum activity was at pH 5.5 on most occasions and the reaction was linear in assays of up to 60 min. The influence of substrate concentration carried out on two occasions had similar results under low and high flow conditions. V_{max} decreased with distance downstream, although the lowest activity was recorded on the eutrophic tributary R. Wiske on both occasions. Ks values were comparable on both occasions at all sites,

except km 107.9, where values were almost double that of the other sites on both occasions.

xi. Spatial variability of PMEase was greater at two upstream tributaries than at a site located in the middle reaches of the main river. Peat drainage and iron deposits were notable influences in samples with the high rates of filtered and total acid PMEase. However, comparison of samples taken mid-stream from a tributary and the main river showed no significant difference in variability.

xii. In a survey carried out during the period of highest PMEase activity, the ratio of PDEase : PMEase decreased on passing downstream

xiii. Mean acid total PMEase decreased on passing downstream whereas neutral and alkaline total PMEase had high values in the middle reaches of the river. At most sites, acid and neutral PMEase were closely correlated but showed a poor relationship with alkaline PMEase suggesting differences in the origin of acid and alkaline PMEases.

xiv. Acid total PMEase was positively correlated with Flow, FOP and A320 and negatively correlated with conductivity at most sites, with the exception of km - 1.9, 86.1. Neutral and alkaline total PMEase had variable relationships with environmental variables at the sites surveyed.

xv. A model using recalculated laboratory data to predict rates of PMEase under ambient field conditions closely matched observed rates from experiments carried out at field conditions. This data suggests that temperature rather than pH is the more important factor influencing rates of PMEase activity in the Swale-Ouse.

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Appendix

Variability of P fractions

Mean concentration ($\mu g\ L^{-1}$ P), standard error and coefficient of variation of three P fractions, from km -2.5, between August 1995 and March 1996 (n=3).

Date		FRP	FTP	TP
16/08/95	Mean	7.2	16.7	18.8
	S.E.	0	0.4	1.2
	C.V.	0	2.5	6.4
12/09/95	Mean	28.9	6.9	20.9
	S.E.	27.5	0.2	0.3
	C.V.	26.6	3.2	1.6
17/10/95	Mean	21.7	7.2	18.9
	S.E.	22.1	0.9	2.1
	C.V.	26.5	12.2	11.1
14/11/95	Mean	21.6	7.9	16.4
	S.E.	19.1	0.4	0.3
	C.V.	20.9	5.2	1.9
18/12/95	Mean	13.1	10.6	10.4
	S.E.	13.6	0.2	0.3
	C.V.	17.3	1.5	2.6
19/01/96	Mean	20.8	20.9	20.2
	S.E.	26.3	0.3	0.2
	C.V.	21.9	1.4	1.1
06/03/96	Mean	10.3	1.1	7.2
	S.E.	11.1	0.3	0.2
	C.V.	10.2	24.2	2.4

Mean concentration ($\mu g\ L^{-1}$ P), standard error and coefficient of variation of three P fractions, from km 10.9, between August 1995 and March 1996 (n=3).

Date		FRP	FTP	TP
16/08/95	Mean	6.5	15.1	20.4
	S.E.	0	0.5	0.1
	C.V.	0	3.2	0.4
12/09/95	Mean	5.2	37.0	31.1
	S.E.	0.3	7.0	0.3
	C.V.	5.3	19.0	1.0
17/10/95	Mean	4.7	14.4	16.9
	S.E.	0.2	1.2	0.5
	C.V.	3.9	8.4	2.8
14/11/95	Mean	6.7	14.0	17.1
	S.E.	0.3	0.4	0.6
	C.V.	4.3	3.2	3.4
18/12/95	Mean	9.7	8.6	10.3
	S.E.	0.1	0.1	0.1
	C.V.	1.2	0.9	0.9
19/01/96	Mean	21.6	23.2	25.0
	S.E.	1.0	0.4	0.2
	C.V.	4.4	1.7	0.9
06/03/96	Mean	0.1	5.4	6.3
	S.E.	0	0.1	0.2
	C.V.	0	2.5	2.4

Mean concentration ($\mu g\ L^{-1}$ P), standard error and coefficient of variation of three P fractions, from km 49.9, between August 1995 and March 1996 (n=3).

Date		FRP	FTP	TP
16/08/95	Mean	250.6	310.6	328.8
	S.E.	3.2	3.0	1.5
	C.V.	1.3	1.0	0.4
12/09/95	Mean	94.2	107.8	130.7
	S.E.	1.7	0.6	0.7
	C.V.	1.8	0.6	0.5
17/10/95	Mean	284.9	308.8	313.9
	S.E.	3.0	5.8	4.8
	C.V.	1.1	1.9	1.5
14/11/95	Mean	101.7	115.9	125.6
	S.E.	1.2	1.2	0.7
	C.V.	1.2	1.1	0.6
18/12/95	Mean	128.8	137.5	144.9
	S.E.	4.8	2.5	3.2
	C.V.	3.7	1.8	2.2
19/01/96	Mean	82.8	123.9	128.1
	S.E.	2.5	8.6	3.7
	C.V.	3.0	6.9	2.9
06/03/96	Mean	39.5	47.8	56.4
	S.E.	1.0	2.0	1.6
	C.V.	2.5	4.1	2.9

Mean concentration ($\mu g\ L^{-1}$ P), standard error and coefficient of variation of three P fractions, from km 107.9, between August 1995 and March 1996 (n=3).

Date		FRP	FTP	TP
18/08/95	Mean	166.2	401.0	449.0
	S.E.	1.0	20.9	17.6
	C.V.	0.6	5.2	3.9
14/09/95	Mean	192.4	756.9	762.8
	S.E.	159.4	13.6	86.1
	C.V.	82.9	1.8	11.3
19/10/95	Mean	174.2	146.1	174.9
	S.E.	9.9	21.8	20.4
	C.V.	5.7	14.9	11.7
22/11/95	Mean	186.9	202.8	223.2
	S.E.	20.4	24.8	40.3
	C.V.	10.9	12.2	18.1
20/12/95	Mean	279.4	233.4	278.2
	S.E.	17.1	23.8	70.5
	C.V.	6.1	10.2	25.3
17/01/96	Mean	148.5	183.1	231.5
	S.E.	28.2	42.9	21.4
	C.V.	19.0	23.4	9.3
09/03/96	Mean	128.3	129.8	149.0
	S.E.	1.1	3.1	2.2
	C.V.	0.8	2.4	1.5
16/04/96	Mean	309.5	315.1	328.6
	S.E.	9.0	5.5	9.6
	C.V.	2.9	1.7	2.9

Mean concentration ($\mu g\ L^{-1}$ P), standard error and coefficient of variation of three P fractions, from km 145.0, between August 1995 and March 1996 (n=3).

Date		FRP	FTP	TP
18/08/95	Mean	544.7	1265.3	1990.7
	S.E.	14.0	41.7	25.5
	C.V.	2.6	3.3	1.3
14/09/95	Mean	128.1	1254.0	1352.3
	S.E.	18.3	8.9	23.6
	C.V.	14.3	0.7	1.7
19/10/95	Mean	261.7	269.4	285.4
	S.E.	5.7	11.1	3.1
	C.V.	2.2	4.1	1.1
22/11/95	Mean	319.2	343.9	382.1
	S.E.	4.6	6.9	3.3
	C.V.	1.5	2.0	0.9
20/12/95	Mean	448.7	389.4	422.6
	S.E.	15.4	6.1	2.7
	C.V.	3.4	1.6	0.6
16/04/96	Mean	721.9	737.8	789.6
	S.E.	13.3	3.7	32.6
	C.V.	1.8	0.5	4.1

Mean concentration ($\mu g\ L^{-1}$ P), standard error and coefficient of variation of three P fractions, from km –1.9, 86.1, between August 1995 and March 1996 (n=3).

Date		FRP	FTP	TP
18/08/95	Mean	1291.8	2977.9	3051.5
	S.E.	13.2	32.7	53.5
	C.V.	1.0	1.1	1.8
14/09/95	Mean	1093.0	1828.6	2069.7
	S.E.	29.9	121.2	264.1
	C.V.	2.7	6.6	12.8
19/10/95	Mean	971.7	952.0	998.7
	S.E.	14.5	13.3	21.4
	C.V.	1.5	1.4	2.1
22/11/95	Mean	1131.0	1207.6	1239.1
	S.E.	17.3	19.7	25.0
	C.V.	1.5	1.6	2.0
20/12/95	Mean	983.6	1256.0	1271.5
	S.E.	14.8	14.1	14.0
	C.V.	1.5	1.1	1.1
17/01/96	Mean	2491.3	3203.9	3303.7
	S.E.	12.3	9.1	47.2
	C.V.	0.5	0.3	1.4
16/04/96	Mean	1040.4	1055.5	1064.0
	S.E.	71.1	74.9	81.7
	C.V.	6.8	7.1	7.7