Phosphorus and its biological effect in lowland rivers

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by

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to my mother and father, for all their love

ABSTRACT

Phosphorus and its biological effect in lowland rivers

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Eastern England is mostly rural with extensive arable farming. It is one of the driest areas of the UK, receiving approximately two thirds of the national rainfall average. The rivers in this region are generally considered sluggish with high nutrient content.

This study examined the sources and seasonal pattern of phosphorus concentrations in these lowland rivers. Biological effect of phosphorus (measured as soluble reactive phosphorus, SRP) was investigated in algae; specifically the response of diatom species and periphyton biomass to elevated levels of phosphorus.

The rivers in this part of the UK are routinely monitored by the Anglian Region of the Environment Agency. Routine monitoring data for SRP were used to indentify high and low phosphorus rivers. Overall, concentrations ranged between <10 $\mu g\ l^{-1}$ to over 10,000 $\mu g\ l^{-1}$ SRP with values significantly skewed towards the lower end of this range. For 1991-5, 21% of sampled river stretches had 100 $\mu g\ l^{-1}$ SRP or below, 49% between 100-500 $\mu g\ l^{-1}$ and 30% between 500-1000 $\mu g\ l^{-1}$. The ratio of nitrogen to phosphorus (total oxidised nitrogen to soluble reactive phosphorus by mass) for the upper reaches of eight rivers indicated that 83 % of sites on these rivers during summer months were potentially phosphorus-limited when the boundary ratio of 10:1 was applied.

Effluent and geology were found to influence instream SRP concentrations. Rivers on chalk geologies had significantly lower phosphorus concentrations than those on other geologies. A significant relationship between effluent load and instream load was found in the small headwater streams of the River Welland (receiving below 5 kg SRP per day)($r^2=0.58$, $p<0.05$) and eight other rivers within the Region ($r^2=0.83$, $p<0.05$). Phosphorus load in some stream sites however could not be accounted for by upstream input of sewage effluent; thus diffuse or agricultural point source was presumed to be responsible. The percentage contribution of each potential source was estimated for the upper Welland in Leicestershire (the most intensively sampled catchment) as 43% point source, 27% diffuse and 30% background.

Diatom species composition changed with SRP concentration. Multivariate analysis of diatom results showed that species composition at sites with less than 50 $\mu g\ l^{-1}$ SRP differed from species assemblage above this concentration. Stream velocity had greater influence on diatom assemblage above 50 $\mu g\ l^{-1}$ SRP. The Shannon index of diversity (H') showed no correlation between diatom species richness and phosphorus concentration. The Diatom Quality Index responded to differences in diatom assemblage with phosphorus concentration only below approximately 100 $\mu g\ l^{-1}$ SRP. Biomass of *Cladophora* and biofilm (measured as chlorophyll 'a') showed restricted growth under 100 $\mu g\ l^{-1}$. Biofilm biomass decreased linearly with increasing stream velocity and *Cladophora* growth increased linearly with increasing velocity within the range of 0.1 to 0.2 $m\ s^{-1}$ mean summer stream velocity.

Low phosphorus stream and rivers (<100 $\mu g\ l^{-1}$ SRP) in this region were those receiving little or no effluents and located on chalk geologies. Algal investigation suggested that the diatom and *Cladophora* communities of such streams would be influenced at this level of phosphorus concentration both in terms of biomass and species composition.

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SRP	soluble reactive phosphorus
DRP	dissolved reactive phosphorus
TP	total phosphorus
TDP	total dissolved phosphorus
DOP	dissolved organic phosphorus
DIP	dissolved inorganic phosphorus
DCP	dissolved condensed phosphorus
PRP	particulate reactive phosphorus
DIN	dissolved inorganic nitrogen
TON	total oxidised nitrogen
STW	sewage treatment works
DWF	dry weather flow
PE	person equivalent
NGR	national grid reference
CPOM	coarse particulate organic matter
FPOM	fine particulate organic matter
DQI	Diatom Quality Index
SPI	Specific Pollution Sensitivity Index
ID	Indice Diatomique
CEC	Commission for Economical Community index
S&S	Steinberg and Schiefele index
GDI	Generic Diatom Index
WMS	weighted mean sensitivity

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CHAPTER 1 INTRODUCTION: THE PROBLEM OF EUTROPHICATION

1.1 The problem in lakes

Ecological systems resist change and move towards a state of equilibrium during succession (Odum, 1971). In this way an ecosystem develops increased control over the physical environment through self-regulation or homeostasis which not only increases energetic efficiency but provides protection against perturbations. Homeostasis is achieved by a mature ecosystem within the framework of its physical environment, i.e. the set of existing abiotic conditions. There will usually be a single limiting factor to growth at any one time for each biological component of the ecosystem. The nature of this factor will depend on the environment and of course the requirements of each species. In freshwater systems, factors controlling primary production are most often light and nutrients. In lakes, where light is not usually limiting, nutrients are responsible for such control (Golterman and de Oude, 1991; Gibson, 1997). Light may however limit growth in lakes where phytoplankton blooms become sufficiently dense to cause self-shading.

Photosynthesising cells require several macro- and micronutrients for the production of protoplasm. Those which limit productivity are those that are scarcest in the environment in relation to demand by the photosynthesising cell (Moss, 1998). Phosphorus fulfils this condition most often since it is one of the scarcest elements in the lithosphere and is required in high amounts by living organisms relative to its supply. Similarly nitrogen is an important macronutrient and although it has an unlimited atmospheric supply, its availability to natural systems can be limiting because of the difficulty in converting the inert gas to a stable ion.

The process of natural eutrophication as referred to above has been accelerated by human activities within catchments, adding nutrients to freshwater at levels far exceeding natural concentrations. This so-called 'cultural eutrophication' can significantly disrupt the equilibria of a natural lake by releasing species from nutrient limitation (Stumm and Morgan, 1981). Addition of key nutrients like phosphorus and nitrogen allows autotrophic production to continue until another factor, usually light in the case of lakes, limits further proliferation.

Eutrophication causes both chemical and biological changes (Golterman and de Oude, 1991). The most apparent manifestation is the cyanobacterial bloom in cases of chronic enrichment. Such organisms replace the diatoms and green algae of a 'normal' lake phytoplankton community. Macrophytes may initially increase in biomass but in the

long term they are often replaced by a vigorous planktonic community. Decomposition of the large standing crop of primary producers uses up oxygen often depleting the water column in summer. Fish will be deleteriously affected by reduced oxygen and also by loss of macrophytes required for spawning and refuge (Harper, 1992).

1.2 Nutrient sources and transport

Phosphorus in a pristine river environment comes from the weathering of phosphatic rock within catchment and the deposition of airborne mineral and organic particles (Mainstone *et al.*, 1994). Anthropogenic activity in catchments has added to these background levels through the discharge of human and industrial effluents to rivers. Such discrete inputs are referred to as point sources. Elevated nutrients in diffuse sources such as land runoff are usually caused by agricultural activity chiefly ploughing, fertilising and stock excreta.

Secondary treatment of human sewage involves the removal of organic material through bacterial oxidation (Harper, 1992). This conventional treatment leaves behind oxidised elemental products of microbial decomposition such as nitrogen and phosphorus. The amount of phosphorus removed from final effluent is estimated as 30-40% (Sas, 1989). Since both nutrients are soluble, sewage treatment works (STWs) deliver high concentrations of nitrogen and phosphorus to the receiving watercourse. Approximately half of the phosphorus in final effluent is derived from sodium tripolyphosphate, a cleaning agent added to detergents (Moss, 1998).

A proportion of sewage effluent is comprised of industrial effluent. Such waste waters are chemically variable and dependent on the industrial activity involved. Industries known to deliver effluents of high nutrient content are those concerned with food and drink processing and the production of fertilizers and cleaning materials. Metal finishing operations are also known to use phosphorus solutions (Morse *et al.*, 1993; Harper, 1992).

Runoff and leaching from agricultural land causes the delivery of nitrate to surface waters. Terrestrial plant productivity responds to increases in the supply of available nitrogen hence nitrate fertilizer usage has increased a hundred fold in the UK between 1950 and 1980 to sustain intensification of production (Heathwaite, 1993). Nitrate is soluble and vulnerable to leaching so that the potential for loss from land to water has long been recognised. Conversely, only a very small fraction of phosphorus is held in soil solution due to its strong affinity for adsorption sites on soil particles (Mainstone *et al.*, 1996). It was traditionally thought by the agricultural community to be sufficiently immobilised in soils for losses to be of minor consequence (Haygarth, 1997; Catt *et al.*,

1998). There is evidence in Europe and North America however, that fertilizer application over the years has meant the phosphorus-binding capacity of cultivated soils has been exceeded, causing enrichment of waterbodies by phosphorus as well as nitrate (Sharpley *et al.*, 1994; Sharpley and Rekolainen, 1997).

Sewage effluent is considered to contribute more nutrients overall to surface waters than agriculture (Mason, 1996; Morse *et al.*, 1993). At the catchment scale however, the impact of either is very much dependent on land use, population size and industrial activity.

1.3 Effects of eutrophication in rivers

Most research into freshwater eutrophication has to date been directed towards lakes and reservoirs because of the severe amenity, health and economic consequences (Harper, 1992). Elevated levels of nutrients in rivers promote increases in photosynthesizer biomass and community changes similar to lakes. The main primary producers in flowing waters, however, are attached algae and macrophytes as opposed to the phytoplankton found in lentic environments. Enrichment causes certain species of algae and macrophyte to flourish and displace other less vigorous species that were more able to compete under conditions of nutrient limitation. The resultant river environment is one of reduced floral diversity dominated by extensive stands of 'nuisance' species. A prime example of a 'nuisance' species is the filamentous attached alga, *Cladophora glomerata* (Whitton, 1970). Although part of normal riverine flora, this species has become increasingly evident in nutrient-impacted rivers and is probably the most noticeable symptom of enrichment. Certain species of macrophyte are able to compete aggressively and overcrowd river channels. *Potamogeton pectinatus* is one example of an obligate eutrophic species (Haslam, 1978). Phytoplankton communities are naturally supported in rivers, usually in sluggish lower reaches where instream conditions are more lentic than lotic. Nuisance blooms of green algae and less frequently cyanobacteria have been attributed to high nutrient loads. Their occurrences have usually coincided with periods of low flow and high temperature (Cartwright *et al.*, 1993; Rose and Balbi, 1997).

River eutrophication also results in changes further up the food chain. A change in the plant and algal communities essentially means that there is a dietary change for the invertebrate and fish communities. An increase in vegetative biomass also means elevated levels of decomposition which may cause a reduction in oxygen even in flowing waters.

1.4 Nutrient limitation of plant growth: phosphorus or nitrogen?

Phosphorus is most frequently the determining nutrient against which changes in biomass and species of stream and lake ecosystems are measured; the fundamental reason being that phosphorus is in relatively shortest supply in the environment. It is however important to establish that this is in fact the case given that nitrogen, if not the limiting nutrient *per se*, may be limiting at certain times of the year, for example, when denitrification processes occur at a high rate (Mainstone *et al.*, 1994).

Phosphorus is generally considered to be of primary importance in lakes. Vollenweider (1968) examined studies of lake nutrient dynamics and concluded that primary productivity is largely determined by nitrogen and phosphorus with phosphorus being 'predominant over nitrogen as the limiting factor'. Schindler (1971) performed a series of classical experiments that led to a confirmation of this original conclusion. Elser *et al.* (1990), however, surveyed the limnological literature to distinguish the relative roles of nitrogen and phosphorus in constraining algal growth. The greatest algal growth response was achieved through addition of both nutrients as opposed to either individually. He considered that nitrogen should not entirely be relegated to a secondary limiting position and that more attention should be paid to both of these nutrients in future research.

The relative significance of phosphorus and nitrogen can be established from comparison of instream measurements to the relative quantities required by a photosynthesizing cell. The ratio of atomic weights for nitrogen and phosphorus taken from the equation for the composition of algal protoplasm is 16:1. Other studies have experimentally determined this ratio by comparison of algal chlorophyll 'a' with ambient concentrations of both nutrients. In streams, nitrogen was limiting below a ratio of 7:1 N:P and phosphorus limiting above 17:1 N:P (Chessman *et al.*, 1992). Forsberg and Ryding (1980) concurred with the ratio beyond which phosphorus is limiting but their data for Swedish lakes suggested that below 10:1 N:P, nitrogen was limiting to growth. Moss *et al.* (1988) calculated the amount of phosphorus and nitrogen entering upstream of Hoveton Bridge on the River Bure from diffuse and point sources. The nutrient budget indicated that 563 tonnes of nitrogen and 20.6 tonnes of phosphorus was imported from the catchment upstream of this point on the Bure. This equates to a N:P ratio of 26:1 by weight which is much higher than ratios quoted by other authors. Nutrient losses from the river system due to biotic uptake and sedimentation of particulates were not accounted for. This ratio would suggest that phosphorus would be most likely limiting to algal growth however phytoplankton cells were found to be severely nitrogen depleted and nitrate was often undetected in the Bure

during summer months. This discrepancy may be due to the budget not having taken into account downstream nutrient removal. Extrapolation of an N:P ratio value from nutrient budget data should therefore be viewed with some caution.

The N:P ratio in sewage effluent is low (4N:1P by weight) due to high concentrations of SRP. This can under certain circumstances shift a waterbody with high biomass from phosphorus to nitrogen limitation. Agricultural runoff is considered to be of a higher ratio, usually between 30:1 and 50:1 (Heathwaite, 1993).

There are fewer nutrient studies where nitrogen as opposed to phosphorus has been implicated as limiting. Periphytic growth response to nitrogen enrichment has been reported in streams when inorganic nitrogen (ammonium and nitrate) concentrations have been manipulated below 60 $\mu g\ l^{-1}$ (Triska *et al.*, 1989a). Other authors have failed to elicit a response when inorganic nitrogen was increased above 50 $\mu g\ l^{-1}$ (Newbold 1992). Ram and Plotkin (1983, cited by Cartwright *et al.*, 1993) found that nitrogen was just as frequently limiting as was phosphorus in a Connecticut river. This they established by algal bioassay and N:P ratio. Nitrogen limitation was due to the low N:P ratio in raw and treated wastewaters discharged to the river. Lohman and Piscu (1992) similarly found nitrogen to be limiting during late summer/early autumn for *Cladophora glomerata* in the Columbia River, Montana. The ratio of dissolved inorganic nitrogen to SRP was <4:1 during this period. This low ratio was attributed to geology since catchment soil provided a rich source of phosphorus to the streams.

Experimental studies determining stream nutrient limitation have most successfully been performed in pristine environments not subject to anthropogenic influence. Stretches of a tundra river in Alaska have been experimentally enriched with phosphorus since 1983 (Peterson *et al.*, 1985; Peterson *et al.*, 1993; Harvey *et al.*, 1998). SRP concentrations have been consistently increased from ambient levels of <5 $\mu g\ l^{-1}$ to approximately 15 $\mu g\ l^{-1}$. All trophic levels within the experimental stream reaches responded by a dramatic increase in production and indicated a strong 'bottom up' response to enhanced phosphorus levels. Nitrate was added to prevent nitrogen limitation which was being taken up at an elevated rate owing to the increase in available phosphorus. At high discharge, elevated phosphorus concentration was observed as far downstream as 10 km from the point of entry. During low flow , most phosphorus was removed within 3-5 km.

Stockner and Shortreed (1978) found in stream enrichment experiments in British Columbia that nitrate enrichment of attached algae produced little response whereas phosphate enrichment (from <5 $\mu g\ l^{-1}$ to approximately 18 $\mu g\ l^{-1}$) alone promoted a five fold increase in growth. Both nutrients together gave a seven to eight fold increase.

Following this experiment, trees bordering the stream channels were logged to test the effect of light on the periphyton biomass. Essentially no change occurred, implicating phosphorus as being of primary importance in controlling primary productivity.

The weight of literature presently indicates that phosphorus is more likely to be the limiting nutrient in flowing waters. Evidence arises from lake studies and more recently stream enrichment studies. Of ultimate significance is the ratio of phosphorus to nitrogen in determining the limiting nutrient in any freshwater system.

1.5 What nutrient concentration promotes eutrophication in rivers?

The onset of eutrophication in lakes, as judged by 'nuisance' levels of phytoplankton chlorophyll 'a', has been associated with total phosphorus levels above 35 $\mu g\ l^{-1}$ (Vollenwieder and Kerekes, 1982). 10 $\mu g\ l^{-1}$ phosphorus (measured as either total phosphorus or soluble reactive phosphorus) has been suggested as limiting to algal productivity (Cartwright *et al.*, 1993; Reynolds, 1984; Sas, 1989; Sharpley *et al.*, 1985).

Research into river eutrophication is sparse compared to that for lakes and many research projects have solely focused on *Cladophora glomerata* and the nutrient conditions that initiate its prolific growth in nutrient impacted streams. Pitcairn and Hawkes (1973) observed only modest growth of *Cladophora* below 1000 $\mu g\ l^{-1}$ total inorganic phosphorus in eight rivers in central England. Wong and Clark (1976) found that 60 $\mu g\ l^{-1}$ total phosphorus was the level beyond which maximum growth of this alga would occur. In a Canberra river, growth of *Cladophora* was only slight when nutrient concentrations were lower than 20 $\mu g\ l^{-1}$ orthophosphate and below 200 $\mu g\ l^{-1}$ total inorganic nitrogen (Henley *et al.*, 1980). Dodds *et al.* (1997) collated the results of 200 river sites from the literature and correlated stream variables including nitrogen and phosphorus with benthic chlorophyll 'a', a measure of total attached algal biomass. They found that biomass would be kept below nuisance levels when nutrient concentrations were less than 30 $\mu g\ l^{-1}$ total phosphorus and 350 $\mu g\ l^{-1}$ total inorganic nitrogen. Algal chlorophyll 'a' densities in excess of 100 mg m^{-2} were considered a nuisance. Dodds *et al.* (1998) considered 75 $\mu g\ l^{-1}$ total phosphorus to be the boundary between mesotrophy and eutrophy in streams. This was concluded after comparison of stream benthic and sestonic chlorophyll 'a' measurements with total phosphorus and total nitrogen taken from previously published data. This was a broad brush approach to categorizing trophic boundaries. Cumulative distribution of values for chlorophyll 'a' and nutrient data were essentially divided into thirds with each third representing oligotrophy, mesotrophy and eutrophy.

Aquatic vascular plants respond to variation in nutrient level by changes in community assemblage and biomass. Correspondence of such changes with absolute nutrient concentrations in rivers is poorly represented in the literature. This may in part be due to most studies of macrophytes being lacustrine. Also, rooted macrophytes are able to extract nutrients from both the water column and the sediment which adds a further potentially confounding dimension. Not only are different rooted plant species able to make use of either or both sources, but river sediment phosphorus content is spatially heterogeneous. This heterogeneity is due to phosphorus sorption being particle-size dependent. Finer particles like clays have a greater sorption capacity than sands, for example, and the distribution of substrate type is determined by stream turbulence and velocity. Hence river sediment phosphorus is more patchy in contrast to lake sediments which are more homogeneous. Nevertheless, changes to the vascular plant community have been ascribed to eutrophication. Haslam (1978) described species commonly associated with varying degrees of enrichment and cites *Potamogeton pectinatus* as being particularly tolerant to this type of pollution. This species best correlates with a water soluble reactive phosphorus concentration of 300-1200 $\mu g\ l^{-1}$ and a sediment pore water soluble reactive phosphorus concentration of 2000-3000 $\mu g\ l^{-1}$ (Haslam used water chemical data supplied by the former River Authorities and River Purification Board). The author also observed that a eutrophic site may have 4-7 species, a fairly clean clay river 5-8 species whilst an exceptionally clean river may be expected to support 10-18 species. Carbiener *et al.* (1990) conducted an extensive study of macrophytes in reaches along the upper Rhine valley. The sites chosen displayed varying trophic status but similar hydrochemical characteristics. They found that eutrophic species predominated above 10-24 $\mu g\ l^{-1}$ soluble reactive phosphorus.

The above studies have examined macroflora in the context of the perceived shift from a healthy, diverse community to one degraded and dominated by few species and high biomass. The 'degraded' river is ultimately a subjective assessment in the same way as levels of 'nuisance' biomass and 'reduced diversity' are necessarily subjective too. The best assessment would undoubtedly be comparison with historical data and a river's biological condition prior to anthropogenic pollution. Such records, however, are scarce.

1.6 Aims, hypotheses and predictions

The overall objective is to *understand the biological responses to elevated nutrient levels in lowland rivers and streams.* Biological responses were examined as biomass and species composition of attached algae. The nutrient used as indicator of eutrophication was phosphorus (measured as soluble reactive phosphorus), after comparison of both phosphorus and nitrogen concentrations.

In lowland regions, very low phosphorus concentrations (approaching levels of detection) in rivers are rarely if ever observed. Anthropogenic activity has increased phosphorus concentrations in excess of background levels. Background levels themselves may also be high due to lowland geology. The aim of this study was to determine the major source of phosphorus to study rivers and examine the relationship between elevated phosphorus concentration and algal species and biomass.

Null Hypotheses:

a). *Background phosphorus levels in lowland rivers will be the same as those recorded from undisturbed catchments elsewhere.*

b). *Elevated phosphorus levels in lowland rivers affected by human activities cannot be attributed to one source.*

c). *Diatom species assemblage will change with increases in phosphorus concentration throughout the range of phosphorus concentrations found in lowland rivers.*

d). *Algal biomass will linearly increase with increasing phosphorus concentration throughout the range of concentrations found in lowland rivers.*

Predictions:

1). Diffuse source origins (e.g. agriculture) will be detectable as elevated stream concentrations

2). Point sources will be detectable as downstream increases in concentration

3). Instream phosphorus composition and seasonal pattern of flux will reflect source

Phosphorus concentrations elevated through human activity will have consequences for the attached algae community. Within the range of phosphorus concentrations found in lowland rivers:

4). Microalgal species assemblages will change with increasing phosphorus concentration up to an identifiable concentration beyond which assemblage change will not be as a result of phosphorus concentration

5). Macroalgae and microalgae biomass will increase linearly with increasing phosphorus concentration up to an identifiable concentration beyond which biomass change will not be as a result of phosphorus concentration

6). Macroalgae will displace microalgae with increasing phosphorus concentration

These predictions were tested in lowland rivers. Chapters 3 and 4 consider the levels, sources and behaviour of phosphorus in the study rivers. Subsequent biological investigation (Chapters 5 and 6) examines the relationship between phosphorus and algal species and their biomass. Chapter 7 discusses the link between algae and phosphorus results and inferences that can be made about lowland rivers in eastern England.

CHAPTER 2 PHOSPHORUS CHEMISTRY AND ALGAL GROWTH IN RIVERS

Rivers are responsible for the continual flux of soluble and particulate matter from the terrestrial environment to the sea. River water is made up of variable proportions of subsurface water and ground water in addition to overground runoff from the catchment. Chemical composition is the result of interactions between rainfall, soil and vegetation. The range of variation caused by these three factors, both in composition and quantity, is extensive. Unlike sea water, which is remarkably constant throughout the oceans, freshwater is considerably more variable in its composition (Stumm and Morgan, 1981). Pollution of freshwater through addition of anthropogenically derived nutrients has caused further disparity in the chemical composition of streams and rivers.

Much research has been directed towards nutrient delivery and instream transformations. This chapter reviews the literature relating to riverine phosphorus chemistry and delivery of this nutrient from the catchment. The response of the algal component of the biological community to nutrient levels is examined separately.

2.1 Phosphorus chemistry and catchment export

2.1.1 Forms of phosphorus in water and their analysis

Phosphorus exists in soluble and particulate forms in water, and as both inorganic and organic ions and molecules. The fraction of a water sample composed of bioavailable phosphorus is important since it describes the quantity of phosphorus readily available for uptake and ultilization by primary producers. Algal bioassay is the most precise way of estimating this bioavailable phosphorus fraction; however this technique is slow and labour intensive and not suitable for large scale sampling and monitoring programmes (McKelvie *et al.*, 1995). Phosphorus species are instead operationally defined and classified according to the procedure used for analysis rather than the precise molecular form.

Phosphorus in nature has one stable oxidation state, PO_4^{3-}, which is commonly termed orthophosphate. This is recognised as the most readily bioavailable phosphorus form. Chemical analysis of all phosphorus species in natural waters is based upon the reaction of orthophosphate with molybdate to produce phosphomolybdenum blue (MEWAM, 1980). Soluble reactive phosphorus (SRP) is the operational determinand synonymous with orthophosphate. The soluble/dissolved fraction is arbitrarily defined as phosphorus that has passed through a 0.45 μm membrane filter. SRP determination therefore requires that a water sample is filtered prior to analysis with molybdate. SRP

may also be referred to as dissolved reactive phosphorus (DRP) or molybdate reactive phophorus (MRP) in the literature. The other common analytical species is total phosphorus (TP). Total phosphorus is measured by acid digestion of a water sample to convert all forms of phosphorus to orthophosphate which is then measured as soluble reactive phosphorus. The sample is not filtered. Total phosphorus includes all forms of phosphorus, both dissolved (orthophosphate, inorganic polyphosphates, condensed phosphates and dissolved organic phosphorus) and particulate matter in suspension. The various forms of particulate phosphorus include organically-bound phosphorus, phosphorus adsorbed onto clays/metal oxides, phosphorus occluded into the lattice structure of clay/metal oxides, phosphorus associated with amorphous metal oxides and the primary phosphorus found in particles of igneous rock such as apatite (Froelich, 1988; Holtan *et al.*, 1988).

Determination of other phosphorus fractions can be achieved through combination of filtration, acid digestion, acid hydrolysis and derivation (Appendix 2, Figure 1). Total reactive phosphorus (TRP), for example, is determined in the same way as SRP but without filtration whereas acid digestion of a filtered sample provides an estimate of total dissolved phosphorus (TDP). Acid hydrolysis of a filtered sample determines the fraction comprising dissolved inorganic phosphorus (DIP). Dissolved organic phosphorus (DOP) can then be derived from the difference between TDP and DIP.

The biological equivalence of these analytical species is not absolute. The term 'reactive phosphorus' is used instead of orthophosphate since it is not just orthophosphate that is measured. Reactive phosphorus may also include a small fraction of condensed phosphates and some of the more labile organic phosphorus, both of which can be hydrolysed during chemical determination (House and Casey, 1989). Orthophosphate however is not the only bioavailable phosphorus species although it is traditionally held to be the most significant. Non-molybdate-reactive components of dissolved organic compounds, condensed phosphates and labile phosphorus loosely sorbed onto colloidal particulates can become available to organisms like algae and bacteria that produce extracellular enzymes such as alkaline phosphatase. Bioavailable phosphorus may therefore be underestimated by SRP.

Several studies have investigated the equivalence of SRP to bioavailable phosphorus. Twinch and Breen (1982) found SRP to underestimate bioavailable phosphorus at low concentrations and overestimate it at SRP concentrations above 0.02 mg l^{-1}. Bioavailable phosphorus exceeded SRP by over 10% in 65-70% of samples examined from 39 Canadian river and lake samples (Bradford and Peters, 1987). Some studies however, have found SRP and bioavailable phosphorus to be equivalent (Nürnberg and Peters, 1984).

Bradford and Peters (1987) compared concentrations of bioavailable phosphorus determined through algal bioassay against phosphorus in several chemically-determined phosphorus fractions. These fractions included total phosphorus, total soluble phosphorus, total reactive phosphorus, SRP and a combination of initial SRP plus enzyme-hydrolyzable phosphorus and/or exposure to ultraviolet radiation. Water samples for analysis were taken from lakes and rivers with total phosphorus values ranging from 9 to 250 $\mu g\ l^{-1}$. The results of biological and chemical analysis were analysed as three subsets; lakes and rivers with <30 $\mu g\ l^{-1}$ of TP, lakes with >30 $\mu g\ l^{-1}$ of TP and rivers with >30 $\mu g\ l^{-1}$ of TP. Regression analysis showed that the fraction most statistically representative of bioavailable phosphorus in rivers (r^2=0.95) and lakes (r^2=0.98) over 30 $\mu g\ l^{-1}$ of TP was total soluble phosphorus. Below 30 $\mu g\ l^{-1}$, total reactive phosphorus explained 73% of the total variation in bioassay phosphorus. The latter fraction is determined in the same way as SRP but on an unfiltered sample.

Phosphorus speciation and transformation in natural waters is complex and not fully understood. Operationally defined species are extensively used in eutrophication studies reflecting the practicalities of such analysis over the alternative method, algal bioassay. These determinands are however limited in terms of their absolute biological relevance.

2.1.2 Phosphate bonding to particulate matter

The gradual reduction in phosphorus concentration downstream of a point source indicates the storage and transformation of this nutrient. Demand by instream organisms is such that a phosphate ion may be used repeatedly from source to estuary unlike other elements such as Mg, K, Na, and Cl which may pass through a system virtually unaffected. This downstream movement is conceptually referred to as spiralling and is essentially cycling with a longitudinal downstream dimension (Webster and Patten, 1979). Thus a phosphate ion may be taken up and assimilated into the cells of algae (including cyanobacteria), heterotrophic microbes, macrophytes, bryophytes and riparian plants. Abiotic processes such as physical adsorption and chemical bonding by inorganic and organic particulates will also account for some phosphorus removal. Reintroduction of the atom into the stream water may occur through excretion, desorption or dissolution of chemical bonds and release following cell lysis of dead primary producers and consumers.

The majority of phosphorus in rivers is transported bound to inorganic and organic particles as opposed to its soluble inorganic state. Dissolved phosphorus is estimated to account for only 5-10% of the total phosphorus transported to the oceans (Froelich, 1988). The nature of particulate matter and the attachment between different

components of particulate matter and phosphate ions is of significance to the understanding of removal and downstream transport.

Mineral particles such as clays are of particular importance due to their chemical interaction with ions. They have large surface areas in comparison to their size which provides for a greater number of sites for ion exchange (Viner, 1987). Clay particles have an excess negative charge which attracts cations, adsorbing them to the surface. Free phosphates can be adsorbed directly onto clay particles at sites of positive charge. Adsorbed anions such as phosphate are commonly present in smaller quantities than cations since negative charges on clay particles generally predominate (Brady, 1984). Fe^{2+} and Al^{3+} are the most commonly adsorbed cations, particularly in the form of their insoluble hydrated oxides, due to their prominence in the earth's crust. Other metal cations can similarly be held. Hydroxides of iron and aluminium adsorbed onto clays act as a sponge for anions and cations and have a high affinity for phosphate (Viner, 1987). They can also bind phosphate as distinct complexes to form various metal-phosphate minerals (Golterman, 1988). These complexes may be crystalline in structure or else amorphous, and of no strict lattice structure. The latter are believed to have higher sorptive capacities than crystalline complexes (Parfitt, 1988; Meyer, 1979; Holtan *et al.*, 1988).

Organic phosphorus compounds are mostly found in particulate form (Holtan *et al.*, 1988). This particulate fraction consists chiefly of cellular phosphorus-based compounds within a variety of microorganisms. During decomposition these organic compounds (a variety of phosphate esters and organic acids) are released and become part of the dissolved phosphorus fraction (Hooper, 1973). Phosphate can also be indirectly adsorbed to organic particles as a result of binding to iron and aluminium. These metals are thought to become associated with organic particles in a similar way to the coating of metal hydroxides on clay particles (Böstrom, 1982). Such compounds are referred to as humic-iron-phosphate complexes and very little is known about how phosphate, once adsorbed, might be released. Sharpley (1985) considered that organic particles transport a larger fraction of adsorbed phosphorus than inorganic particulates.

2.1.3 Phosphate buffering

Uptake or sorption of phosphate ions by sediment, whether benthic or suspended, are believed to control dissolved phosphate concentrations in water at some near-constant value regardless of phosphorus inputs and biological removal (Froelich, 1988). Evidence for this comes from dilution of suspensions of riverine or estuarine sediments by varying quantities of distilled water or sea water and the constancy of dissolved phosphorus values (Fox *et al.*, 1989). The term put forward to describe this

is the phosphate buffer mechanism, analogous to pH buffering. A large pool of phosphate therefore exists sorbed onto mineral particles that, under conditions promoting desorption, is released to the water column. This process is reversible with an increase in phosphorus concentration in solution causing adsorption. The phosphorus in natural flowing waters is buffered but given the natural fluctuations (biological production and decomposition, mineral weathering and nutrient spiralling), let alone anthropogenic sources, it seems likely that equilibrium would be a short-lived phenomenon.

Phosphorus bound to sediment particles or complexes is mobilized (desorption, dissolution of precipitates and complexes, ligand exchange and enzymatic hydrolysis of organic matter) and then transferred to the pool of dissolved phosphate in the pore water (Böstrom *et al.*, 1988). (Pore water is the water that occupies the spaces between sediment particles). Here the dissolved phosphorus may be transferred to another fraction before it has chance to be transported away from the sediment. For example, organic phosphates hydrolysed by microorganisms may quickly be sorbed by iron complexes, phosphate released from inorganic species may be taken up by microorganisms and, below pH 5.5, part of the phosphate is bound into calcium minerals.

Mechanisms responsible for transport from pore water to water column will differ between lentic and lotic waters. Physical mixing through stream turbulence will play a more important part in rivers and will be most significant during and just after storm events. Phosphorus is also transported over the sediment/water boundary through diffusion and bioturbation from burrowing animals and human activity (boats). The effect of changing redox potential responsible for the reduction of iron (III) to iron (II) and the subsequent release of phosphorus is unlikely to be as important a process in river sediments as it is in lakes (Casey and Farr, 1982). The upper 4 cm of river sediment is most actively involved in transport, mixing processes and storage (Hill, 1982; Svendsen and Kronvang, 1993).

Sediment particle size, organic content and its iron and aluminium content are the main factors influencing phosphorus storage (Klotz, 1985). Fine-grained silty sediments were found to have a higher buffering capacity than coarse sediments in Bear Brook (Hubbard Brook Experimental Forest) (Meyer, 1979). A comparison of two Ontario rivers' sediments by Hill (1982), similarly showed that the higher buffering capacity of one was attributed to finer substrate particle size and organic content. A finer sediment is more likely to have a higher clay and metal oxide content which would result in this enhanced ability to trap phosphorus.

Storage of phosphorus within the sediment potentially reduces eutrophication by reducing phosphorus bioavailability. Net retention depends on the settling rate of particulate phosphorus to the sediment, and on the specific sediment retention capacity. Sediments do not provide a limitless sink for phosphorus storage and in line with the buffering model a reduction in ambient phosphorus concentrations in the water will promote net phosphorus release from the suspended and bottom sediments. This is evident from the instances where point-sources of phosphorus have been reduced and a parallel reduction in stream water phosphorus has not materialised (Marsden, 1989). For example, phosphate stripping at a large STW on the River Ant, Norfolk Broads, failed to reduce phosphorus loading in Barton Broad to the extent anticipated. The lake sediments were found to be relatively rich in total phosphorus and calculation of a nutrient budget for Barton Broad revealed a net annual release of phosphorus (Phillips, in Harper, 1992).

The extent of release will depend on the water phosphorus concentration and the length of time for which the sediment has been exposed to a level of phosphorus concentration. Sediments of three Australian lakes studied by Slater and Boag (1978) similar in composition differed in phosphate concentration – one was relatively higher and thus had a reduced phosphorus sorption capacity. This was attributed to its lengthy exposure to a point source.

Fox *et al.* (1989) examined sediment composition downstream of a STW on the River Stour, Kent, to determine the possible role it plays in altering river phosphorus concentrations. Three sites located 1 km, 10 km and 16 km downstream from the STW were chosen for investigation. Chemical analysis of the sediment cores in the first instance reflected the change in catchment geology downstream. The sediment of the uppermost site had the higher clay mineral content and the highest surface area of sediment (m^2g^{-1}). The furthest downstream site had a relatively high percentage of CaO and labile-phosphorus as a result of the typical chalk-stream character of the river. The upper site sediment displayed the highest concentrations of iron bound phosphorus and iron oxide and also the lowest rate and extent of desorption of phosphorus in laboratory experiment. The reverse was true for sediment from the lowest site which released phosphorus more readily and in greatest quantity. This difference reflected the greater strength of bonding between phosphate anions adsorbed onto iron hydroxides and phosphate bound as $FePO_4$ as opposed to the weaker association of labile-phosphorus which was found in highest quantity at the lowest site. Fox *et al.* (1989) inferred from this that although the higher content of clay mineral in sediment will in most instances pre-dispose it to higher uptake of phosphate, it is perhaps the fractional breakdown which is more revealing in terms of bonding strengths and potential rates of

release. In terms of management strategy, they concluded that a reduction in the phosphorus loading of the River Stour through phosphorus removal at this particular STW was unlikely to initiate a significant release of phosphorus from the sediment of the most eutrophicated area that lies directly downstream given the strength of phosphorus bonding at this site.

Phosphorus export has been found to be maximal during storm events. Pommel and Dorioz (1997) found that about 90% of particulate phosphorus and 60% of annual TDP was exported in the six largest storm flows in the Lake Léman catchment, France. During stable low-flow periods, 90% of the TP measured was in the form of TDP. Similarly, Kronvang *et al.* (1996) estimated that 28% of particulate phosphorus loss took place during 2% of the year in a Danish arable catchment.

2.1.4 Export of phosphorus from land to water

Phosphorus pathways from soil to water include surface runoff (overland flow) and subsurface runoff (leaching)(Figure 2.1). Subsurface runoff can be of two types; phosphorus that passes through the soil profile reaching the stream or river without entering the water-table and phosphorus that flows directly through the soil and into the water-table. The latter is subsequently discharged to the river or stream as seepage (Morgan, 1997).

The bulk of phosphorus exported as surface runoff is associated with particulate matter rather than soluble phosphorus. This may be phosphorus that is adsorbed onto colloidal particulates or else organic matter in the form of plant fragments. Intuitively, there is size selectivity in exported material since the particulates that require less energy such as clays and silt are preferentially mobilised over heavier sand particulates (Heathwaite, 1997). The phosphorus from subsurface runoff is more likely to be soluble and such losses are usually considered smaller than overland runoff. This is attributed to likely retention by soil particles as the phosphorus ion descends through the soil profile. The presence of a tile-drainage system in a field however can substantially increase the contribution made by subsurface runoff to phosphorus export. The drains provide a speedy passage from land to receiving water and prematurely remove the phosphorus ion from the profile and potential uptake by soil particles (Morgan, 1997). Grant *et al.* (1996) found that fine particles in addition to soluble phosphorus are transported from topsoil to drainage water giving rise to drainage water rich in sediment associated phosphorus. This occurs due to the presence of macropores in soils that have a high clay or loam content.

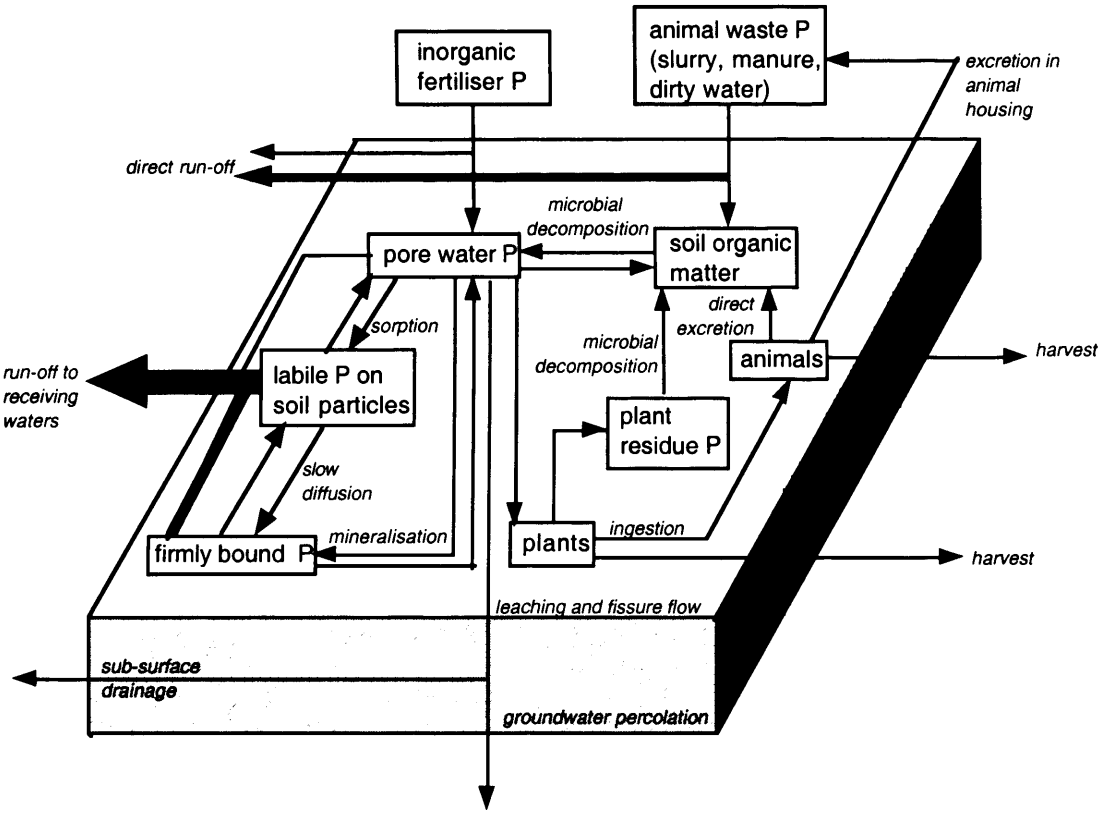


Figure 2.1 Phosphorus behaviour in soils (from Mainstone *et al.*, 1996). Width of line indicates importance of transport route; broken lines refer to minor pathways or those of unknown importance.

The factors controlling phosphorus loss from land are highly site-specific. Phosphorus export is determined by a variety of soil properties, cropping characteristics, fertilization regime and the annual pattern and amount of rainfall (Table 2.1).

Table 2.1 Factors responsible for controlling phosphorus loss at various geographical/organisational scales (from Edwards and Withers, 1998)

Scale	Leaching	Erosion	Leaching and erosion
Profile	Sorption properties, pH, electrolyte concentration, soil:solution ratio	Aggregate stability Soil texture	
Field	Soil mineralogy % saturation/P index Artificial drainage	Extent and nature of crop cover Cultivation practices	Rainfall intensity Antecedent moisture content
Farm	Farm category P surplus	Proximity to river Slope Field boundary conditions	Soil type Farm type
Catchment			Climate Land use Soil type, relief Farm types and numbers

Land use can greatly affect the erosive potential of rain. Loss of phosphorus as surface runoff was found to be greatest from arable land and least from low-intensity grassland (Lennox *et al.*, 1997). Greater amounts of particulate phosphorus were transported from cropped wheat watersheds than from native grass, due to increased erosion where removal of vegetation had reduced the stability and integrity of the soil (Sharpley and Smith, 1990). The direction of drilling (sowing) of crops was found to affect the transport of sediment from land. Erosion was much less when winter wheat was drilled along contour lines than when drilled up and down the slope (Hansen *et al.*, 1996). Surface runoff also increased where compaction by machinery during seed bed preparation had reduced the soil's ability to allow infiltration of ponded water.

Extent of phosphorus loss from land is in part determined by the amount that is applied in the form of fertilizer. Nitrogen and potassium fertilizers are readily accessible to crop roots and the uptake of such fertilizer by crops is estimated to be as high as 80% (Morgan, 1997). This is not the case with phosphorus fertilizer which after dissolution into the soil water is quickly fixed by soil particles (the chemical and physical fixation of phosphorus in soils is as varied and complex as that in water). The amount of soluble phosphorus therefore available for crop nutrition is dependent on the reversal of

such reactions and subsequent release into soil water. Inevitably, the amount of phosphorus fertilizer applied to arable land is in excess to allow for this.

2.2 Nutrient control of attached algal growth in flowing waters

The following section reviews the literature relating the abundance and community structure of attached algae in rivers to trophic status, in the context of other prevailing biotic and abiotic influences. In lakes, the ubiquitous algae are the phytoplankton as opposed to the attached algae which can only proliferate in the shallower littoral margins. The relationship between standing crop of phytoplankton and nutrient status in lakes is relatively easier to establish given the absence of flow. Here the biomass of algae per unit volume of water is proportional to the initial mass of the limiting nutrient per unit volume. Biomass per unit volume subsequently increases as soluble limiting nutrient per unit volume is incorporated into biomass. The linear nature of rivers however, increases the diversity of abiotic factors that can influence instream productivity and growth form of algae so that light penetration, substrate, depth, water velocity, temperature, sloughing and invertebrate grazing act in association with nutrient concentration.

The members of the autotrophic community of rivers have various names. Attached algae, both the macro- and microscopic forms, are generically referred to as periphyton. This term does not include the heterotrophic component. Algae may also be described by their occurrence on stone (epilithon), macrophyte (epiphyton) and fine sediment (epipelon). Biofilm more specifically refers to attached microorganisms such as the autotrophic algae and heterotrophic bacteria, fungi and protozoa that inhabit the slimy film (polysaccharide matrix) which coats submerged surfaces. For example, epilithic biofilm refers only to the microorganisms attached to stone and excludes macroscopic filamentous algae.

2.2.1 Uptake and release of phosphorus by stream periphyton

Biogenic additions of both dissolved fractions (DOP and DIP) occur as a result of lysis following cell death of riverine oganisms, animal excretion and as a by-product of metabolizing algal and bacterial cells. The precise nature of excretion and products following lysis are not certain but are most likely to be organic phosphate esters and perhaps phosphonate compounds. Cembella (1984a, 1984b) reviewed the literature relating to the uptake of phosphorus by microalgae. The phosphate ion enters the space between the plasmalemma boundary and the cell wall by diffusion. This boundary surrounds the organelles within the confines of the cell and is a semipermeable barrier to the entry of nutrient ions. The ion then passes into the plasmalemma for intracellular

utilization. The precise nature of intracellular transport is not entirely known but evidence suggests that this process is actively mediated against an electrochemical gradient requiring metabolic energy.

Micro- and macroalgal cells are capable of 'luxury uptake' whereby surplus phosphorus is accumulated within the plasmalemma as polyphosphate in excess of that required for growth. Uptake is therefore not only a function of external concentrations but also of the amount of phosphate in reserve.

Phosphate groups can be released from a broad spectrum of dissolved inorganic and organic phosphorus sources through extracellular enzymatic hydrolysis by phosphatase. Such hydrolysis is achieved at the cell surface. Phosphatase is considered a broad spectrum enzyme since phosphate is known to be released from a wide variety of phosphate monoesters. The polyphosphate reserve pool need not necessarily be depleted before sources other than DIP are utilized.

Sand-Jensen (1983) equated the periphytic community to a microcosm in which autotrophic and heterotrophic processes occur within a boundary layer. Transport of nutrients is by diffusion from the stream water across the community/water interface and into this microcosm. Since molecular diffusion is a slow process, algal uptake generally exceeds the rate of diffusion thus establishing a concentration gradient from the boundary or interface to the innermost layers of the periphytic film or mat. As the distance from the interface increases, the phosphorus concentration progressively declines. Naturally, phosphorus concentration and stream velocity can affect the rate of diffusion by altering the gradient. The author suggests that at the outset of periphytic growth on a substrate the relationship between water quality parameters and biomass change is more apparent. At a later stage of development when the community has become thicker and biologically more complex, internal turnover of nutrients complicates this relationship.

2.2.2 Nutrients and the growth of *Cladophora*

Cladophora proliferation is one of the most noticeable outcomes of enrichment of temperate rivers and streams. This has initiated a number of studies looking at the relationship between this species and growth-promoting nutrient levels.

Pitcairn and Hawkes (1973) examined the relationship between the mean annual dry weight of *Cladophora glomerata* in a number of English rivers and mean annual total phosphorus concentrations during the *Cladophora* growing season (March to November). The survey results supported a general positive correlation (r=0.54). A few sites produced anomalous results such that small stands in some instances were

located in high phosphorus stream water and extensive *Cladophora* biomass was found in low phosphorus areas. Their results suggested that *Cladophora* growth is restricted in rivers with a total inorganic phosphorus concentration of less than 1000 $\mu g l^{-1}$. Mean annual variation was the only level at which a relationship was found; seasonal variation in standing crop could not be correlated with corresponding changes in nutrient status.

Wharfe and Taylor (1984) surveyed the distribution and abundance of macrophytes and *Cladophora* upstream and downstream of a large STW on the Great Stour, Kent, between 1978 and 1982. The principal aim was to determine whether phosphate stripping was necessary at this STW in the near future given the expected rise in population. An increase in effluent processing would have inevitably led to higher phosphorus concentrations and may have caused unacceptable growths of *Cladophora* downstream of this STW. This concern stemmed from the period between 1956-1964 when the old STW had become over loaded and the effluent quality poor. As a result, *Cladophora* proliferated and replaced the previously healthy macrophyte community. In 1964, effluent quality was improved due to the commissioning of the then present treatment works and rooted macrophytes became re-established although vigorous seasonal growths of *Cladophora* still occurred. The annual mean orthophosphate concentrations during the study period (1978-1982) were above 1000 $\mu g\ l^{-1}$ downstream of the STW and *Cladophora* only dominated the flora at the nearest of the four downstream survey sites. This suggested that local conditions like river width and depth, flow, shading and bed substrate were more important in determining abundance and distribution. Furthermore, Wharfe and Taylor (1984) considered that this concentration was above the 'level at which further increases would have an appreciable effect on the growth rate and hence the accumulation of *Cladophora*'. They therefore rejected the proposal that phosphate removal from final effluent would be necessary since they considered that a rise in phosphorus concentrations would have no perceivable effect on *Cladophora* abundance. The unacceptable levels of *Cladophora* growth between 1956-1964 may have occurred as a result of other components of the effluent such as high concentrations of ammoniacal nitrogen and dissolved organic matter. Alternatively, the authors suggest that high levels of suspended solids may have placed *Cladophora* at a competitive advantage over rooted macrophytes.

Marsden *et al.* (1997) assessed the relationship between mean SRP concentrations (presumed to be annual mean) and abundance of *Cladophora* in the Forth Catchment, Scotland. Abundance of *Cladophora* was measured as percentage cover. The relationship was not statistically proven but showed a broad correlation. At SRP concentrations below 20 $\mu g\ l^{-1}$ little algal growth was recorded; between 20-100 $\mu g\ l^{-1}$

most sites recorded low algal abundance (<40%) but occasional high values did occur; while at sites with mean SRP values in excess of 100 μg l^{-1} low abundance was unusual and cover ranged between 30% to 80%. The use of percentage cover in this survey is not a precise measure of algal biomass however this factor is negated to some extent by the large sample size (approximately 100 samples).

Wong and Clark (1976) observed a direct link between total phosphorus in stream water and the total phosphorus content of *Cladophora glomerata* and *Potamogeton pectinatus* tissue (r=0.87) for six rivers in Ontario. They suggest that this relationship allows for the prediction of phosphorus in the water column where the total phosphorus content of the plant is known based on the empirical correlation between these two. This correlation is useful for predicting average stream nutrient concentrations over a period of time without lengthy sampling since the phosphorus content in plant tissue is less affected by daily fluctuations in phosphorus concentration. The authors determined the critical or growth controlling phosphorus level within the *Cladophora* tissue as 1.6 mg P/gram dry weight which equates to a stream water concentration of 60 μg l^{-1} phosphorus.

Henley *et al.* (1980) examined the effect of nutrients on algal growth in the Murrumbidgee River, Canberra. Upstream of sewage effluent inputs from this city, orthophosphate was mostly below 20 μg l^{-1} and growth of *Cladophora* was only slight. Below Canberra, growth became luxuriant.

Freeman (1986) used physiological indicators of nitrogen and phosphorus deficiency to determine the role of these nutrients in proliferation of *Cladophora* in the Manawatu River, New Zealand. Channel concentrations of phosphorus were generally below 30 μg l^{-1} dissolved reactive phosphorus (DRP). Physiological assay indicated that nitrate was in excess of requirement throughout the period of study. DRP concentrations of 4-5 μg l^{-1} were found to limit *Cladophora* growth during a sustained low-flow period.

2.2.3 Synergy of nutrients with other moderators of stream periphyton

Some of the above studies have alluded to the effect of other stream factors acting in conjunction with nutrients to influence growth. Whitford and Schumacher (1964) were among the first to establish the synergistic affect of current and phosphorus on algal growth due most probably to the enhancement of the nutrient concentration gradient between cell surface and surrounding water. They found that the uptake of P^{32} in two species of algae (*Spirogyra and Oedogonium*) was linearly related to current speed up to 40 cm s^{-1}.

Horner *et al.* (1981) demonstrated that flow increases up to 50 cm s^{-1} enhanced diatom accumulation, when SRP exceeded 40-50 μg l^{-1}, in a natural stream channel in Washington, USA. At lower phosphorus concentrations, accumulation was largely a function of phosphorus, with no positive velocity effect in evidence. The algae for this research were sampled from initially bare rock surfaces that had been colonised after they had been introduced into the stream sites.

A further study undertaken by Horner *et al.* (1983) sought to verify these field observations in the controlled environment of laboratory streams. The dominant algae grown within these artificial streams were the filamentous forms *Mougeotia* (green) and *Phormidium* or *Lyngbya* (cyanobacteria). The results showed that periphyton chlorophyll 'a' increased in proportion to SRP up to 25 μg l^{-1}. Beyond this value, biomass increase occurred up to a maximum of 75 μg l^{-1} but was less pronounced. The authors suggest that this was due to phosphorus saturation which most likely occurred between 15-25 μg l^{-1}. A distinct increase in biomass was observed as stream velocity was adjusted from 5 to 25 cm s^{-1}. Beyond 25 cm s^{-1}, accrual of biomass was not a function of stream velocity. In summary, the overwhelming factor influencing biomass accrual was SRP concentration in the range 2-75 μg l^{-1} where stream velocity varied between 5 and 75 cm s^{-1}.

This same research group later set out to establish the biomass level of periphyton which constitutes a nuisance, analogous to a eutrophic state in lakes (Welch *et al.*, 1988). The previous two studies had made use of introduced substrate and artificial streams. The premise for this piece of research was that, from a management point of view, experimental data should be gathered from *in situ* substrate with a naturally developed periphytic community. Using data from six Washington streams they found that periphyton biomass could not be related to either ambient SRP or ammonium or nitrate-nitrogen concentrations. The authors suggested that other factors, such as shading, likely nitrogen-limitation and heavy grazing pressure in several of the six streams would possibly account for the scatter in the data. Additionally, a review of literature by the authors suggested that a nuisance biomass of filamentous periphytic algae may be represented by a biomass level above 100-150 mg of chlorophyll 'a' per m^2 (mg chla m^{-2}). Below this range, filamentous coverage was found to be less than 20% in twenty six studies performed in the USA and Sweden.

Welch *et al.* (1992) set out to test the mechanistic model put forward as part of earlier research conducted in artificial stream channels (Horner *et al.* 1983). This model predicted periphytic biomass as a function of phosphorus concentration, nutrient uptake, velocity, light and temperature and was tested in 1992 on samples taken up and downstream of point sources in seven New Zealand streams. From a management

perspective, they wanted to know how effective this model was in predicting biomass in natural streams. The phosphorus concentrations in these 26 stream sites ranged between 2 and 2670 $\mu g\ l^{-1}$ (mean DRP of 306.9 $\mu g\ l^{-1}$). The model predicted that eighteen of the sites would have biomass appoaching the chlorophyll 'a' maximum of 1000 mg chlorophyll 'a' m^{-2}. Observed biomass however was found to be approaching this high level at only five sites. In total, only seven sites downstream of point sources out of nineteen exceeded the proposed aesthetic nuisance level of 100-150 mg chlorophyll 'a' m^{-2} (Welch *et al.*, 1988). Overestimation of biomass by the model was put down to high densities of invertebrate grazers and/or sub-optimal physical conditions such as shading, unstable substrata and very high turbulence.

Dodds *et al.* (1997) suggested that periphyton biomass falls below nuisance levels of 100 mg chlorophyll 'a' m^{-2} when mean in-stream total nitrogen and total phosphorus concentrations are maintained below 350 $\mu g\ l^{-1}$ and 30 $\mu g\ l^{-1}$. This was determined from analysis of a global dataset comprising chlorophyll 'a' values and nutrient data from 27 studies conducted in North America, Europe and New Zealand. They found that DIN and SRP were poor predictors of periphyton chlorophyll 'a'.

The influence of light on periphyton can be significant in small streams under dense canopy. Steinman (1992) increased the level of irradiance in a heavily shaded oligotophic stream. The periphytic community responded by a significant increase in biomass. However this biomass increase was only observed when snail populations were drastically reduced. In this instance, herbivory prevented the positive effects of increased irradiance becoming manifest in biomass.

Rosemond (1993) demonstrated that algal biomass and productivity are constrained by irradiance, nutrients and herbivory. The effect of each individual factor within this multifactorial experiment was not detectable; only means from treatments in which all three factors were manipulated were significantly different from controls. The ambient phosphorus concentration was $\leq 7\ \mu g\ l^{-1}$ phosphorus (control concentration) which was raised to 35 $\mu g\ l^{-1}$ during the experiment.

Bothwell (1988) found that temperature accounted for 90% of annual variability in growth of a thin diatom dominated biofilm when phosphorus concentrations were saturating (1.2-5.4 $\mu g\ l^{-1}$ phosphorus). Light, under phosphorus saturation, showed a less significant correlation with growth rate accounting for 34% of the observed variation. Under phosphorus limiting conditions ($< 1\ \mu g\ l^{-1}$ phosphorus) light exerted no influence on growth rate. The critical nutrient concentrations in this experiment were very low and referred only to saturating phosphorus levels in respect to a thin biofilm.

Higher concentrations of phosphorus would have promoted development of an overstorey.

Grazing by aquatic insects can have a profound influence on micro- and macroalgae evidenced by a decrease in biomass and/or a change in taxonomic composition and community structure. Walton *et al.* (1995) examined the effect on stream algae of grazing by the large caddisfly larva *Dicosmoecus gilvipes* in laboratory channels. The effect of grazing was examined in relation to in-stream SRP concentrations of 2, 5 and 10 $\mu g\ l^{-1}$. Grazers reduced both filamentous and non-filamentous algae by 90%. Grazing rates increased with progressive enrichment due to the increased biomass available to grazers. The authors suggest that in a natural stream, the grazing population would increase to accommodate the increased food production. In this experiment, the larva population was maintained at a constant level hence the effect of biomass loss through grazing decreased with increasing enrichment. The ungrazed periphyton community was diverse with no particular taxa dominating. Grazing caused an increase in the filamentous cyanobacteria *Phormidium* and a reduction in other taxa primarily the diatoms *Synedra*, *Gomphonema* and *Fragilaria*. This suggests that the larvae selectively consumed diatoms in preference to *Phormidium*. The authors suggested that this filamentous alga may have been unpalatable. Alternatively selective grazing may have been a function of vulnerability such that stalked diatoms like *Gomphonema* are more susceptible to grazers since they have an elevated position above the substrate. *Cladophora* did not develop within the channels although dominant within the algal inoculum used to seed the channels.

2.2.4 Instream removal of phosphorus by autotrophs

Meyer (1979) found that beds of bryophyte (dominated by liverwort) removed phosphorus from solution as a pulse of enriched stream water passed over. A non-quantified proportion of this uptake was attributed to epiphytic algae. Several studies have indicated that macrophytes are not as efficient nutrient traps as the microscopic plant community. Ball and Hooper (1963) found that uptake by *Chara*, *Fontinalis* and *Potamogeton* was far slower than by periphyton. Similarly, in the south of England, the dense macrophyte stands found in chalk streams did not remove significant amounts of phosphate when uptake was compared to concentrations of phosphorus in through-flow (Casey and Newton 1972). This last point emphasises that significant uptake in terms of a biotic compartment may not equate to significant removal of phosphorus from the stream water.

Newbold *et al.*, (1981) constructed a mathematical model to determine spiralling length. This describes the distance travelled by a phosphate ion from being a dissolved

available nutrient, through incorporation into living tissue and consequently several links in the food chain, until eventual release. This model was later applied by the same workers (Newbold *et al.*, 1983) to a Tennessee stream in order to measure the spiralling lengths of different biotic compartments. The study stream had very low ambient concentrations of SRP, 4 $\mu g\ l^{-1}$. A slug of radioactive $^{34}PO_4$ was added to the stream water and spiralling length was estimated at 190 m. Uptake length, or the distance travelled by the phosphorus ion in the dissolved available form (prior to uptake) was 165 m of this 190m. Transport by particulate matter (coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM)), including benthic and suspended algae and microbes, accounted for 25 m of the spiral length. The authors calculated that the combined consumer and predator community accounted for 25% of the standing stock of exchangeable phosphorus in the stream. Due to a low drift rate, this joint community was highly retentive with a turnover time of 152 days. Unfortunately the study did not also measure abiotic retention by suspended and benthic sediment.

Paul and Duthie (1989) demonstrated that, in an oligotrophic stream, well-developed epilithic communities utilised more phosphorus than immature ones. This was apparent from the decrease in spiralling length from 30.4 m (immature community) to 14.1 m (well developed communities). Furthermore it was the overlying layer of the biofilm with its higher density of actively metabolizing cells that was responsible for most of the uptake of phosphate. The epilithon in the study stream was dominated by bacterial cells and the diatom *Tabellaria flocculosa*.

Several general observations can be made about the capacity for individual stream retention of phosphorus. The uptake of this nutrient and the subsequent reduction in downstream concentrations is in general terms dependent on the diversity and size of the photosynthesizing community. This in turn is reliant on type of stream bed and the degree of anthropogenic influence on channel morphology. The examples described above are studies of nutrient poor streams. McColl (1974) pointed out that, where a stream is overloaded or saturated, breakthrough of nutrients and enrichment downstream will occur. Hence phosphorus enrichment studies are confined to upper, nutrient-limited reaches in order to achieve a noticeable affect.

This chapter has examined the nature of phosphorus in freshwater in relation to speciation and interactions with other elements and particulate matter. Factors controlling its export from land were also considered. The combination of these physical and chemical processes influence both phosphorus levels and bioavailability of this nutrient to instream photosynthesizing organisms. The utilization of phosphorus by different components of the algal community was also examined here, within the

context of other growth controlling factors. The proceeding two chapters investigate phosphorus levels in streams and rivers across the Anglian Region, the sources and the effect source has on load and species within the water column.

CHAPTER 3 STUDY RIVERS AND RESEARCH METHODOLOGIES

3.1 Study Region

The rivers studied in this research lie in the Anglian Region of the Environment Agency - East Anglia and the surrounding counties of Lincolnshire, Cambridgeshire and Leicestershire. This region is part of the driest area of the UK receiving approximately two thirds of the national rainfall average. This translates to about one third of the national average if considered as effective rainfall (net rainfall once evaporation has been accounted for). An important hydrological feature of East Anglia is therefore its vulnerability to drought and low river flows (NRA, 1994).

Geologically, the region is broadly divided north to south by a swathe of cretaceous chalk outcrop (Figure 3.1). This provides natural underground aquifers for maintaining base flow in many of the region's rivers. In addition these aquifers supply about half of the region's public water supply. About 30% of effective rainfall infiltrates into these underground reserves.

This area of England is mostly rural with arable farming as the main agricultural activity. There is a population of six million across this region; a significant proportion of which is contained in the major cities and towns. Urban areas, for example, account for only 1% of the Ely Ouse catchment area which is the largest watershed in the region with towns such as Thetford, Bury St Edmunds, Newmarket and Mildenhall.

3.2 Regional-scale investigation

An initial assessment of the rivers across the region was made to gain an overall picture of phosphorus concentrations and likely influencing factors. Phosphorus measured as SRP was compared with geology, size of effluent entering above the point of sampling and stream size for 345 of the approximately 1100 Environment Agency's routinely-monitored sites. An annual mean soluble reactive phosphorus concentration was calculated from 1995 data. Sites on the river Bure were not included in the dataset since phosphorus stripping at sewage treatment works on this river has resulted in low soluble reactive phosphorus values in relation to size of effluent source. Similarly, sites directly downstream of waterbodies were left out as they would give low results. Solid geology underlying each river and stream was determined from a UK map of solid geology, 1:63360 (Institute of Geological Sciences, 1979). Figure 3.1. gives a simplified version of the geological landscape of this region. The geology of each river was identified as either clay, chalk (including oolite), cornbrash, greensand, Norwich

crag or mixed where no particular geological form was dominant. The cumulative dry weather flow (m^3 day^{-1}) input of effluent from sewage works was calculated for upstream of every sampling point. Stream order (Strahler, 1952 in Gordon *et al.*, 1992) was used as a measure of size and determined from a 1:250000 river flow map (DOE, 1979).

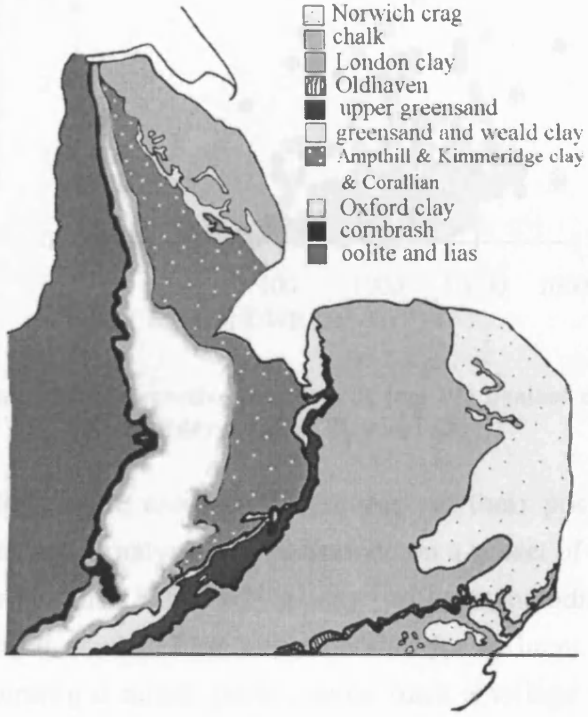


Figure 3.1 Solid geology of study region (after Institute of Geological Sciences, 1979)

Figure 3.2 shows the comparison of mean annual SRP levels against DWF effluent input. There is a general trend of increase in stream concentrations with greater effluent size. Some of the scatter of data points may be attributed to the fact that effluent inputs were additive upstream. Hence no account was made for natural instream removal of this nutrient such that an effluent point source entering several kilometres upstream will have a reduced phosphorus content by the time it reaches the point of sampling. This would imply overestimation of the size of effluent reaching a sample site and is indicated by the bias of data points towards the x-axis.

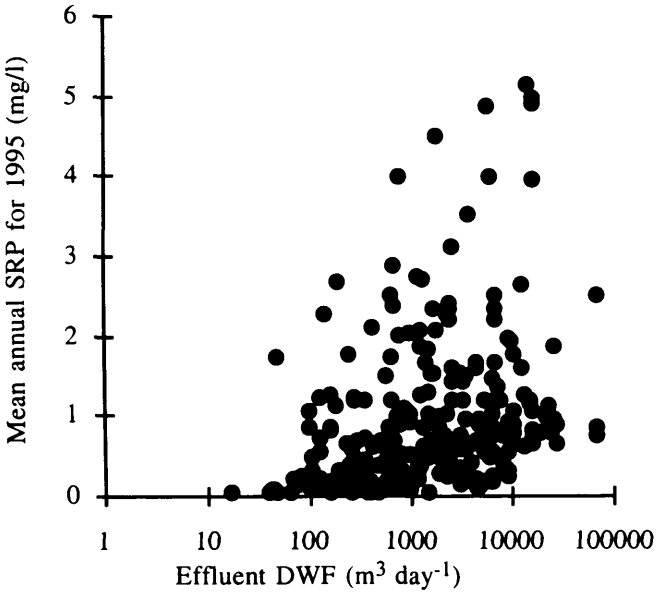


Figure 3.2 Mean annual soluble reactive phosphorus (mg l^{-1}) against cumulative upstream effluent (dry weather flow m^3 day^{-1})

Differences in geology were examined in respect to their possible influence on phosphorus concentration. Analysis was performed on a subset of the data containing only those sites with less than 500 DWF m^3 day^{-1} effluent including 52 sites with no upstream effluent input at all. This cut off value for effluent DWF was chosen arbitrarily as representing a minor point source from a village or small town. A Kruskal-Wallis test was applied to the dataset to determine whether there were any discernible differences in the phosphorus levels of rivers on different geologies, grouped as three categories; 'clay,' 'chalk' and 'other'. The results (Table 3.1) indicate that rivers flowing over chalk bedrock are more likely to have lower levels of this nutrient. The mean values for SRP were considerably lower for chalk than for the other two geological categories. Potentially, geology may influence the choice of agricultural land usage within a catchment which may in itself affect the nature and size of diffuse delivery of phosphorus.

Table 3.1 Application of Kruskal-Wallis test to geological categories

Dataset	Kruskal-Wallis value	P	n	mean SRP (mg l^{-1}) on 'clay' (n=60)	mean SRP (mg l^{-1}) on 'chalk' (n=57)	mean SRP (mg l^{-1}) on 'other' (n=25)
<500 DWF	H=22.3	P<0.001	n=142	0.46	0.21	0.52

First and second order streams made up 67% of the total number receiving no STW effluent. These had an average of 0.245 mg l^{-1} SRP. This compares to an average value of 0.977 mg l^{-1} SRP for first and second order streams receiving STW effluent. Fourth and fifth order watercourses received more sizeable effluent having on average

11358 DWF m^3 day^{-1}. The mean annual instream SRP concentration was 0.844 mg l^{-1}.

The levels of phosphorus within these rivers are influenced by the solid geology overwhich the river flows and point source inputs from STWs. Low phosphorus watercourses are more likely to receive little or no effluent input and are most likely to be found on chalk geologies. They will be most frequently found in the middle and upper reaches of rivers.

3.3 Research Strategy

The levels of phosphorus within the rivers of this eastern region are routinely monitored once monthly by the Environment Agency (EA). This frequency of data gathering is sufficient for monitoring general levels of this nutrient but does not allow for more in-depth consideration of temporal and spatial fluctuation. Augmenting EA sample frequency was considered a prudent way of achieving a larger dataset from which phosphorus dynamics could be described. Study rivers were chosen to provide a range of phosphorus levels. The preliminary survey described in section 3.2 indicated that most low phosphorus rivers are located within the headwaters. The selection process had also to take into account the potential differences in instream phosphorus associated with chalk geology.

Nine rivers were selected for further study. Eight of these rivers were paired and chosen for having contrasting high and low phosphorus concentrations. Each pair was located within the same catchment and catchments were selected to reflect a broad geographical and geological range. Paired rivers included the Waithe Beck and Great Eau in Lincolnshire, the Wensum and Bure in Norfolk, Little Ouse and Wissey in Norfolk/Cambridgeshire and the Deben and Alde in Suffolk. Three EA routine sample sites were selected on each river in the upper-middle reaches. One of the three sites chosen on the Great Eau was located on one of its tributaries, the Long Eau. Similarly, one of the three sites chosen on the Little Ouse was located on the River Sapiston just before its confluence with the Little Ouse (refer to Appendix 1). The ninth river, the River Welland in Leicestershire and Northamptonshire, was chosen for more intense sampling. EA routine sampling frequency was augmented by inserting an extra sample in between their sample collection to give fortnightly sample intervals. Figure 3.3 details the location of each river; Table 3.2 the sites and sample frequencies

Figure 3.3 Map of study rivers

Table 3.2 Sample sites and frequencies of water sampling (excluding the River Welland, refer to Table 3.3)

River	Site	NGR	This project sampling	EA routine sampling	Sample frequency per month overall	Total no. of samples Mar '95 to Nov '95	
						SRP	TP
Little Ouse	Blo Norton Ford	TM012791	✓	✓	2	15	9
Little Ouse	Knettishall Rd Br	TL956807	✓	✓	2	15	9
Sapiston	Euston Road Bridge	TL893802	✓		1	6	9
Wissey	Necton Common Rd Br	TF896083	✓	✓	2	16	8
Wissey	N. Pickenham Br Houghton Lane	TF866067	✓	✓	2	16	8
Wissey	Bodney Rd Bridge	TL829988	✓	✓	2	16	8
Wensum	Sculthorpe Mill	TF893304	✓	✓	2	15	7
Wensum	Great Ryburgh Bridge	TF964274	✓	✓	2	16	7
Wensum	Billingford Bridge	TG004202	✓	✓	2	16	7
Bure	Moor Hall Ford	TG100311	✓	✓	2	14	9
Bure	Saxthorpe Mill	TG115303	✓	✓	2	19	9
Bure	Ingworth Bridge	TG193292	✓	✓	2	19	9
Deben	A1120 Rd Br,Ashfield	TM203615	✓	✓	2	15	8
Deben	Glevering Bridge	TM295566	✓	✓	2	15	9
Deben	Ufford Bridge	TM300519	✓	✓	3	28	9
Alde	Bruisyard Arch	TM334656		✓	1	10	4
Alde	Farnham Bridge	TM360601		✓	1	10	4
Alde	Langham Bridge	TM375581		✓	1	10	4
Great Eau	Cloves Bridge	TF468905	✓	✓	3	24	17
Great Eau	Withern Bridge	TF425826	✓	✓	2	15	9
Long Eau	Three Bridges	TF439881	✓	✓	2	15	9
Waithe Beck	Thorganby Bridge	TF208976	✓		1	7	9
Waithe Beck	Brigsley Bridge	TA252016	✓	✓	2	16	8
Waithe Beck	Tetney	TA313006	✓	✓	2	16	8

The upper Welland catchment (Figure 3.4) in Leicestershire was sampled spatially and temporally with greater intensity. Thirty-two sites, most of which are not normally sampled by the EA, were selected along five tributary streams together with the upper reaches of the Welland itself. Sites were chosen to reflect a range of influences and phosphorus concentrations, and to provide a range of sample frequencies (Table 3.3).

Figure 3.4 Sampling points on the upper Welland

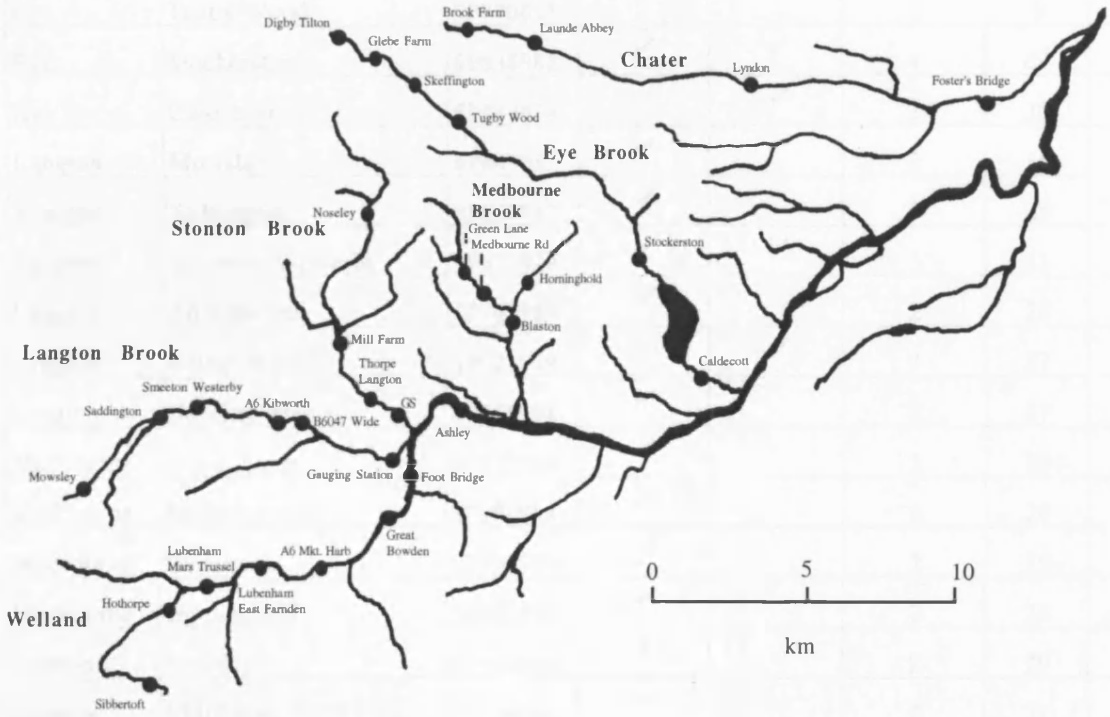


Table 3.3 Sampling sites and frequency of water sampling for the Welland catchment

Stream	Site	NGR	This project sampling	EA Routine sampling	Sample frequency per month	Total no. of samples Mar '95 to July '96	
						SRP	TP
Chater	Brook Farm	SK782053	✓		2	30	31
Chater	LaundeAbbey	SK801045	✓		2	29	31
Chater	Lyndon	SK908036	✓		2	28	30
Chater	Foster's Bridge	SK960030	✓		2	29	29
Eye	Digby Tilton	SK734048	✓		2	26	30
Eye	Glebe Farm	SK742042	✓		2	31	31
Eye	Skeffington	SK754034	✓		2	30	31
Eye	Tugby Wood	SP770023			1	9	9
Eye	Stockerston	SP838982	✓	✓	4	69	54
Eye	Caldecott	SP867934		✓	2	35	16
Langton	Mowsley	SP645887	✓		2	16	17
Langton	Saddington	SP660913	✓		2	28	30
Langton	Smeeton Westerby	SP677923	✓	✓	3	43	33
Langton	A6 Kibworth	SP705923	✓		2	24	29
Langton	B6047 Wide	SP721919	✓	✓	3	37	30
Langton	Gauging Station	SP755908	✓		2	27	32
Medbourne	Green Lane	SP779969	✓		2	30	33
Medbourne	Medbourne Rd	SP794958	✓		2	29	31
Medbourne	Blaston	SP796954	✓		2	28	30
Medbourne	Horninghold	SP802968	✓		2	28	30
Stonton	Noseley	SP744989	✓		2	29	32
Stonton	Mill Farm, S. Wyville	SP734946	✓		2	28	30
Stonton	Thorpe Langton Ford	SP743929	✓		2	27	30
Stonton	Welham, Green Lane	SP759919	✓		2	28	30
Welland	Sibbertoft	SP664834	✓	✓	4	54	44
Welland	Hothorpe	SP670854	✓		2	30	32
Welland	Lubenham, M. Trussel	SP697864		✓	2	32	15
Welland	Lubenham, E. Farndon	SP705870	✓		2	28	29
Welland	A6 Mkt. Harb.	SP739872	✓		2	27	29
Welland	Great Bowden	SP750883	✓	✓	4	56	44
Welland	Foot Bridge, Green Lane	SP759901	✓		2	26	33
Welland	Ashley	SP792918	✓	✓	3	43	30

3.4 Geological outline of study rivers

The Little Ouse and Sapiston lie in the south east of the Ely Ouse catchment. This eastern area of the catchment is more elevated than the lower lying fenland basin to the west. Both rivers flow over a surface geology composed of boulder clay overlying a bedrock of chalk for most of their lengths. The Wissey lies in the north east of the Ely Ouse catchment. This river rises on boulder clay overlying chalk. Surface geology changes in the middle and lower reaches to chalk. The sampling points in this study lay within the boulder clay influenced upper reaches.

The Deben, for most of its length, cuts through boulder clay and flows over a bed of sand and gravel. The heavy boulder clay surface geology within the catchment though means that there is very little interaction with the sand and gravel aquifers below ground and flows are relatively flashy. The result is a river that has naturally low flows during dry conditions. The Alde catchment is boulder clay in the upper reaches and crag sand and gravels in its lower reaches.

The Bure is located to the north of the Yare catchment. The upper reaches flow through a region of sand and gravel over chalk and Norwich crag (sand and gravel). The surface geology of the southern area of the Yare catchment is principally boulder clay. The northern areas, including the upper reaches of the Bure, Wensum and Ant, are on comparatively more permeable geologies and as a result have relatively higher and more stable baseflows. The River Wensum is located in the north west of the Yare catchment. Outcrops of sands and gravels overlay either boulder clay or brickearth in the upper reaches.

The Waithe Beck and Great Eau are located on the Lincolnshire coast within the Louth coastal catchment. This catchment is made up of small parallel sub-catchments of which Waithe Beck and Great Eau are two out of a total of seven. These rivers have very different upland and lowland areas. There is a chalk outcrop over much of the western part of the Louth Coastal catchment which extends as far as Brigsley on the Waithe Beck and about 4 km from the source of the Great Eau. The central and eastern areas are covered by boulder clay and alluvium. This lowland area has been significantly modified by man to enable arable farming. In the absence of gravity and pumped drainage systems and tidal inundation protection, this region would otherwise be tidal marsh.

The River Welland is a lowland clay river extending from the headwaters near Market Harborough, Leicestershire to the Wash Estuary along the Lincolnshire coast. In the

upper reaches, the Welland runs in a bed of alluvium overlying lower lias clay. This surface geology of alluvium is replaced in the midde and lower reaches by middle and upper lias.

3.5 Sample collection and chemical analyses

Water samples for chemical analyses were taken from stream or river sites directly where access could be gained or else with the aid of a bucket and rope. Care was taken to avoid disturbing bottom sediments which may have contaminated the sample and altered the level of phosphorus. Water was removed as far as possible from the middle of the channel, mid-water column. Samples were decanted or taken directly into polyethyleneterephthalate bottles (phosphate may readily absorb onto the walls of normal plastic bottles). The bottles were then transported to the cold room (4 $^{\circ}$C), Environment Agency, Peterborough to await transfer to laboratory for analysis. Chemical analysis for total phosphorus and soluble reactive phosphorus was performed using automated UV digestion and a Skalar Air Segmented Flow Analyser.

Methods used for chemical determination are described in Appendix 2.

CHAPTER 4 PHOSPHORUS IN LOWLAND RIVERS: LEVELS, SOURCES AND BEHAVIOUR

4.1 Introduction

The results of the sampling strategy outlined in the previous chapter are presented here. Soluble phosphorus data are analysed to establish the sources of this nutrient to the Region's rivers and the effect source has on seasonal changes in concentration. The first section examines Regional levels over a five year period and compares soluble phosphorus to nitrogen concentrations using a subset of this dataset to qualify the use of phosphorus as a measure of trophic status.

4.2 Regional nutrient levels

SRP data between the years 1991 and 1995 were averaged for 997 sites within the regional dataset. The frequency distribution shown in Figure 4.1 is divided into classes of 100 $\mu g\ l^{-1}$. This was expected to approximate a log normal distribution since the eutrophic nature of many of the Region's rivers would imply a predominance of sites with SRP over 100 $\mu g\ l^{-1}$. The graph however shows an asymmetrical distribution with the highest frequency of results at 100 $\mu g\ l^{-1}$ or below. Figure 4.2 examines the same dataset in more detail with phosphorus values only extending up to 1000 $\mu g\ l^{-1}$. SRP shows a skewed normal distribution, the highest percentage frequencies falling equally within the 51-60 and 61-70 $\mu g\ l^{-1}$ classes. Twenty one percent of these sites occurred at 100 $\mu g\ l^{-1}$ or below; 23% between 100-250; 26% between 250-500; 16% between 500-750 and 14% between 750-1000. The lower limit of detection is 50 $\mu g\ l^{-1}$ SRP for most routine EA samples, which suggests that this peak is misleading because an unknown proportion of results represent lower values. This might also explain the high percentage frequency of results between the 51 to 70 $\mu g\ l^{-1}$ range.

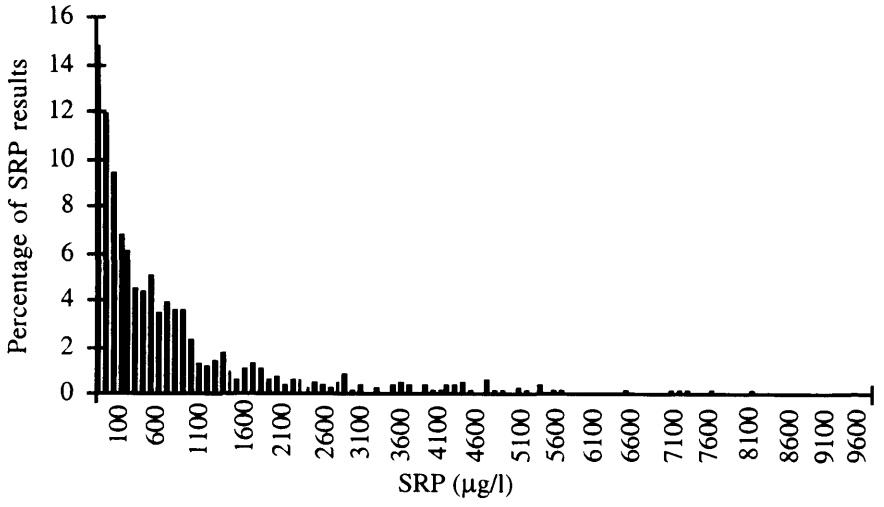


Figure 4.1 Frequency distribution of 1991-95 averaged site data for SRP

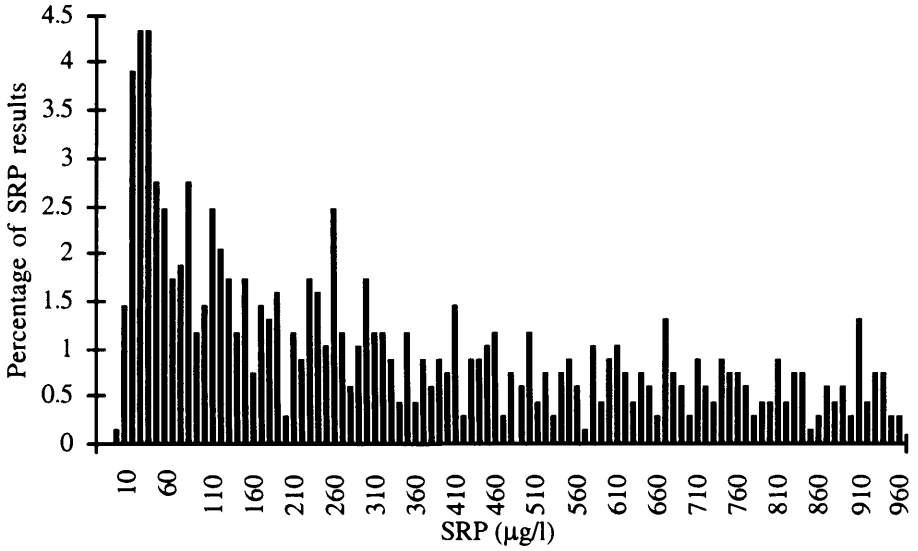


Figure 4.2 Frequency distribution of 1991-95 averaged site data for SRP up to 1000 µg l^{-1}

It is important to confirm at the outset that phosphorus as opposed to nitrogen is the limiting nutrient in these study rivers. The chemical data shown in Figures 4.3 and 4.4 are from the rivers Waithe Beck and Great Eau in Lincolnshire, Wensum and Bure in Norfolk, Little Ouse and Wissey in Norfolk/Cambridgeshire and the Deben and Alde in Suffolk between 1991 to 1995. The lines drawn on these graphs correspond to a nitrogen:phosphorus ratio of 10:1 which takes account of the variation in ratios reported in the literature (Kelly, 1998a). Below a ratio of 10:1, nitrogen is more likely to be limiting and above 10:1, phosphorus is more likely to be limiting. During autumn and winter months (Figure 4.3), 95% of samples taken from these river sites were thus probably phosphorus limited. The increase in summer phosphorus levels due to declining summer flow increases the number of potentially nitrogen limited samples. In Figure 4.4, 83% of samples were phosphorus limited. Overall this would indicate that the rivers are mostly phosphorus limited. In reality, there is a point along the x-axis at which SRP concentrations are so high as to be saturating. This would also apply to nitrogen values regardless of ratio. At this point, other factors become limiting to algal growth, such as light.

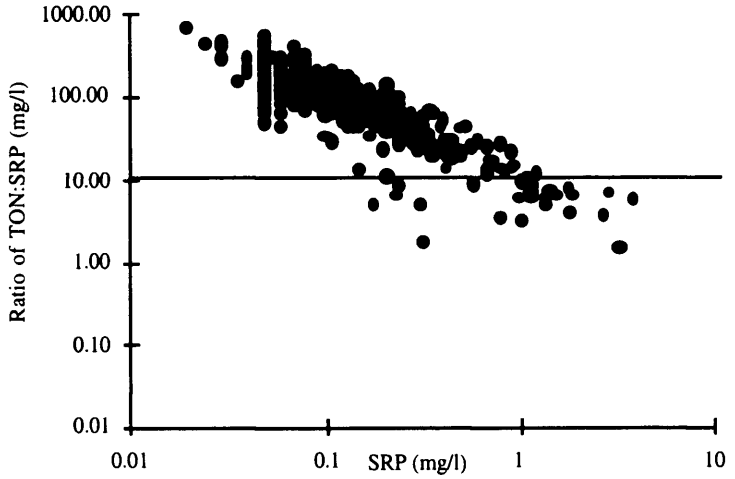


Figure 4.3 Ratio of total oxidised nitrogen (TON) and SRP during autumn/winter months, October to March (n=523)

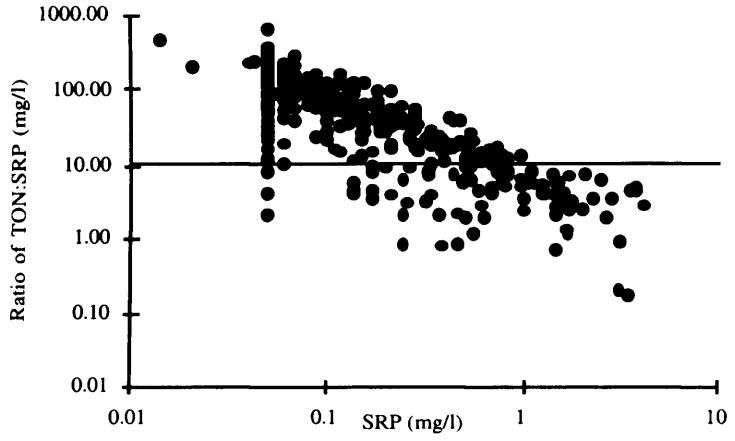


Figure 4.4. Ratio of total oxidised nitrogen (TON) and SRP during spring/summer months, April to September (n=543)

4.3 Sources of phosphorus

4.3.1 Inter-catchment comparison

Initial comparison of the Region's rivers with factors potentially influencing phosphorus levels had suggested that sewage effluent was a likely source (Chapter 3). The influence of sewage effluent was therefore considered in seven rivers within four of the Region's catchments; Wensum and Bure in Norfolk, Little Ouse and Wissey also in Norfolk, Deben and Alde in Suffolk and Waithe Beck and Great Eau in Lincolnshire. Paired rivers are within the same catchment area and one is of high phosphorus concentration and the other low.

Comparison was made between SRP load per day ('measured load') and point source load per day ('estimated load'). Point source load was estimated from the mean annual concentration of SRP ($\mu g\ l^{-1}$) for each effluent source and the dry weather flow

(cumecs). These data existed for all effluents entering the rivers except for those discharging into the upper Wissey. In this instance, effluent load was approximated from the value given for total population equivalent (PE) given (500g of phosphorus per person per annum). The error caused by using the two different methods for establishing effluent load was estimated by comparison: Figure 4.5 shows that these two methods are reasonably comparable (r^2=0.81, $p<0.05$). Point source load was summed along the length of each stream. Phosphorus concentrations were converted to loadings by multiplying concentration ($\mu g\ l^{-1}$) by flow ($l\ s^{-1}$) for each site. The results of these comparisons for each river's three sites are represented graphically (Figures 4.6 to 4.13). The scale for the y-axis for each graph is different reflecting the broad range of loadings in the study rivers.

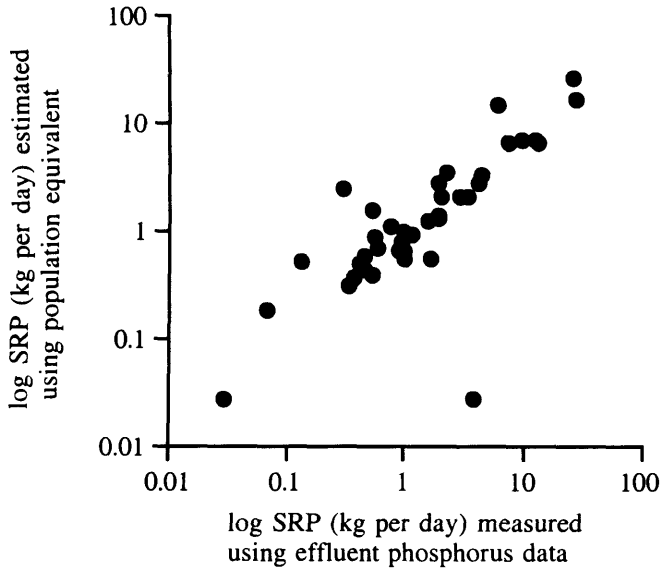


Figure 4.5 Comparison of measuring effluent load using 'population equivalent' and effluent phosphorus data

Load was calculated using an average monthly value for stream discharge and SRP concentration (n=2). The validity of using those data is supported when all instream and point source load data are compared (Figure 4.6). The relationship is statistically significant (r^2=0.83, $p<0.05$) although it is evident from the graph that it is the high load data points that contribute most to this relationship. The fact that this relationship is not 1:1 indicates that point source load is to some extent overestimating instream load.

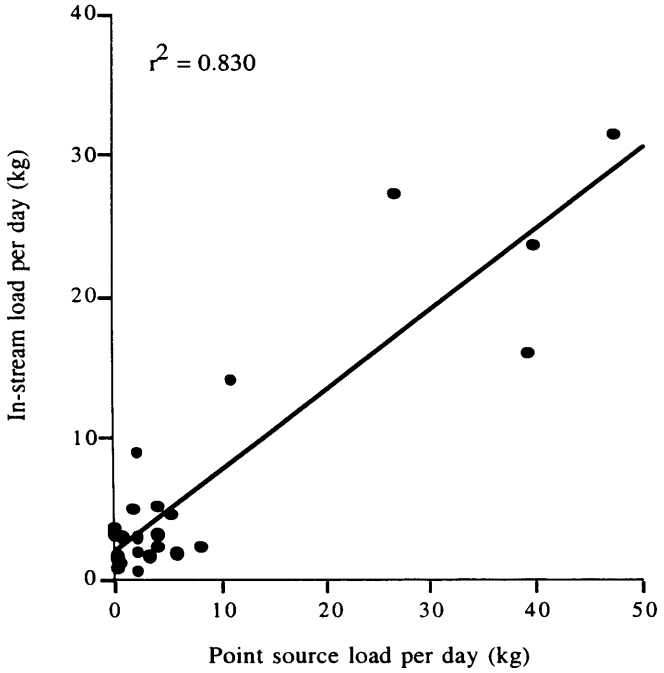


Figure 4.6 The relationship between estimated load and measured load

The SRP load along the Bure (Figure 4.7) broadly reflected the input of sewage from a number of small village STWs in its upper reaches. The load within the River Wensum (Figure 4.8), likewise, reflected sewage input. The sharp increase in load downstream of Fakenham ('19.25 km') was mostly due to this town's STW which serves a population of 11,761. The SRP load of the rivers Little Ouse and Wissey (Figures 4.9 and 4.10) were similarly related to effluent discharge; along the Little Ouse there are villages with population equivalents (p.e.) of between 144 and 2031. The Wissey receives effluents from Necton, Swaffham and Watton STWs with 2797, 5258 and 10,835 p.e. respectively. The sampling point on the River Sapiston was just above the river's confluence with the Little Ouse at Euston (Appendix 1, Figure 1); a distance of 29.95 km from the Sapiston's source. The STWs along its length serve a total p.e. of 17,896. The discrepancy between instream load and point source load may in part be accounted for by biotic removal since the bulk of STWs are situated closer to the source than to the point of sampling.

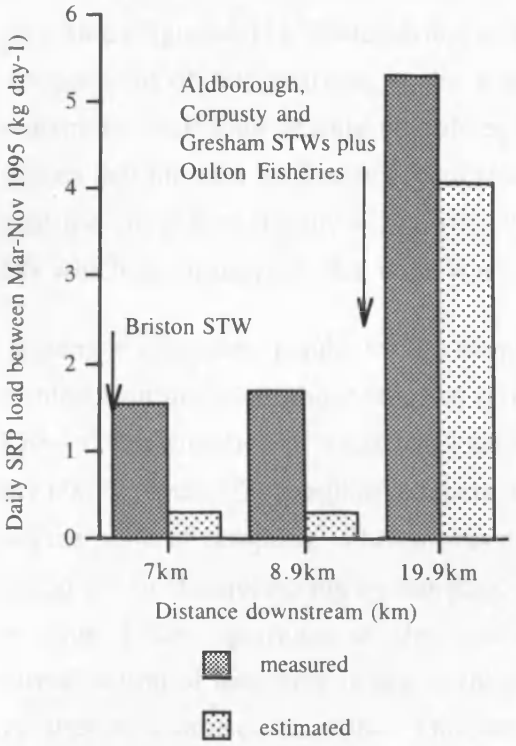


Figure 4.7 River Bure downstream changes in SRP load

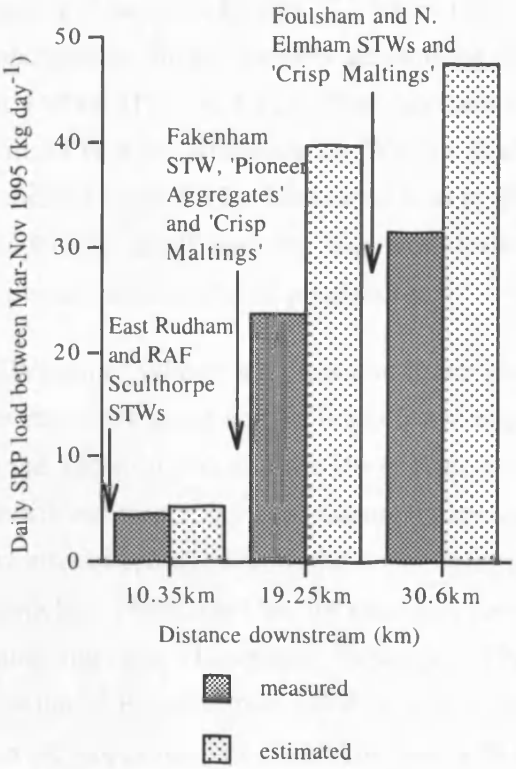


Figure 4.8 River Wensum downstream changes in SRP load

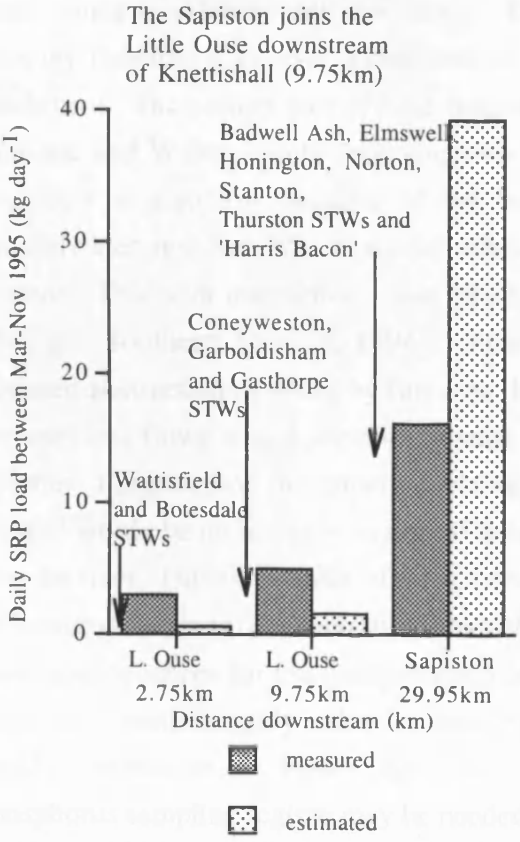


Figure 4.9 Rivers Little Ouse and Sapiston downstream changes in SRP

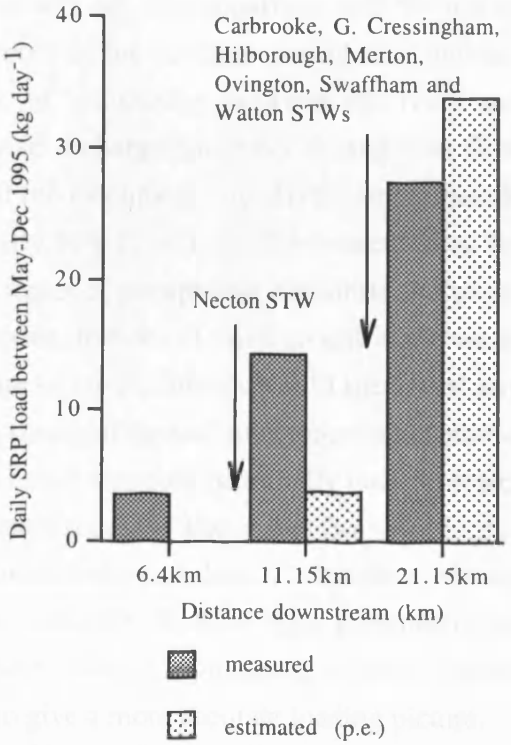


Figure 4.10 River Wissey downstream changes in SRP load

Point source load overestimated measured load at Langham Bridge (22.5 km) on the River Alde (Figure 4.11). Effluent-derived phosphorus did not entirely account for the instream load of this nutrient in the River Deben (Figure 4.12) The increase in downstream SRP load despite no subsequent STW after Binbrook on Waithe Beck suggests that nutrient load comes from elsewhere (Figure 4.13). Measured load at the top of the Great Eau (Figure 4.14) was considerably larger than the load from Driby STW which again suggests that there was an unidentified source of phosphorus.

Comparison of loading results for the Rivers Wensum, Wissey and Sapiston illustrated the impact on instream phosphorus of rivers receiving point source loads from large STWs. Overestimation of measured load in the Sapiston and also the River Alde was due to biotic uptake of phosphorus where the effluent source lay at a distance upstream from the point of sampling. Phosphorus may also be removed from the water column through physical sequestering by inorganic particles. The River Ore, for example, joins the Alde 2 km upstream of the last sampling site (Langham Bridge). The overestimation of load here is due to the position of Framlingham STW which is the largest point source on the Alde. This lies in the upper reaches of the Ore and with a p.e. of 2900.

The Deben is known to be a river that has naturally low flows during dry periods and at other times a rather spatey discharge. This is due to both rainfall and catchment geology (boulder clay over a bed rock of sand and gravel) with chalk in the lower catchment. The eastern part of East Anglia is very dry in comparison with the rest of England and Wales, barely receiving two thirds of the national average for rainfall. Baseflow is also low because of the lack of interaction between the river and groundwater reserves which would otherwise recharge the river during low flow periods. This poor interaction is the result of the catchment's relatively impermeable geology (Southern Science, 1994). Naturally low flow is further exacerbated by licensed abstraction of water by farmers. In terms of phosphorus equilibria therefore, summer low flows would serve to increase concentrations of this nutrient in the water column. Furthermore, the impermeable nature of the catchment would mean that any rainfall would be more likely to run off land, transporting sediment bound phosphorus into the river. Diffuse sources of phosphorus could therefore potentially have a greater influence on instream concentrations than effluent sources. The spatey flow would also have consequences for the interpretation of mass balanced data. Calculation of load relies on a mean monthly value for both flow and SRP. Both of these parameters are highly variable in the Deben given its spatey nature. Therefore a more regular phosphorus sampling regime may be needed to give a more accurate loading picture.

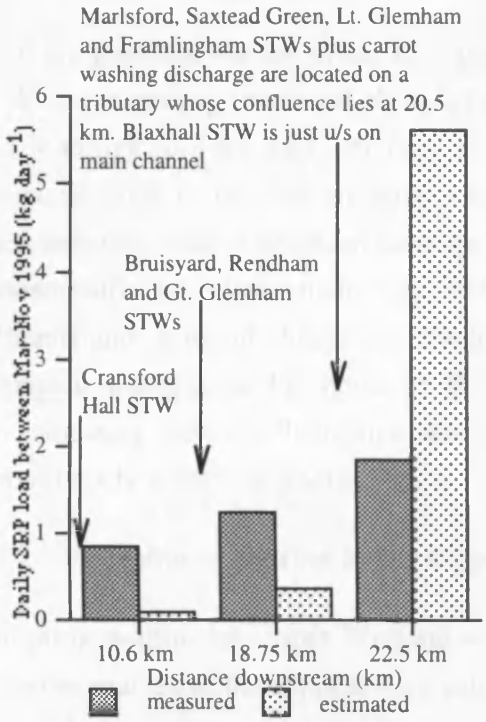


Figure 4.11 River Alde downstream changes in SRP load

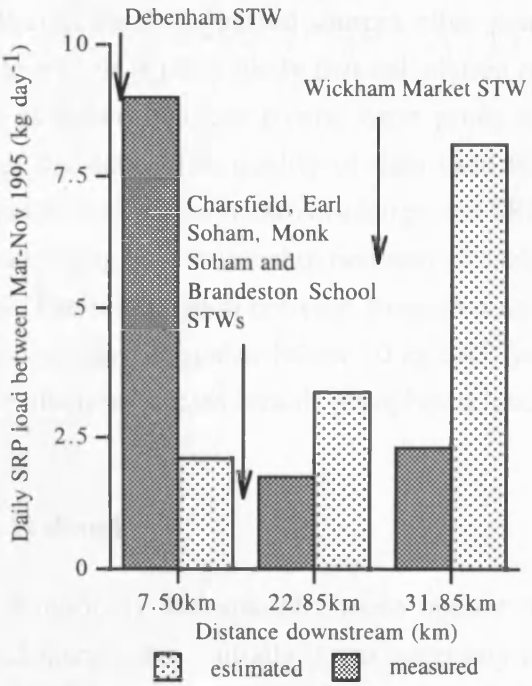


Figure 4.12 River Deben downstream changes in SRP load

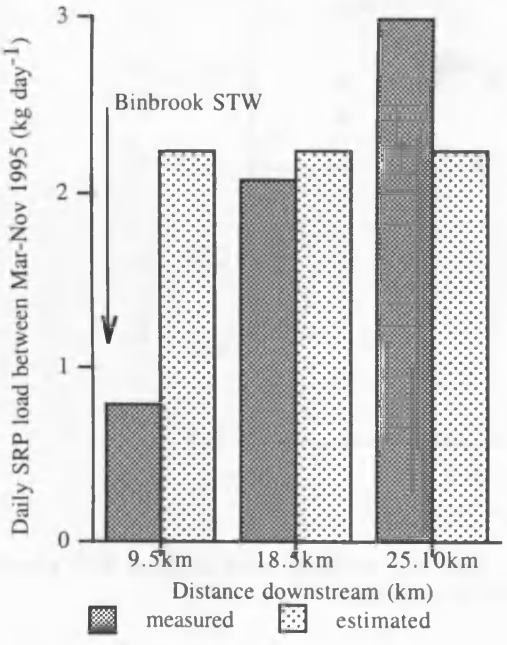


Figure 4.13 Waithe Beck downstream changes in SRP

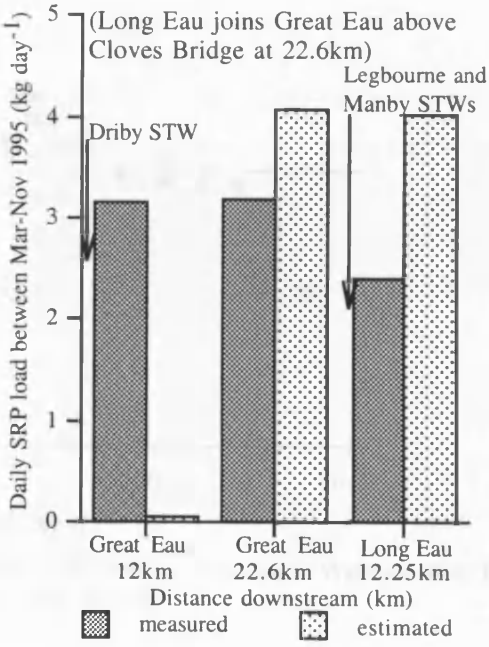


Figure 4.14 Great Eau and Long Eau downstream changes in SRP load

Load comparisons for the Great Eau and Waithe Beck suggested sources other than STWs were causing increased phosphorus levels. It is more likely that calculation of low loadings (0-3 kg SRP per day) such as found in these rivers, were prone to inaccuracy due to the lack of sensitivity of the data. The quality of data used for intercatchment load comparison (average monthly values for stream discharge and SRP concentration, n=2) was satisfactory for establishing the relationship between sizeable effluents and instream phosphorus loading. The relationship between measured and estimated load (Figure 4.6) however shows considerable scatter below 10 kg SRP per day indicating that, if effluent significantly influenced stream soluble phosphorus load, it could not be established here.

4.3.2 Phosphorus sources in the Upper Welland

Sampling within the Upper Welland was temporally and spatially more intense to examine sources of phosphorus on a subcatchment scale. Initially it was necessary to establish the limiting nutrient. The ratio of N:P for nine sites in the Upper Welland over summer 1995 (Figure 4.15) indicates that, at concentrations below 1000 $\mu g\ l^{-1}$ SRP, conditions are mostly phosphorus-limiting.

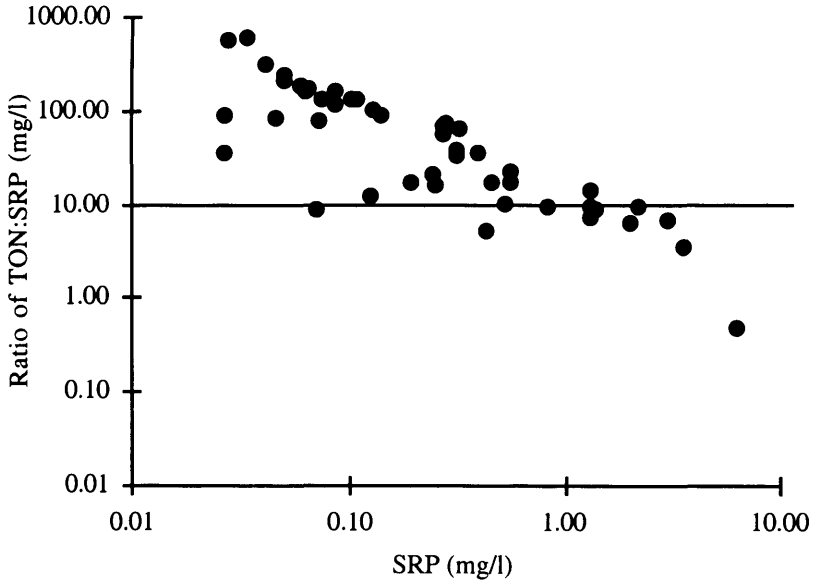


Figure 4.15 Ratio of total oxidised nitrogen (TON) and SRP at nine upper Welland sites for April to October, 1995 (n=48)

The same procedure was used as in section 4.3.1 for comparison of instream load of phosphorus with point source load. A measure of background load was also estimated for each sample. This was calculated from the mean SRP load (expressed as kg km^{-2} ann^{-1}) obtained for four sites without sewage effluent impact. These sites were Digby, Tilton (Eye), Glebe Farm (Eye), Green Lane, Hallaton (Medbourne) and Brook Farm

(Chater); all were located within 3.5 km of the stream's source. The catchment area upstream of these sites was predominantly pasture with no point sources. Figure 4.16 shows the correlation obtained between the load at these sites and the upstream catchment area. The mean value obtained was 3.26 kg km^{-2} ann^{-1} which was used as the background runoff in all subsequent calculations.

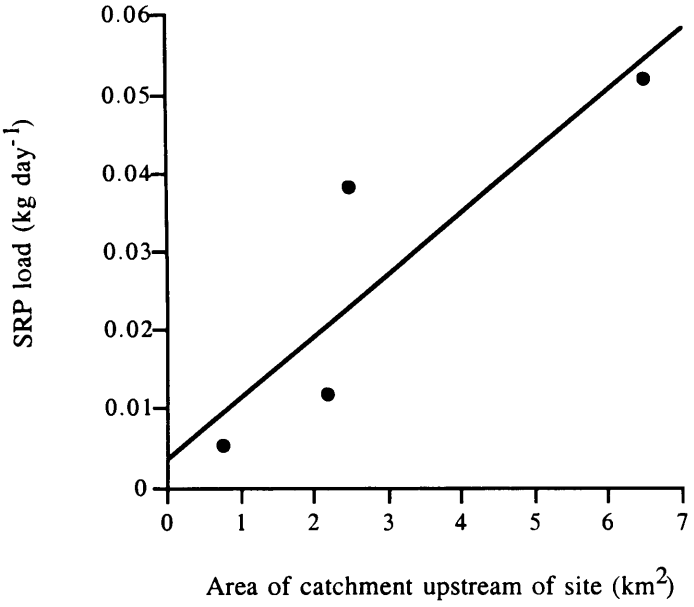


Figure 4.16 Relationship between catchment area and SRP load for four non-impacted sites

Phosphorus concentrations were converted to loadings by multiplying concentration (μg l^{-1}) by flow (l s^{-1}) for each site. Point source load was estimated from the mean annual concentration of SRP (mg l^{-1}) for each effluent and the dry weather flow (cumecs). Where neither of these values existed within the data, effluent load was approximated from the value given for total population equivalent. Point source load is cumulative along the length of each stream.

Stream phosphorus loading in Eye Brook, Langton Brook and the Welland itself reflected inputs of this nutrient from STWs. The impact of two small village STWs at Belton and Tilton on Eye Brook is clearly demonstrated (Figure 4.17). The marked decrease in loading at the furthest downstream site compared to upstream effluent inputs is due to nutrient retention in Eye Brook reservoir. Kibworth STW on Langton Brook (Figure 4.18) and Market Harborough STW on the River Welland (Figure 4.19) are the two largest point sources within the upper catchment serving communities of 4,511 and 19,011 respectively. The confluences of Stonton Brook and Langton Brook with the Welland occur just upstream of the last sample site (at 24.7 km). The point source load at this site therefore includes the effluent inputs from these two streams.

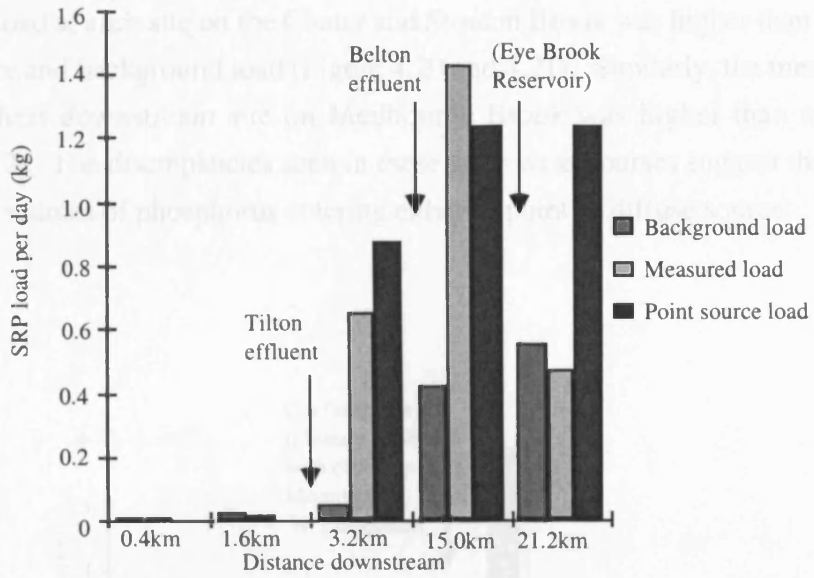


Figure 4.17 Eye Brook downstream changes in SRP load

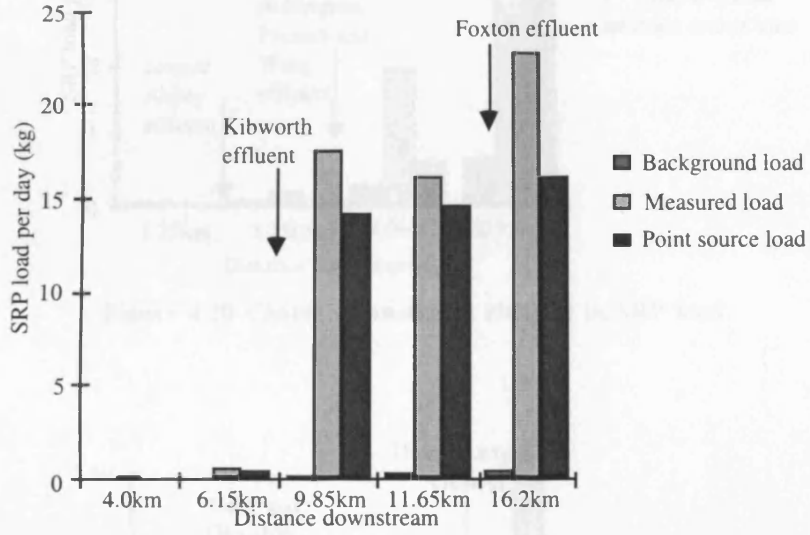


Figure 4.18 Langton Brook downstream changes in SRP load

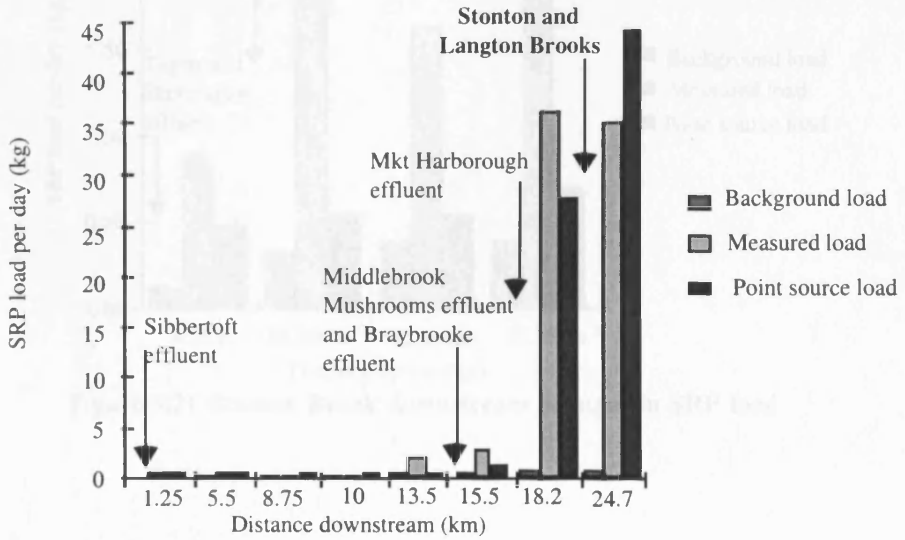


Figure 4.19 Welland downstream changes in SRP load

Measured load at each site on the Chater and Stonton Brook was higher than the sum of point source and background load (Figure 4.20 and 4.21). Similarly, the measured load at the furthest downstream site on Medbourne Brook was higher than anticipated. (Figure 4.22). The discrepancies seen in these three watercourses suggest that there are alternative sources of phosphorus entering either as point or diffuse source.

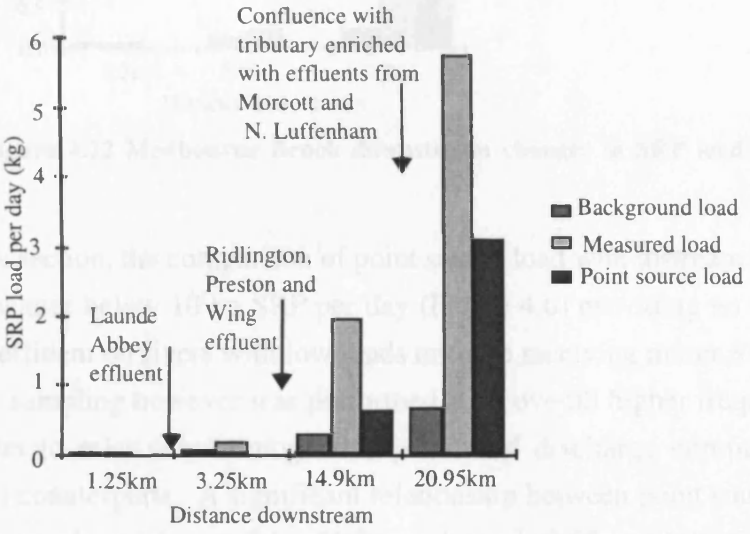


Figure 4.20 Chater downstream changes in SRP load

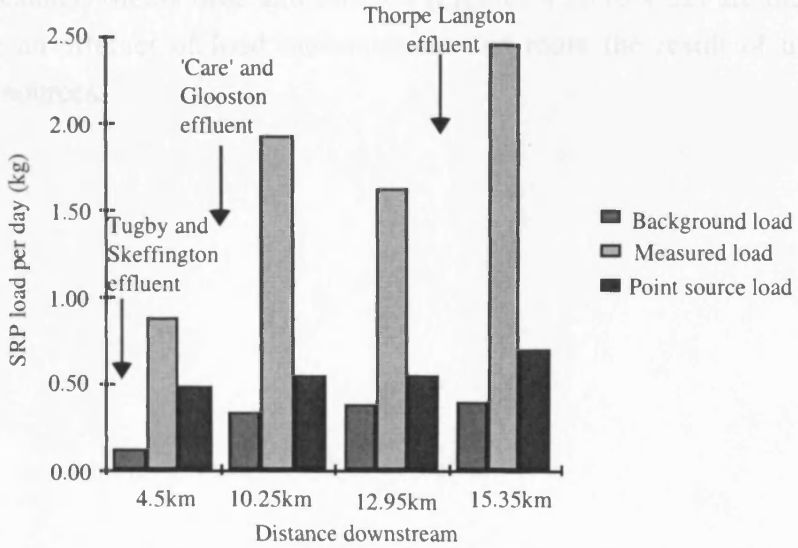


Figure 4.21 Stonton Brook downstream changes in SRP load

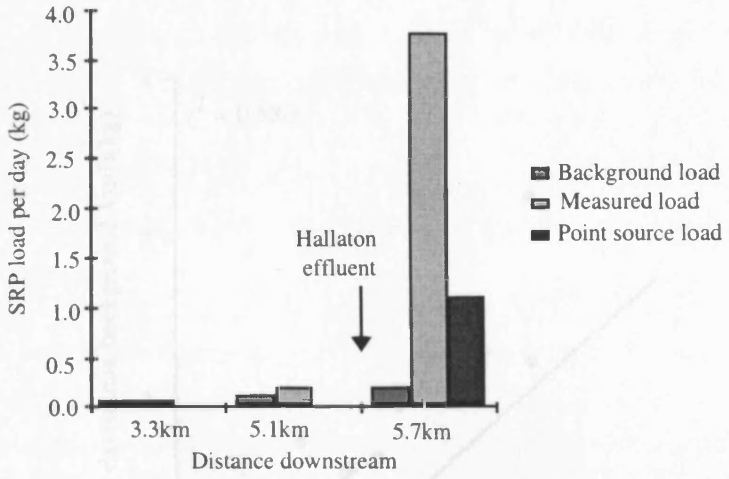


Figure 4.22 Medbourne Brook downstream changes in SRP load

In the previous section, the comparison of point source load with instream load showed considerable scatter below 10 kg SRP per day (Figure 4.6) providing no evidence for the impact of effluent on rivers with low loads or those receiving minor STW effluent. Subcatchment sampling however was performed at an overall higher frequency and in streams subject to relatively homogenous pattern of discharge compared to their intercatchment counterparts. A significant relationship between point source load and instream load was found below 5 kg SRP per day (r^2=0.58, $p<0.05$)(Figure 4.23). Figure 4.24 shows the relationship using the whole dataset. The proportion of instream load comprising background load was accounted for by subtraction. The discrepancies seen in the Chater, Medbourne and Stonton (Figures 4.20 to 4.22) are therefore less likely to be an artefact of load measurement and more the result of unidentified phosphorus sources.

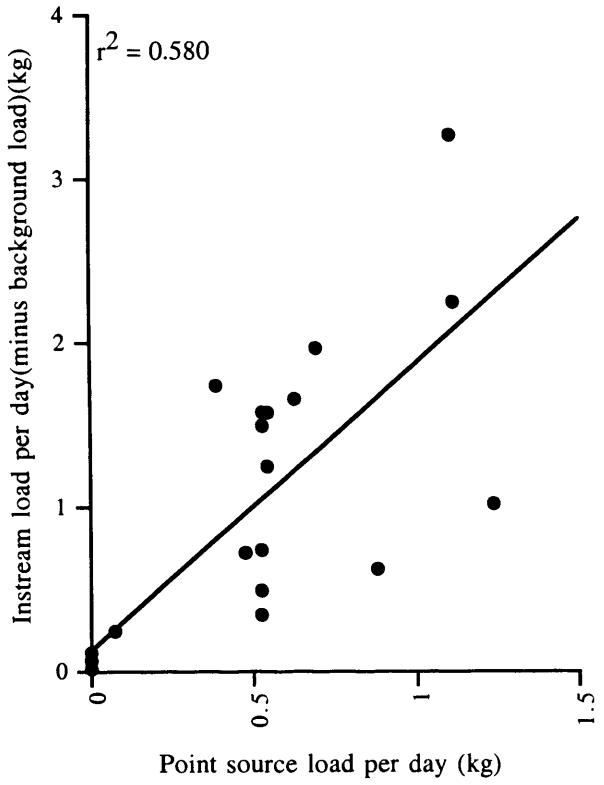


Figure 4.23 The relationship between point source load and instream load (minus background load) for values below 5 kg per day

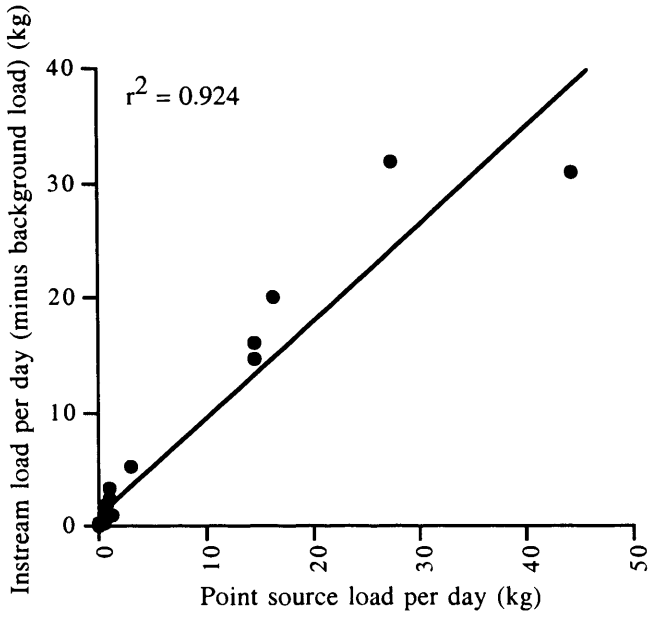


Figure 4.24 The relationship between point source load and instream load (minus background load) in Upper Welland streams

Agricultural land use within the upper Welland was examined to discover any possible linkage between agricultural activity and elevated phosphorus levels not attributable to known point sources. MAFF agricultural census data were used for this purpose. A census of farming activity is performed annually by MAFF and the data released in a summarised format relating to groups of farms or agricultural parishes. The data used were supplied by Edinburgh University Data Library who transform parish data into 5 km^2 grid square data.

Agricultural data were examined for the 5 km^2 grid squares shown in Figure 4.25. Grid squares broadly encompass distinct areas of stream although in the middle of the catchment area there is overlap. Patterns of agricultural activity were evaluated individually across the area; cattle, sheep, pigs and poultry farming and cereal and crop farming. Grid square reference numbers are ordered on the x-axis (Figures 4.26 to 4.28) from C7 to W3 which follows the pattern of squares on the map from the north east corner to the south west corner. Stream reaches with unaccountable phosphorus loads are identified by dotted lines and the grid squares that nominally equate to these areas are C5, C6, C1, C2, C3, E2 and S2. Pig farming showed the most differentiation across grid squares (Figure 4.26) with a distinct gradient from the north east to the south west. Cattle and cereal/crop farming was slightly more prominent to the south of the catchment area (Figures 4.27 and 4.28). The average number of pigs in grid squares C5, C6, C1, C2, C3, E2 and S2 was 391 compared to 298 in the other squares. This difference in mean values was not statistically significant. The drawbacks of using these data are that resolution is poor and the exact whereabouts of pig farms within grid squares is not known.

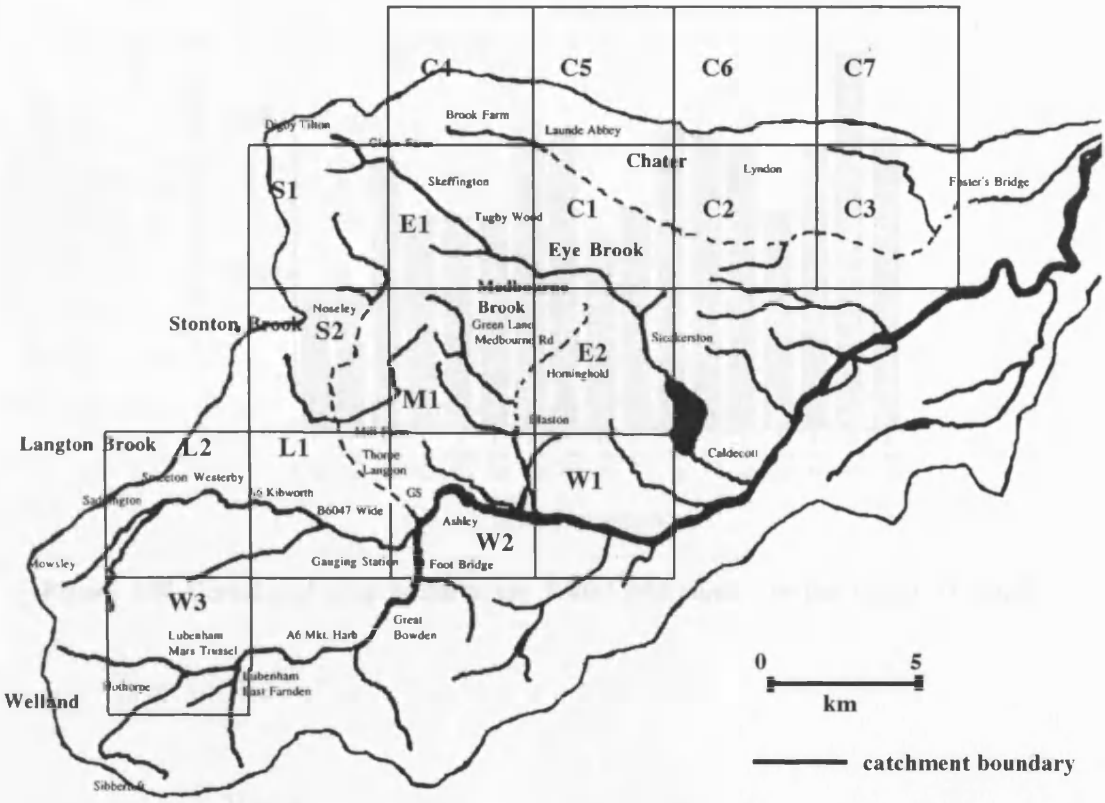


Figure 4.25 Reference 5 km^2 grid squares (Upper Welland) relating to Agricultural Census Data

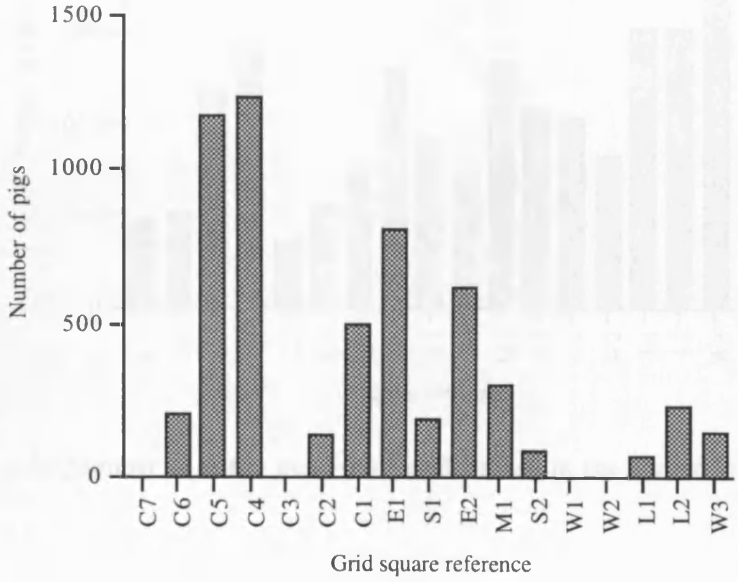


Figure 4.26 Number of pigs per 5 km^2 grid square in the Upper Welland

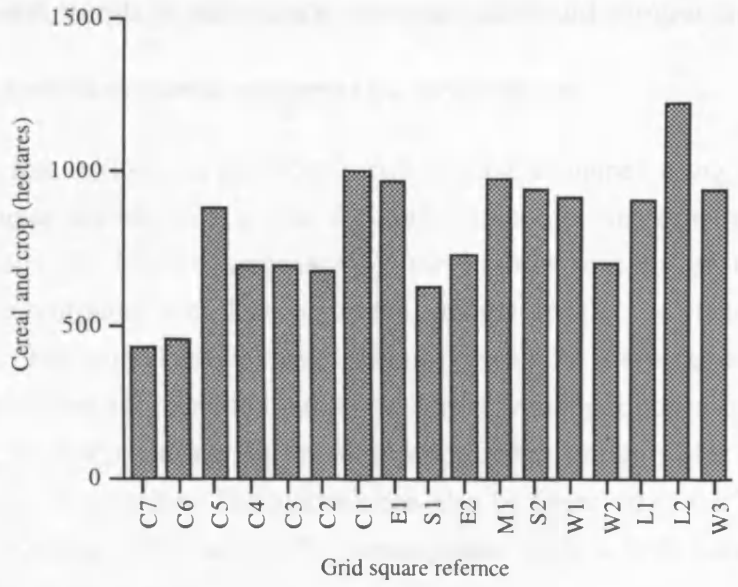


Figure 4.27 Cereal and crop hectares per 5 km^2 grid square in the Upper Welland

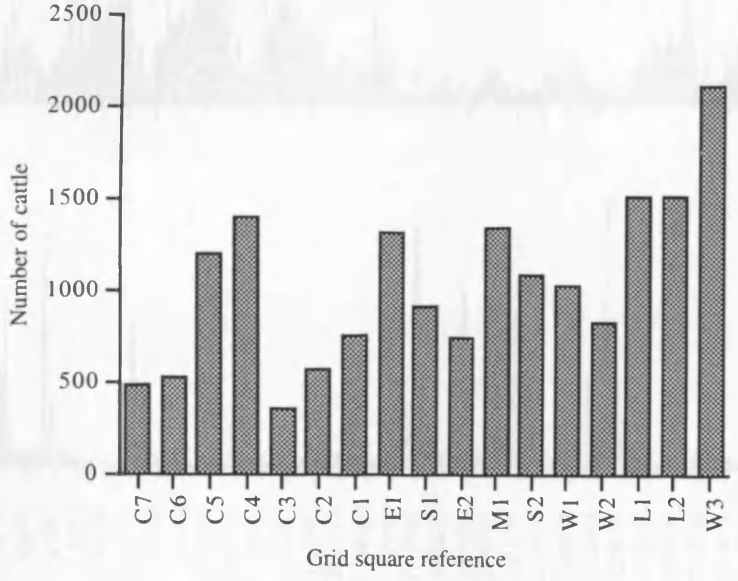


Figure 4.28 Number of cattle per 5 km^2 grid square in the Upper Welland

4.4 Temporal trends in phosphorus concentration and composition

4.4.1 Inter- and intra-annual changes in concentration

Long term changes in flow and phosphorus pattern were examined using 10 year's data for Tinwell (near Stamford) o n the Welland. This site lies downstream of the confluence with the Chater; approaching the middle reaches of the Welland. Phosphorus concentration is high owing to the cumulative effect of effluents from the upper reaches. The annual pattern described in Figure 4.29 is typical of an impacted river. Concentrations are lowered by dilution during winter periods of high discharge and declining discharge during dry months concentrates stream water and levels of phosphorus rise. Inter-annual fluctuations can also be observed in the Tinwell data. High rainfall during 1993 and 1994 corresponds with a reduction in soluble phosphorus during this period.

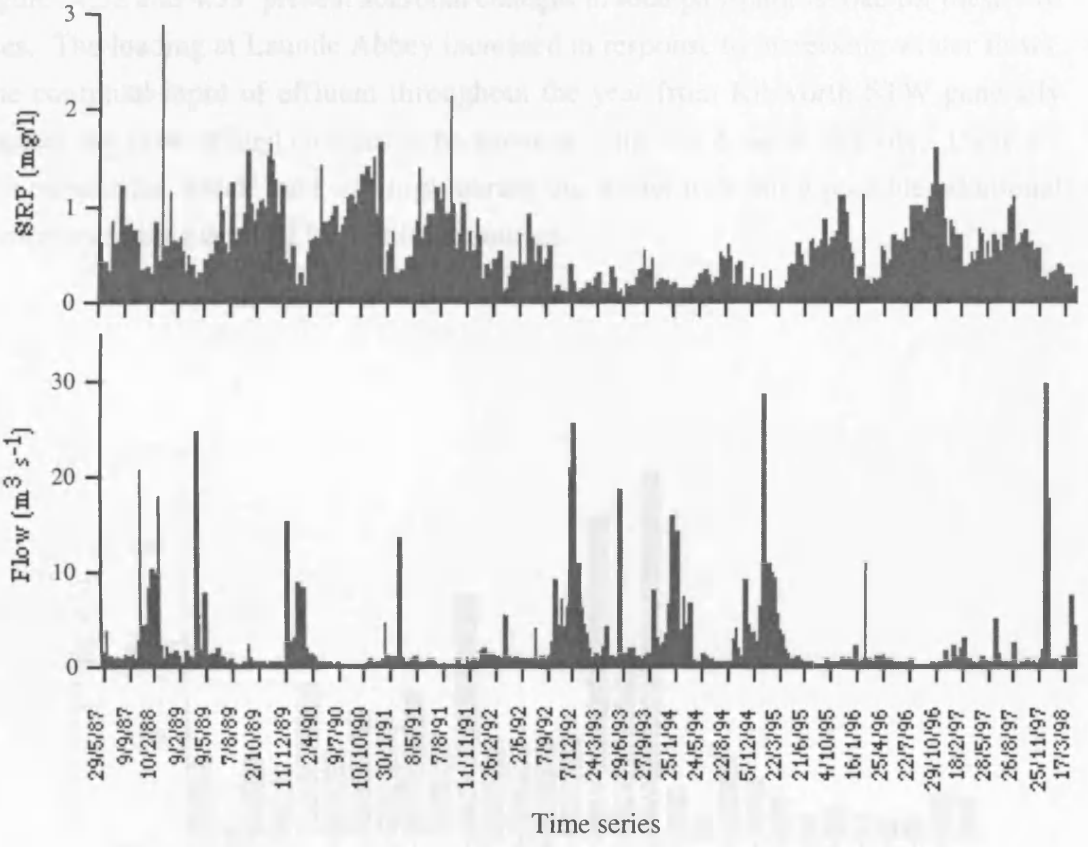


Figure 4.29 Ten year's flow and SRP data at Tinwell, River Welland

Concentrations of phosphorus in natural streams without extraneous sources of nutrient fluctuate less throughout the year than those receiving effluent. Biotic uptake in the summer decreases concentration and sediment entrainment/runoff increases phosphorus concentration during winter months; essentially a reversal of the situation in impacted rivers.

4.4.2 Variations in annual pattern of concentration in relation to source

Variations in annual pattern of phosphorus concentration were observed between those sites downstream of STWs and those free of upstream effluent in the upper Welland. Figure 4.30 shows the pattern of total phosphorus for a non-impacted stream reach, Launde Abbey on the Chater. Phosphorus concentration is on average less in the summer months than winter months during the sampling period 1995 to 1996. Extreme fluctuations in concentration were observed in impacted river sites. The sampling site at the A6 Road Bridge Kibworth (Langton Brook) lies 9.9 km downstream of the source and receives effluent from Kibworth (p.e. 4500). Total phosphorus concentrations increased sharply during summer months as flow fell and remained relatively low throughout most of winter as increased rainfall caused dilution (Figure 4.31)

Figures 4.32 and 4.33 present seasonal changes in total phosphorus load for these two sites. The loading at Launde Abbey increased in response to increasing winter flows. The continual input of effluent throughout the year from Kibworth STW generally masked any flow-related changes in phosphorus at the A6 Road Bridge site. There are two months for which load was high during the winter indicating possible additional phosphorus being derived from diffuse sources.

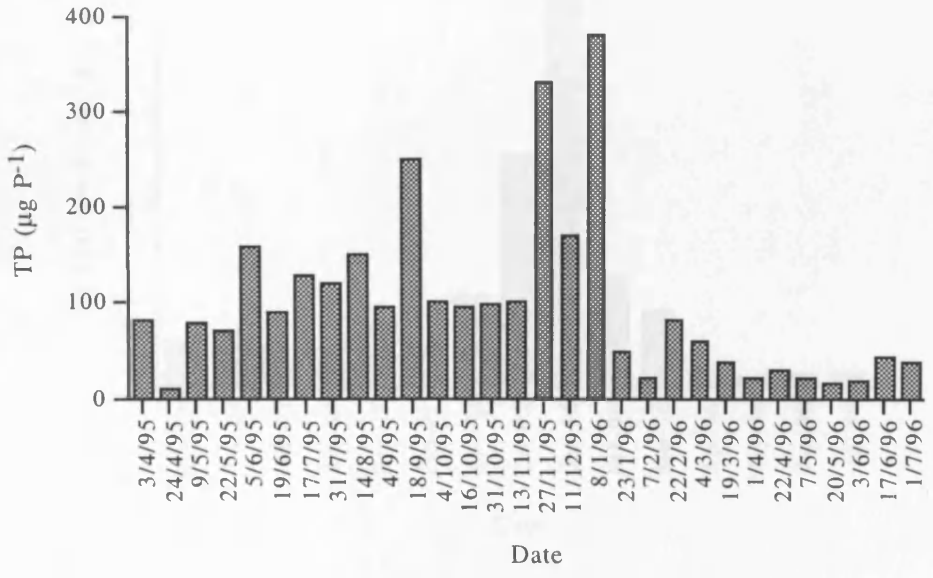


Figure 4.30 Temporal fluctuation in TP concentration at Launde Abbey (Chater)

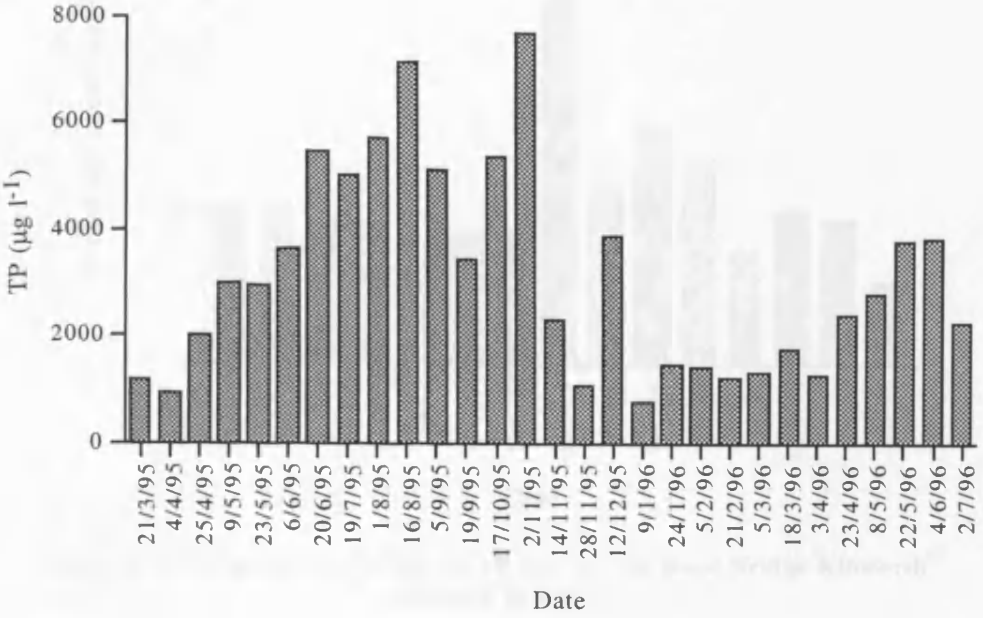


Figure 4.31 Temporal fluctuation in TP concentration at A6 Road Bridge Kibworth (Langton Brook)

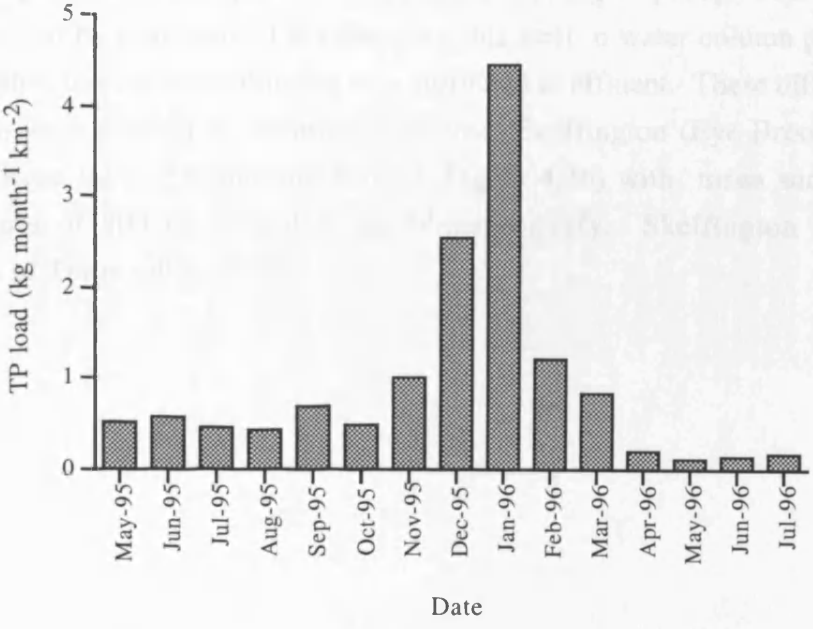


Figure 4.32 Temporal fluctuation in TP load at Launde Abbey (Chater)

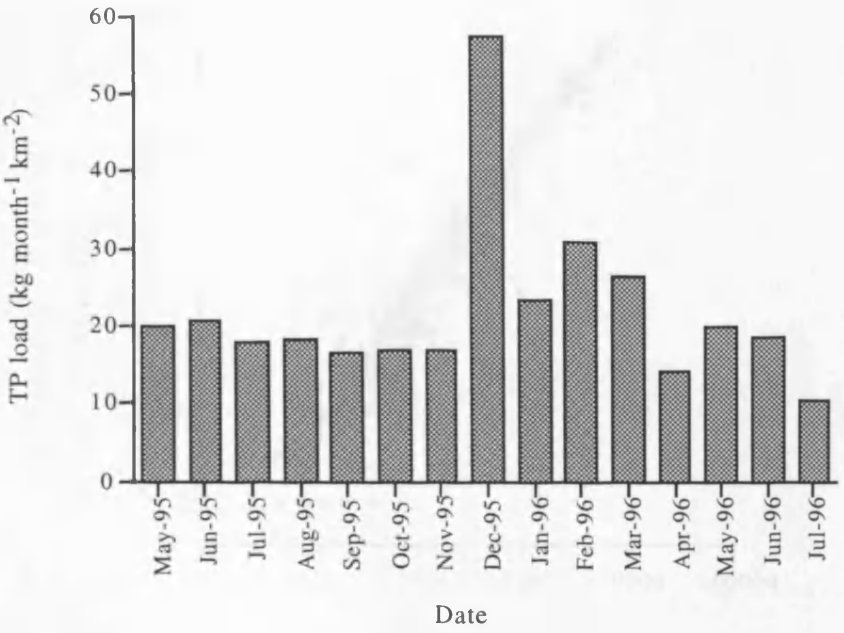


Figure 4.33 Temporal fluctuation in TP load at A6 Road Bridge Kibworth (Langton Brook)

4.4.3 Total phosphorus and its relationship to soluble phosphorus and suspended solids

The fraction of total phosphorus comprising soluble phosphorus increased with higher instream phosphorus concentration (Figure 4.34). Sewage effluent contains more soluble than particulate phosphorus. Sites above 100 μg l^{-1} phosphorus were those mostly impacted by upstream STWs therefore this shift in water column phosphorus from particulate to soluble dominance was attributed to effluent. These differences in composition were evident in chemical data from Skeffington (Eye Brook) (Figure 4.35) and Green Lane (Medbourne Brook) (Figure 4.36) with mean summer SRP concentrations of 809 μg l^{-1} and 13 μg l^{-1} respectively. Skeffington is situated downstream of Tilton village STW.

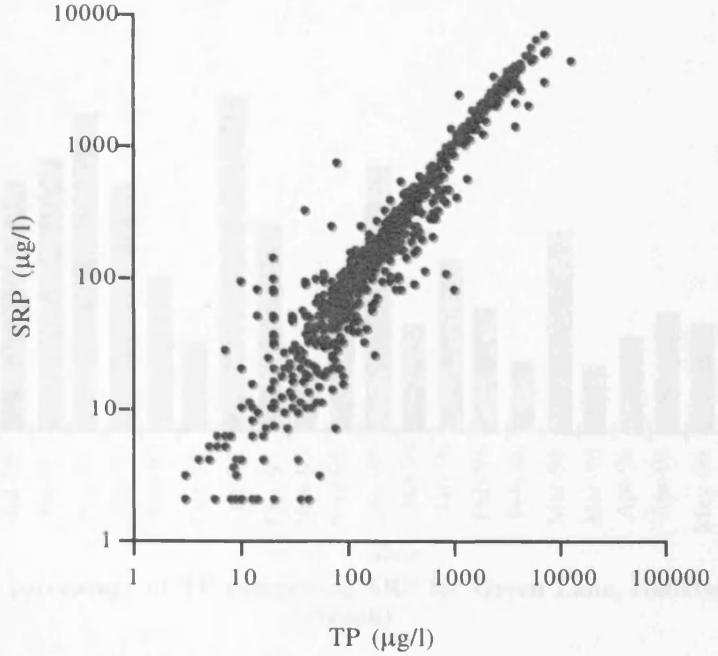


Figure 4.34 SRP against TP data for the period 95/96 for all Welland sites, n=881 (Datapoints above the 1:1 ratio line are erroneous)

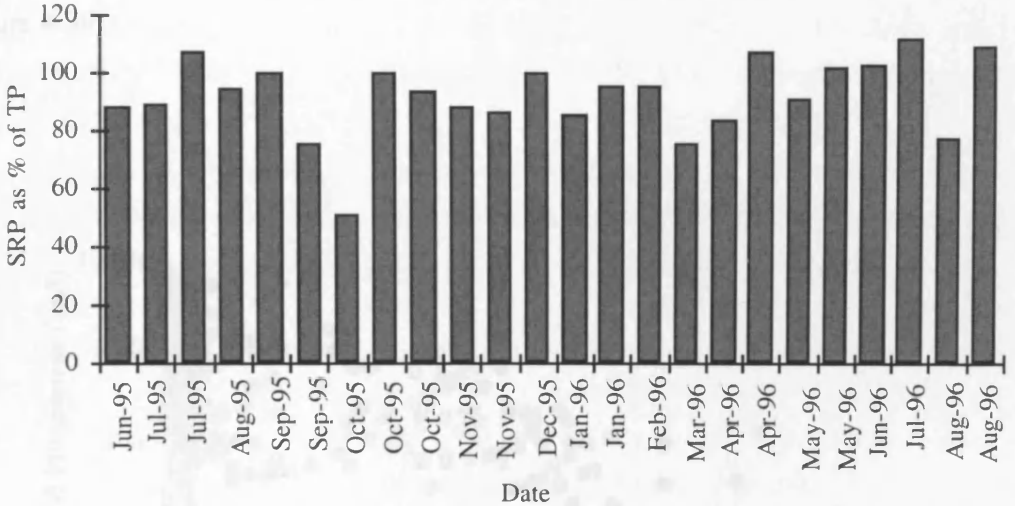


Figure 4.35 The percentage of TP comprising SRP for Skeffington (Eye Brook)

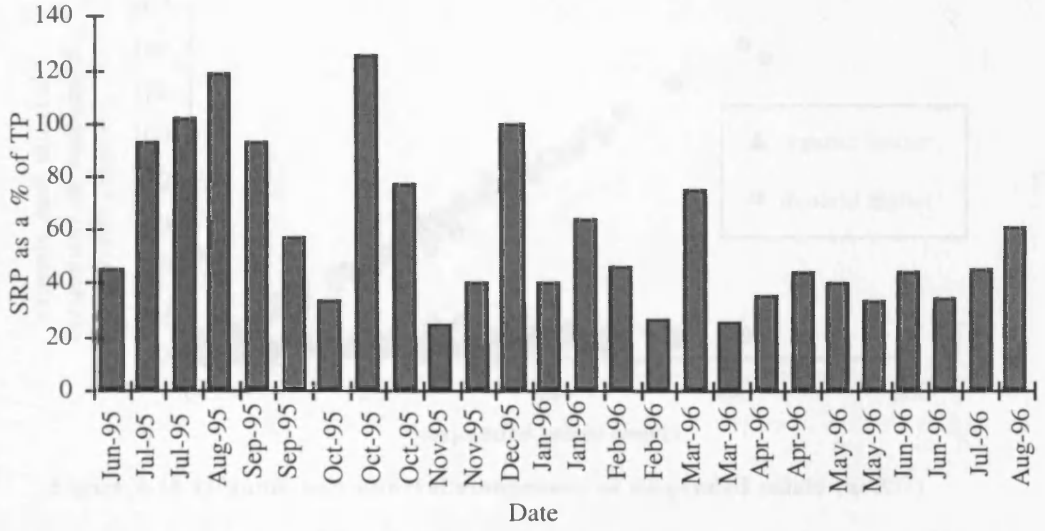


Figure 4.36 The percentage of TP comprising SRP for Green Lane, Hallaton (Medbourne Brook)

The relationship between suspended solid and total phosphorus suggested a tendency towards decreasing total phosphorus at higher suspended solid concentrations (Figure 4.37). Periods of runoff and high discharge as a result of storm events cause increases in particulate matter within the water column. The relationship here would suggest that dilution during these events was reducing total phosphorus concentrations. The mineral as opposed to organic component was responsible for increases in suspended solid (Figure 4.38).

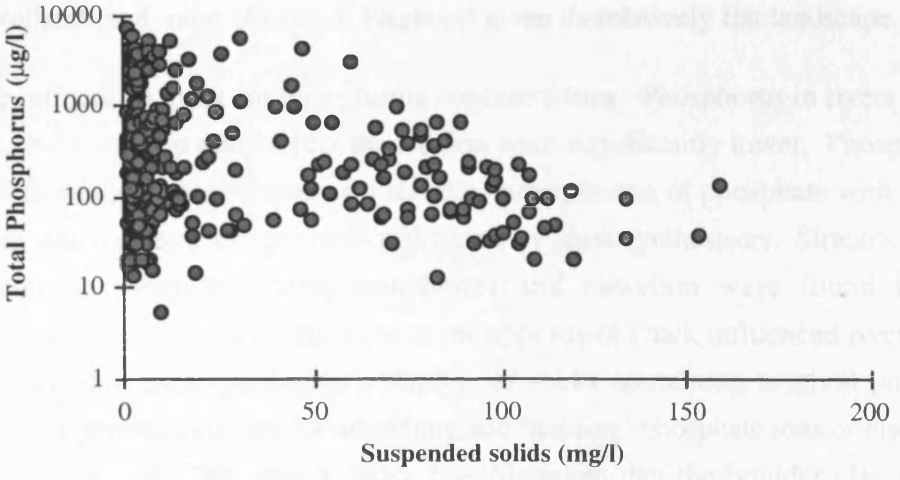


Figure 4.37 TP against suspended solids (n=387)

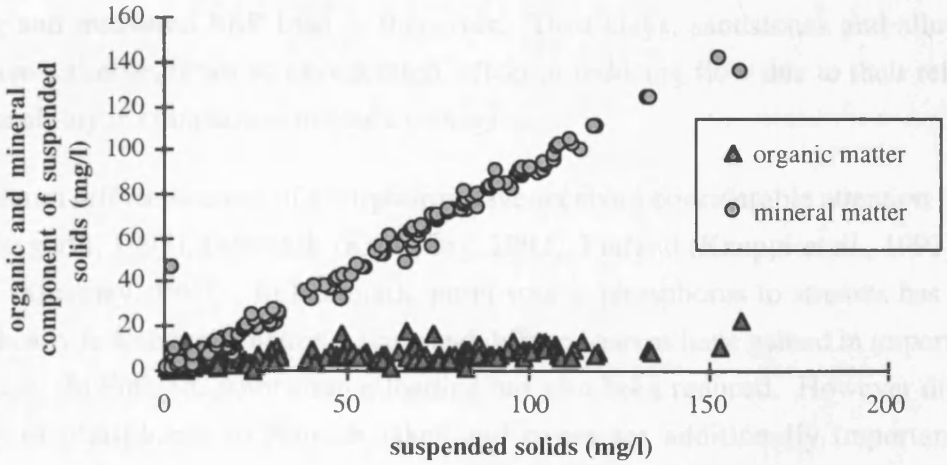


Figure 4.38 Organic and mineral component of suspended solids (n=387)

4.5 Discussion

Background phosphorus levels were calculated for sites at the upper sections of three Welland tributaries; the annual mean SRP concentration for these four sites was 35 µg l^{-1}. This phosphorus concentration is possibly the nearest estimate of 'natural' instream concentrations given that these sites were surrounded by hilly pasture and not undisturbed woodland (the presumed natural state). Meybeck (1982) estimated that the average phosphorus concentration of unpolluted rivers world-wide is 10 µg l^{-1} (soluble reactive phosphorus) or 25 µg l^{-1} (total dissolved phosphorus) which approximates the background concentration calculated for the upper Welland. Furthermore, the background load value of 3.26 kg km^{-2} ann-1 is very low compared to the export values calculated by Dillon and Kirchner (1975) in Southern Ontario; 2.7 kg km^{-2} ann^{-1} for a forested, igneous catchment area to 19.3 kg km^{-2} ann^{-1} for a similar catchment impacted by sewage effluent. A low export value may however be anticipated for the upper Welland (and most of eastern England) given its relatively flat landscape.

Geology influenced instream phosphorus concentrations. Phosphorus in rivers flowing over the chalk outcrop that bisects the Region were significantly lower. Phosphorus is removed from the water column as a result of precipitation of phosphate with calcium carbonate which effectively prevents utilization by photosynthesisers. Streams flowing over geologies such as clays, sandstones and alluvium were found to have approximately twice the concentration of phosphorus of chalk influenced rivers. This may suggest that these geologies comprise of rocks containing original phosphate mineral, have greater capacity for adsorbing and 'holding' phosphate ions or else have a hydrological effect. The latter is quite feasible given that the boulder clay bedrock underlying the River Deben presented an impermeable barrier reducing stream recharge from groundwater reserves. This, in combination with abstraction and low rainfall,

was considered the underlying cause of discrepancies between predicted effluent loading and measured SRP load in this river. Thus clays, sandstones and alluvium may have a similar, if not so exaggerated, effect in reducing flow due to their relative impermeability in comparison to chalk geologies.

Agricultural diffuse sources of phosphorus have received considerable attention in the UK (Haygarth, 1997), Denmark (Kronvang, 1992), Finland (Kauppi *et al.*, 1993) and the US (Sharpley, 1997). In Denmark, point source phosphorus to streams has been methodically reduced on a national scale and diffuse sources have gained in importance as a result. In Finland, point source loading has also been reduced. However diffuse sources of phosphorus to Finnish lakes and rivers are additionally important for geological and climatic reasons. Lake and river waters are soft due to granite bedrock and have naturally low nutrient concentrations. Lakes, in particular, are sensitive to diffuse inputs of nutrient in lowland agricultural areas due to their low buffering capacity and long periods of ice cover. Diffuse sources unquestionably contribute to phosphorus loading of UK streams. However even in an area such as East Anglia which has considerable agricultural activity, effluent sources of phosphorus were most significant.

Elevated phosphorus levels in rivers are the result of 'overloading' such that biological and chemical uptake processes are overwhelmed. Evidence for this comes from the capacity for gradual recovery and reduction in phosphorus concentration downstream of a point source. The amount of phosphorus removed instream is a function of sediment composition (Fox *et al.*, 1989) and diversity and size of the photosynthesizing community (McColl, 1974; Elosegui *et al.*, 1995). Several small headwater streams were as severely impacted by effluent inputs as any of the larger rivers. Phosphorus concentrations in first order streams were elevated by effluents from small village sewage treatment works. For example, the mean annual 1995 SRP concentration at Stockerston (Eye Brook), Horninghold (Medbourne Brook) and Sibbertoft (in the headwaters of the Welland) was 149, 320 and 2190 $\mu g\ l^{-1}$ respectively. This compares to 412, 1053 and 2088 $\mu g\ l^{-1}$ mean SRP measured at Great Ryburgh (River Wensum), Bodney (River Wissey) and Ashley (River Welland). Effluent impact is therefore a size-related phenomenon, nutrient enrichment is not just applicable to high order rivers.

CHAPTER 5 BIOLOGICAL RELATIONSHIP BETWEEN PHOSPHORUS AND LOTIC DIATOM SPECIES

5.1 Introduction

The aim of this chapter is to identify changes in diatom species assemblages with the phosphorus levels encountered in lowland streams. Diatom samples were collected synchronously with water samples for phosphorus determination during 1995 and 1996. Diatom collection was temporally more intensive within Welland catchment streams. Multivariate analyses were applied to epilithic diatom assemblages to examine differences in community composition in relation to phosphorus. Species diversity was compared using the Shannon index of diversity. Two diatom samples at each site were taken during 1995 collection. Diatoms were removed from stone and *Cladophora* filaments to examine the comparability of diatom communities found on each.

Variations in diatom community have been used to indicate levels of river quality. This taxonomic group, in comparison with other stream organisms, has several key qualities which makes it a suitable group to use for river monitoring. Diatoms are ubiquitous within any river and have considerable density per unit area, increasing the accuracy of random collection and counting. Unlike invertebrates they do not have specialised physical habitat niches neither are they influenced as extensively by stream flow (Round, 1993). Their life cycle is rapid and thus reaction to changes in stream water are swift. Higher plants do not have this fast response time. Epilithic diatoms remove inorganic nutrients from the water column only. Nutrient uptake by some higher plants can be from the sediments as well as from the water column. In practical terms, the silicon cell walls used for identification are robust and resistant to damage incurred during collection. Mounted samples can be kept as a permanent record.

Indices and zoning systems have been compiled to interpret pollution levels in terms of diatom community. Descy (1979) produced the 'Indice Diatomique' (Id) to relate diatom assemblage to organic pollution. This was the first index to be based on the Zelinka and Marvan equation published in 1961 (Coste *et al.*, 1991). This formula includes the number of different species plus two further values or ratings; a 'sensitivity index' or ecological tolerance of a species and an 'indicator value' or estimate of each species "ecological amplitude towards pollution" (Descy, 1979). The use of this equation has subsequently been adopted by other workers. It is the scale of species sensitivity based on the author's individual experience that differentiates one index from another. The autecological response of species to pollution is determined by reference to literature and ordination of a worker's own diatom data.

Diatom indices have been used more intensively in continental Europe. Three indices were developed for use in the Artois-Picardie region of France; two are indices based on species and the other is a generic index. These are the Specific Pollution Sensitivity Index (or SPI) compiled by Coste (1982, cited in Prygiel and Coste, 1993), the Commission for Economical Community index or CEC compiled by Descy and Coste (1988, cited in Coste *et al.*, 1991) and the Generic Diatom Index (GDI)(Rumeau and Coste, 1988). The routine biotic method employed throughout France to compliment chemical analysis is macroinvertebrate survey. An alternative method for monitoring water quality within the Artois-Picardie catchment was sought because the river system was considered too disturbed by navigation, dredging and development to enable reliable use of macroinvertebrate sampling. Furthermore, canalisation had increased depth to an extent that net sampling was difficult (Prygiel and Coste, 1993).

The SPI and GDI, like Descy's 'Indice Diatomique', are based on the Zelinka and Marvan equation, the GDI having evolved as a simpler version of the SPI. The number of taxa allocated sensitivity and indicator values for the SPI and GDI are 2035 and 44 species respectively. The CEC was introduced at the outset to compliment the SPI and is based on an index calculated from 208 taxa divided into a double-entry table (Coste *et al.*, 1991). Whereas the GDI is intended for routine monitoring, the other two are used for specific site appraisal. The SPI requires exacting knowledge of diatom taxonomy and is time consuming. These three indices were explicitly developed to assess levels of organic pollution. Prygiel and Coste (1993) in their assessment of six indices considered the above three to correlate well with parameters relating not only to organic pollution but also eutrophication. The GDI faired particularly well and was in their opinion able to estimate water quality reliably given its use of genus as opposed to species.

Zoning systems for water quality classification of river stretches have also been developed. Steinberg and Schiefele (1988) developed a zoning system (S&S) to assess eutrophication of streams and rivers. The zones range from nutrient poor (trophic state I) to heavily polluted sites (trophic state III) with intermediate sites described, for example, as II or II-III. A zone is assigned to a site on the basis of defined assemblages of species as proposed by the authors. Round (1993) also put forward a zoning system to classify diatom communities according to nutrient enrichment in British rivers. This consists of five zones with a number of key species characteristic of each zone. Cox (1995) considered zoning had "too few discriminatory species to be generally applicable". The GDI and SPI, for example, have scales of 1-20 which offer finer resolution. Kelly and Whitton (1995) also found that the zoning method of Steinberg and Scheifele was limited by lack of sensitivity on testing in UK rivers.

Scheifele and Kohman (1993) produced the trophic diatom index (TDI) as a refinement of the zoning system developed by Steinberg and Schiefele (1988) in German rivers. This again is based on the weighted average equation of Zelinka and Marvan (1961) and considers inorganic nutrient pollution separately from organic pollution. The performance of this index was assessed alongside the GDI, SPI and the zoning method of Round (1993) by Kelly *et al.* (1995). The GDI showed the highest correlation and sensitivity to phosphorus concentrations of all the indices tested.

Subsequent to assessing the performance of these indices, Kelly (1996) developed the Diatom Quality Index (DQI) for use in UK rivers. This index primarily correlates diatom assemblage to trophic status (measured as SRP). The extent of organic pollution is also given some consideration within this index such that the proportion of a diatom count composed of species tolerant to organic pollution infers the likely extent of this form of pollution on a percentage scale.

The diatom data collected during this research were interpreted using the DQI. There are two main reasons why this particular index was used instead of the GDI or TDI. Firstly, besides the zoning method of Round (1993) there is no other UK orientated index. Diatom communities and taxa are similar throughout Europe (Round, 1991) however climate, geology and altitude are not. The potential for influence of these large scale factors at stream reach scale can not be ruled out. Secondly, the DQI was developed after consideration of the shortcomings of using the GDI and TDI in UK rivers.

5.2 Methodology

5.2.1 Sample collection

Samples for diatom analysis were taken from fifteen river sites across the Anglian Region at the same time as water samples for phosphorus analysis during May to October 1995 (Chapter 4). A total of forty nine diatom samples were taken; thirty two from streams within the Welland catchment and seventeen from the Bure, Wensum, Little Ouse, Wissey, Deben, Great Eau and Waithe Beck. Diatom collection continued in 1996 between the period June to October from streams within the Welland catchment only. A total of thirty four samples were taken for analysis from nine sites. Table 5.1 details all sites and collection dates for 1995 and 1996. Biofilm was removed from five different stones at each site using a toothbrush and was rinsed into a 500ml bottle using distilled water. Cross contamination between sites was avoided by using separate toothbrushes. During 1995 sampling, small strands of *Cladophora* were also collected from several clumps at each site and rinsed into 500ml bottles using distilled water.

Table 5.1 Dates of diatom sampling and sample origin

Regional streams 1995

River	Site	Date	Substrate
Bure	Ingworth Br	24/7/95	stone
Bure	Moor Hall Ford	24/7/95	stone
Deben	Ufford Bridge	25/7/95	*Cladophora*
Deben	Ufford Bridge	30/8/95	*Cladophora*
Deben	Ufford Bridge	30/8/95	stone
Deben	Ufford Bridge	26/9/95	stone
Great Eau	Withern Bridge	11/7/95	*Cladophora*
Great Eau	Withern Bridge	8/8/95	*Cladophora*
Little Ouse	Knettishall Br	25/7/95	*Cladophora*
Waithe Beck	Thorganby Br	11/7/95	*Cladophora*
Waithe Beck	Thorganby Br	8/8/95	stone
Waithe Beck	Thorganby Br	11/9/95	stone
Wensum	Sculthorpe Mill	13/7/95	*Cladophora*
Wensum	Sculthorpe Mill	13/7/95	stone
Wissey	N. Pickenham	13/7/95	*Cladophora*
Wissey	N. Pickenham	9/8/95	*Cladophora*
Wissey	N. Pickenham	9/8/95	stone

upper Welland catchment streams 1995

River	Site	Date	Substrate
Eye	Skeffington	31/7/95	*Cladophora*
Eye	Skeffington	31/7/95	stone
Eye	Stockerston	31/7/95	*Cladophora*
Eye	Stockerston	31/7/95	stone
Eye	Stockerston	29/8/95	*Cladophora*
Eye	Tugby Wood	29/8/95	*Cladophora*
Eye	Skeffington	4/9/95	*Cladophora*
Eye	Stockerston	4/9/95	*Cladophora*
Eye	Skeffington	18/9/95	stone
Eye	Tugby Wood	25/9/95	*Cladophora*
Eye	Tugby Wood	25/9/95	stone
Langton	Gauging St	19/7/95	*Cladophora*
Langton	Gauging St	1/8/95	stone
Langton	Gauging St	1/8/95	*Cladophora*
Medbourne	Green Lane	31/7/95	*Cladophora*
Medbourne	Green Lane	31/7/95	stone
Medbourne	Green Lane	4/9/95	*Cladophora*
Medbourne	Green Lane	18/9/95	*Cladophora*
Medbourne	Green Lane	16/10/95	*Cladophora*
Stonton	Thorpe L'ton	10/5/95	stone
Stonton	Thorpe L'ton	23/5/95	*Cladophora*
Stonton	Thorpe L'ton	1/8/95	*Cladophora*
Stonton	Thorpe L'ton	1/8/95	stone
Stonton	Thorpe L'ton	19/9/95	*Cladophora*
Stonton	Thorpe L'ton	17/10/95	*Cladophora*
Stonton	Thorpe L'ton	17/10/95	stone
Welland	Lubenham	1/8/95	*Cladophora*
Welland	Lubenham	1/8/95	stone
Welland	Lubenham	5/9/95	*Cladophora*
Welland	Lubenham	5/9/95	stone
Welland	Lubenham	19/9/95	*Cladophora*
Welland	Lubenham	17/10/95	*Cladophora*

upper Welland catchment streams 1996

River	Site	Date	Substrate
Eye	GlebeFarm	21/6/96	stone
Eye	Glebe Farm	9/7/96	stone
Eye	Glebe Farm	25/7/96	stone
Eye	Glebe Farm	29/8/96	stone
Eye	Glebe Farm	16/9/96	stone
Eye	Glebe Farm	8/10/96	stone
Eye	Skeffington	9/7/96	stone
Eye	Skeffington	13/8/96	stone
Eye	Skeffington	16/9/96	stone
Langton	Gauging St	9/7/96	stone
Langton	Gauging St	13/8/96	stone
Langton	Gauging St	16/9/96	stone
Langton	Smeeton Westerby	21/6/96	stone
Langton	Smeeton Westerby	9/7/96	stone
Langton	Smeeton Westerby	13/8/96	stone
Langton	Smeeton Westerby	29/8/96	stone
Langton	Smeeton Westerby	16/9/96	stone
Langton	Smeeton Westerby	8/10/96	stone
Medbourne	Green Lane	9/7/96	stone
Medbourne	Green Lane	13/8/96	stone
Medbourne	Green Lane	16/9/96	stone
Stonton	Noseley	9/7/96	stone
Stonton	Noseley	13/8/96	stone
Stonton	Noseley	16/9/96	stone
Welland	Hothorpe	9/7/96	stone
Welland	Hothorpe	13/8/96	stone
Welland	Hothorpe	16/9/96	stone
Welland	Ft Br Green Lane	9/7/96	stone
Welland	Ft Br Green Lane	13/8/96	stone
Welland	Ft Br Green Lane	29/8/96	stone
Welland	Ft Br Green Lane	16/9/96	stone
Welland	Ft Br Green Lane	8/10/96	stone
Welland	Great Bowden	9/7/96	stone
Welland	Great Bowden	16/9/96	stone

5.2.2 Sample preparation

Diatom species are identified by structural differences in their siliceous skeletons. Samples were treated with hot acid to remove organic matter from the diatom cells and destroy other biofilm-associated organisms leaving behind only these silica structures. Diatom samples were digested using the method of Round (1993), as follows:-

Stone samples were allowed to settle and approximately 10ml subsamples extracted for gentle centrifuging to further concentrate the collected material. Vigorous centrifuging was found to cause damage to diatom frustules (silica skeletons). Approximately 0.5ml of concentrated mixture was placed into a beaker. Samples of *Cladophora* were placed directly into beakers for acid digestion. The process of acid digestion then proceeded in the same way for both stone and *Cladophora* samples. An equal volume (2ml) of potassium permanganate and concentrated hydrochloric acid was added to the samples. The beakers were covered with *Nescofilm* (Nippon Shoji Kaisha Ltd.) and placed on a hot plate at 60 $^{\circ}$C until the liquid turned pale yellow and almost translucent. After cooling, the supernatant was removed and distilled water added. This mixture was agitated to remove traces of acid. The sample was then gently centrifuged. At this point the settled material consisted of very fine grey particles. A fraction of this material was drawn off and placed on a cover slip. This was allowed to dry and the cover slip inverted onto *Naphrax*, high resolution mounting medium (Northern Biological Supplies Ltd.). It was preferable to allow the sample to dry on the cover slip as opposed to the glass slide. In this way, the frustules are viewed directly and not through the mountant. Prior to microscope viewing, the *Naphrax* was left to harden on a hot-plate at approximately 40 °C for several hours. Contamination of samples with silt and inorganic fragments was sometimes experienced due to the poor quality of stone substrate found at some sites. Microscopic examination of epilithic samples was necessary prior to final slide preparation to check that diatoms were present in reasonable quantity or to dilute those samples that were too dense.

5.2.3 Diatom identification

Slides were viewed on a *Zeiss Axiovert 100* inverted microscope using a x10 eyepiece and x1000 oil immersion objective. Diatom floras used for identification were Hustedt (1930), Patrick and Reimer (1966, 1975), Belcher and Swale (1976), Barber and Haworth (1981), Krammer and Lange-Bertalot (1988, 1991a,b, 1997) and Kelly (1998b).

The diatom nomenclature of Krammer and Lange-Bertalot (1988, 1991a,b, 1997) was used except in reference to certain species of the genus *Synedra*, *Fragilaria*, *Cymbella*

and *Achnanthes*. Table 5.2 details these revisions. All diatom species identified are listed in Appendix 3.

Table 5.2 Revision of nomenclature used by Krammer and Lange-Bertalot (1991a,b, 1997)

Nomenclature of Krammer and Lange-Bertalot (1991a,b, 1997)	Nomenclature used in this research	Reference for revision
Fragilaria pulchella	*Ctenophora pulchella*	Willams & Round (1987)
Fragilaria capucina var. *vaucheriae*	*Fragilaria vaucheriae*	Round & Willams (1992)
Fragilaria construens	*Staurosira construens*	Round & Willams (1992)
Fragilaria leptostauron	*Staurosirella leptostauron*	Round & Willams (1992)
Fragilaria acus	*Synedra acus*	Round & Willams (1992)
Fragilaria parasitica	*Synedra parasitica*	Round & Willams (1992)
Fragilaria ulna	*Synedra ulna*	Round & Willams (1992)
Fragilaria fasisculata	*Tabularia tabulata*	Round & Willams (1992)
Cymbella caespitosa	*Encyonema caespitosum*	Round *et al.* (1990)
Cymbella prostrata	*Encyonema prostratum*	Round *et al.* (1990)
Cymbella minuta	*Encyonema minutum*	Round *et al.* (1990)
Cymbella silesiaca	*Encyonema silesiacum*	Round *et al.* (1990)
Cymbella sinuata	*Reimeria sinuata*	Kociolek & Stoermer (1987)
Achnanthes delicatula	*Achnanthidium delicatulum*	Czarnecki, D.B. (1994)
Achnanthes lanceolata	*Achnanthidium lanceolatum*	Czarnecki, D.B. (1994)
Achnanthes minutissima	*Achnanthidium minutissimum*	Czarnecki, D.B. (1994)

5.2.4 Methods for analysis

Diatom species diversity was compared between samples using the Shannon diversity index (Magurran, 1988). This index takes into account the number of species, their abundance and the 'evenness' of species abundance within a sample.

TWINSPAN and DECORANA (Hill, 1994) analyses were performed to examine phytosociological groupings of species and the potential factors affecting such groupings independently of the above indices. The Braun-Blanquet percentage scale used for TWINSPAN designated pseudospecies cut levels as follows:

1 0-4%
2 5-25%
3 26-50%
4 51-75%
5 76-100%

This scale was chosen because it takes account of species found at very low abundance, as it was observed that many species occur at low frequency such as *Gyrosigma* and large members of the genera *Nitzschia* and *Cymbella*. Over 50% of most samples were made up of three to four species.

The diatom quality index (DQI) (Kelly, 1996a) uses diatom species composition to indicate phosphorus concentration within a stretch of river or stream. Kelly established relationships between ambient phosphorus levels and 86 diatom taxa in British rivers. Information derived about the range of phosphorus concentration favoured by each taxon provided the basis for determining 'sensitivity' and 'indicator' values. The DQI is computed using values for these two factors in addition to species number and abundance. The equation is based upon the weighted average equation of Zelinka and Marvan (1961):

$$index = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j} \qquad \text{equation 1}$$

where a_j is the abundance (proportion) of species j in the sample, v_j is the indicator value and i_j the pollution sensitivity of species j. The indicator value is a measure on the scale of 1 to 3 of the strength of relationship between taxa and species abundance. The sensitivity value relates to the level of phosphorus concentration at which the species is most abundant. This value is assigned on a scale of 1 to 5.

The DQI requires the identification and enumeration of at least 200 diatom valves. Trophic state is indicated by a value between 1 and 100, with 1 indicating very high phosphorus concentrations and 100 very low concentrations. Equation 1 above was applied to the diatom assemblages for each sample. The value is referred to as the 'weighted mean sensitivity' (WMS). A DQI value for each sample was therefore calculated from:

$$DQI = 100 - [(WMS \times 25) - 25]$$

The extent of organic pollution is also evaluated. Diatom species tolerant of organic pollution are *Gomphonema parvulum*, *Navicula gregaria*, *Navicula lanceolata*, small species of *Navicula* and *Sellaphora* (<12 μm), *Nitzschia palea*, *Tryblionella* and *Psammodictyon* (Kelly, 1996a). The proportion of a diatom count composed of these tolerant species infers the likely extent of organic pollution on a four point scale:

<20% - free of significant organic pollution
21-40% - some evidence of organic pollution
41-60% - organic pollution likely to contribute significantly to eutrophication of site
>61% - site is heavily contaminated with organic pollution

5.3 Results

5.3.1 Multivariate analysis

Figure 5.1 illustrates the results of TWINSPAN analysis of 1996 data. The 1996 diatom dataset was used since the range of SRP results for this years sampling was broader with a greater number of average SRP values below 100 $\mu g\ l^{-1}$. The first division essentially split the 34 samples into those sites with SRP concentrations above 50 $\mu g\ l^{-1}$ (25) and those below 50 $\mu g\ l^{-1}$ SRP (9). The exception was a sample classified as a borderline positive which was included as one of the nine low SRP samples. The ambient SRP concentration for this sample was 280 $\mu g\ l^{-1}$.

TWINSPAN could discern no indicator species for the negative arm of the first division. Examination of the data, however, showed that *Cocconeis pediculus* was present in 18 of the 25 samples at 5% abundance and above. This species was only present in one sample out of the nine on the positive arm and at less than 4% abundance within that particular sample. *Diatoma vulgare* could also qualify as an indicator species. This species was found in 14 of the 25 samples compared to 1 out of nine on the positive arm.

The division between groups I and II was relatively weak with an eigenvalue of 0.167. All of the samples on the negative arm had over 76% abundance of *Rhoicosphenia abbreviata*. The samples on the positive arm were characterised by the species *Achnanthidium lanceolata* (5 to 25%), *Nitzschia dissipata* (0 to 4%), *Melosira varians* (0 to 4%) and over 76% *Amphora pediculus*. The average SRP concentration for group I and II was 1505 and 1351 $\mu g\ l^{-1}$ respectively.

The only indicator species for the lower SRP samples was *Achnanthidium minutissimum* at a level of abundance of 51 to 75%. Examination of the data also indicated that high abundances (over 26%) of *Navicula tripunctata* were only found in samples on this side of the dichotomy.

The eigenvalue for the division between groups III and IV was 0.277 and indicates greater differentiation between these two groups in contrast to groups I and II. The average SRP concentration for group III was 84 $\mu g\ l^{-1}$. This average included the 280 $\mu g\ l^{-1}$ value measured for the sample that TWINSPAN suggested as borderline. Excluding this value, the average SRP concentration would be 19 $\mu g\ l^{-1}$. The five samples in group IV were taken from just one site, Glebe Farm on Eye Brook. This site averaged an SRP concentration of 2.6 $\mu g\ l^{-1}$ for days on which samples were collected.

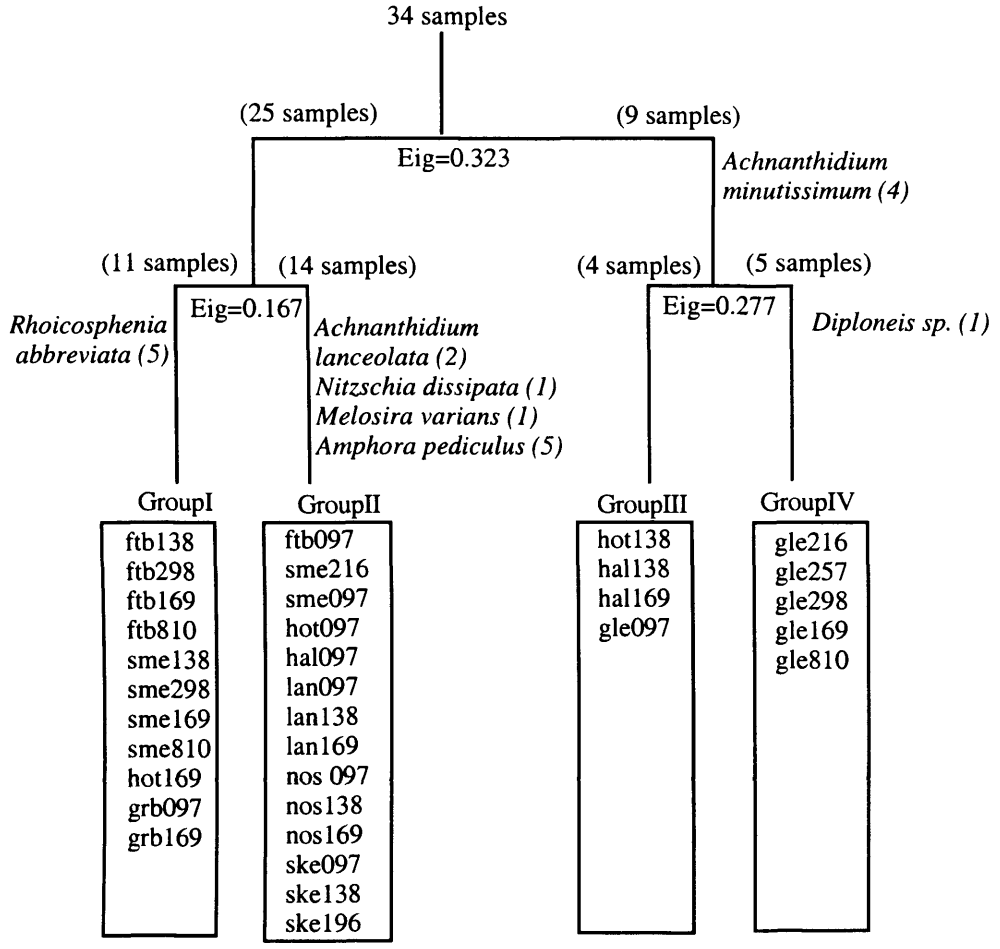


Figure 5.1 TWINSPAN (Hill, 1994) dendrogram of 1996 diatom samples. The indicator species at each division are shown in decreasing order of significance. Pseudospecies cut levels are denoted by brackets. Numbers after the site codes are dates, referring only to the day and month (all samples were collected in 1996). Table 5.1 details the complete list of sites and dates.

Code	Site name	Code	Site name
grb	Great Bowden (Welland)	ftb	Foot Bridge Green Lane (Welland)
hal	Green Lane (Medbourne)	hot	Hothorpe (Welland)
gle	Glebe Farm (Eye)	sme	Smeeton Westerby (Langton)
ske	Skeffington (Eye)	lan	Gauging Station (Langton)
nos	Noseley (Stonton)		

Ordination of the same dataset was performed using DECORANA (Figure 5.2). Groups I to IV, as indicated by TWINSPAN, are enclosed by a line. The positioning of groups III and IV towards the right of the ordination suggests that axis 1 is most probably ambient phosphorus levels with the scale on this axis corresponding to decreasing phosphorus concentration. The eigenvalue for axis 2 is half that of axis 1 but suggests that groups I and II are spatially differentiated, if only weakly, along this axis.

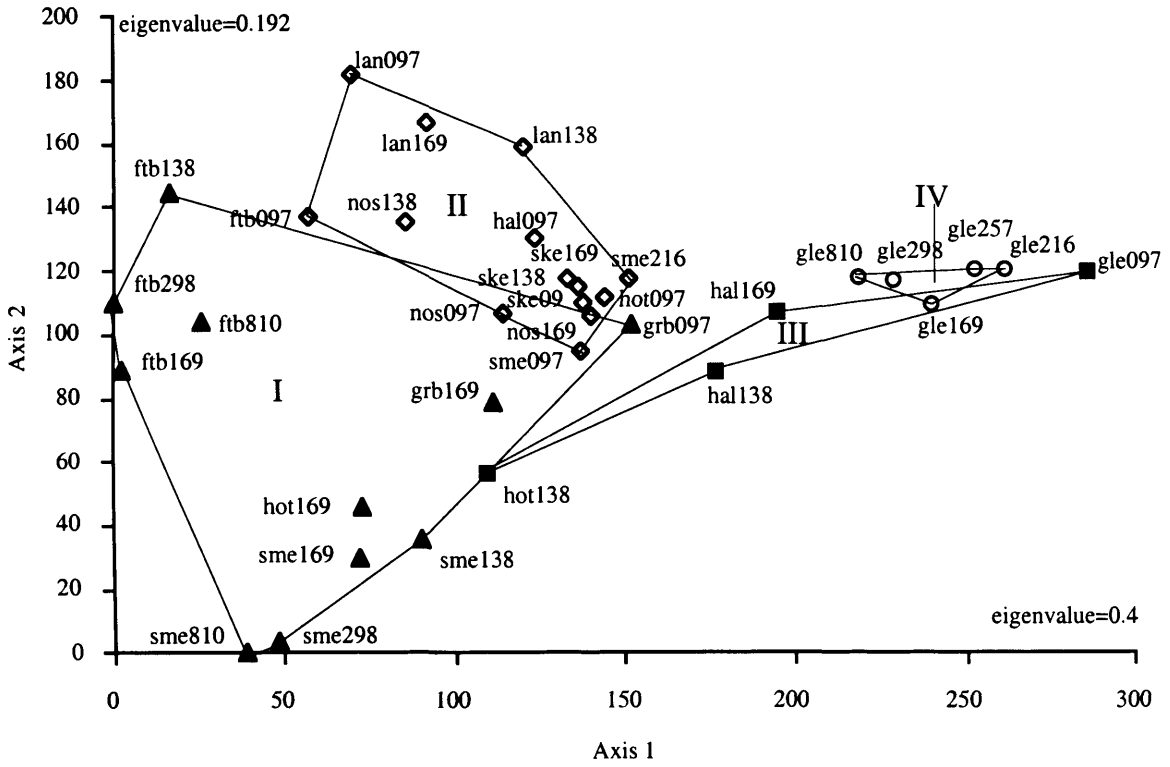


Figure 5.2 Interpretation of 1996 diatom samples using DECORANA (Hill, 1994). Numbers after the site codes are dates, referring only to the day and month (all samples were collected in 1996). Table 5.1 details the complete list of sites and dates.

Code	Site name	Code	Site name
grb	Great Bowden (Welland)	ftb	Foot Bridge Green Lane (Welland)
hal	Green Lane (Medbourne)	hot	Hothorpe (Welland)
gle	Glebe Farm (Eye)	sme	Smeeton Westerby (Langton)
ske	Skeffington (Eye)	lan	Gauging Station (Langton)
nos	Noseley (Stonton)		

The site groups produced by TWINSPAN analysis clearly differentiated between sites of high (>50 $\mu g\ l^{-1}$) and low (<50 $\mu g\ l^{-1}$) SRP concentration. Average SRP values for groups I and II was 1505 and 1351 $\mu g\ l^{-1}$ SRP respectively suggesting that differences in the concentration of phosphorus was unlikely to be the influencing factor in separating sites into these two groups. Furthermore, groups I and II occupy a similar position in relation to Axis 1 (Figure 5.2). Water velocity however may have been the determining factor affecting this division. Samples in group I mostly come from Smeeton Westerby on Langton Brook and the Footbridge on the Welland. These two sites had the highest average stream velocities of all the sites sampled in 1996. The indicator species for this group was *Rhoicosphenia abbreviata*; a diatom recognised more as an epiphyte on *Cladophora* than an epilithic colonizer of stone. This species attaches to substrate via a mucilaginous stalk. The high percentage abundance (over 76%) of this diatom in group I samples may be associated with the observed lush growths of *Cladophora* at these sites. *Cladophora* favours higher stream velocity and as a consequence may have supported a rich colony of epiphytes. This colony, in turn,

may have extended the areas of colonization onto the stone to which the filamentous alga was attached. In contrast, three of the four indicator species for group II were unattached species that live within the biofilm. These species were *Achnanthidium lanceolatum*, *Nitzschia dissipata* and the chain forming *Melosira varians*. The fact that these species are unattached and potentially more vulnerable to high flow may support this theory. Spatial ordination of results (Figure 5.2) shows that Groups I and II are spatially differentiated along Axis 2 with only minor overlap. Axis 2 most probably relates to stream velocity with the scale corresponding to decreasing velocity (ms^{-1}).

Figure 5.2 shows group III positioned between group IV and groups I and II in relation to Axis I. This suggests that phosphorus concentration influenced the segregation of sites into groups III and IV. The position of both groups on Axis 2, however, is sufficiently close to suggest that stream velocity was similar across these sites. The indicator species diffentiating groups III and IV was *Diploneis* sp.

5.3.2 Changes in seasonal abundance

Figures 5.3 to 5.5 represent percentage abundance composition of samples taken on three dates during 1996. Species included are those with abundances consistently above 5%. These more abundant species were used since they represented on average over 90% of all diatoms within samples. The site codes are listed in increasing order of SRP concentration (denoted by the arrow). *Achnanthidium minutissimum* and *Achnanthidium lanceolatum* most clearly prefer opposing ends of the trophic spectrum on all three sampling dates. *Navicula tripunctata* is observed across all sites but is most abundant at lower phosphorus concentrations. The distribution of most species across sites suggests that other factors besides phosphorus concentration are more instrumental in determining their presence and growth.

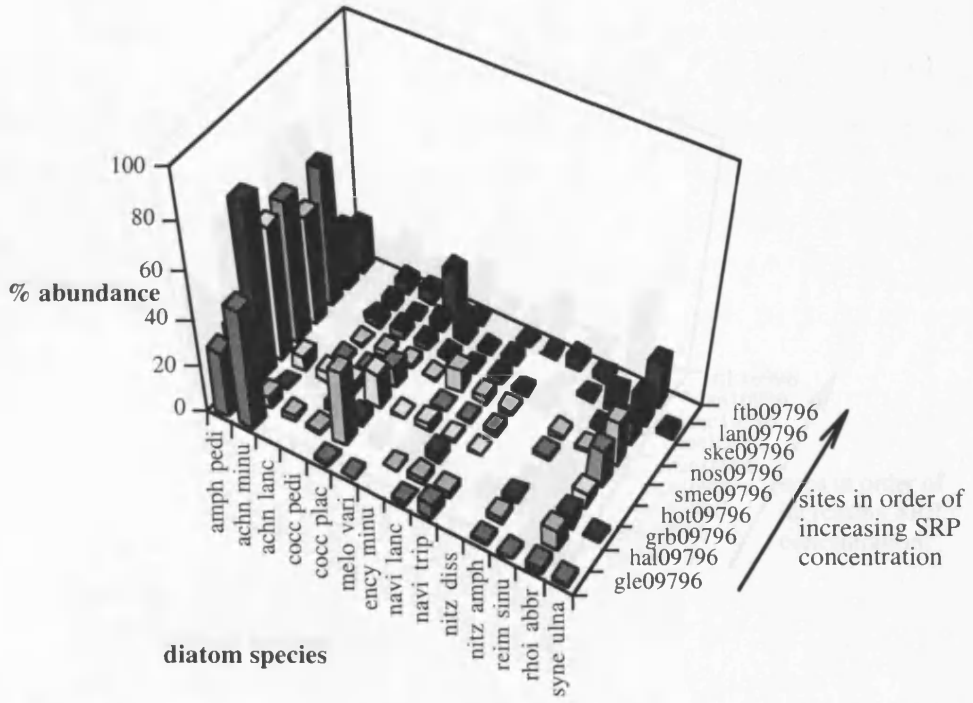


Figure 5.3 Percentage abundance of diatom species at nine sites sampled on 9.7.96. (explanation of site and species code can be found overleaf)

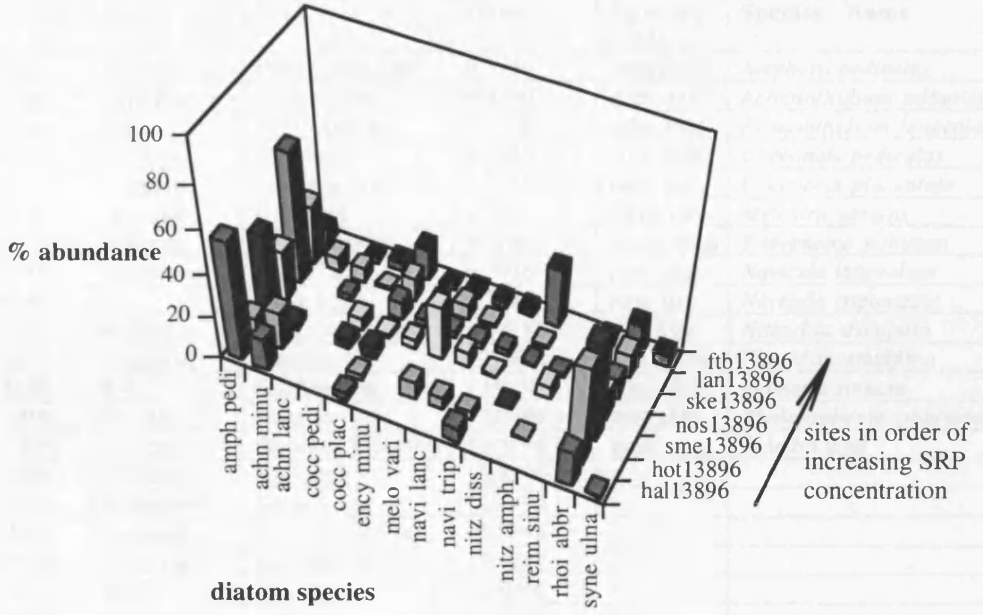


Figure 5.4 Percentage abundance of diatom species at seven sites sampled on 13.8.96. (explanation of site and species code can be found overleaf)

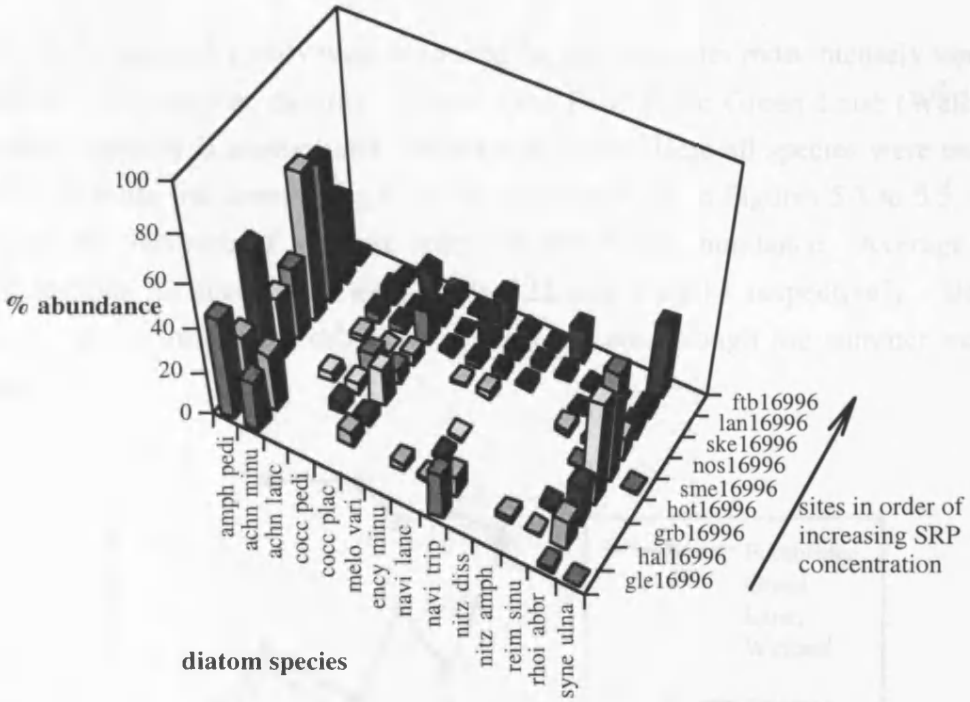


Figure 5.5 Percentage abundance of diatom species at nine sites sampled on 16.9.96. (explanation of site and species code can be found below)

Site Code	River	Site	Date		Species Code	Species Name
ftb09796	Welland	Ft Br Green Lane	9/7/96		amph pedi	*Amphora pediculus*
lan09796	Langton	Gauging St	9/7/96		achn minu	*Achnanthidium minutissimum*
ske09796	Eye	Skeffington	9/7/96		achn lanc	*Achnanthidium lanceolatum*
nos09796	Stonton	Noseley	9/7/96		cocc pedi	*Cocconeis pediculus*
sme09796	Langton	Smeeton Westerby	9/7/96		cocc plac	*Cocconeis placentula*
hot09796	Welland	Hothorpe	9/7/96		melo vari	*Melosira varians*
grb09796	Welland	Great Bowden	9/7/96		cymb minu	*Encyonema minutum*
hal09796	Medbourne	Green Lane	9/7/96		navi lanc	*Navicula lanceolata*
gle09796	Eye	Glebe Farm	9/7/96		navi trip	*Navicula tripunctata*
ftb13896	Welland	Ft Br Green Lane	13/8/96		nitz diss	*Nitzschia dissipata*
lan13896	Langton	Gauging St	13/8/96		nitz amph	Nitzschia amphibia
ske13896	Eye	Skeffington	13/8/96		reim sinu	*Reimeria sinuata*
nos13896	Stonton	Noseley	13/8/96		rhoi abbr	*Rhoicosphenia abbreviata*
sme13896	Langton	Smeeton Westerby	13/8/96		syne ulna	*Synedra ulna*
hot13896	Welland	Hothorpe	13/8/96			
hal13896	Medbourne	Green Lane	13/8/96			
ftb16996	Welland	Ft Br Green Lane	16/9/96			
lan16996	Langton	Gauging St	16/9/96			
ske16996	Eye	Skeffington	16/9/96			
nos16996	Stonton	Noseley	16/9/96			
sme16996	Langton	Smeeton Westerby	16/9/96			
hot16996	Welland	Hothorpe	16/9/96			
grb16996	Welland	Great Bowden	16/9/96			
hal16996	Medbourne	Green Lane	16/9/96			
gle16996	Eye	Glebe Farm	16/9/96			

Seasonal changes in diversity were examined for the three sites most intensely sampled throughout the summer months. These were Footbridge Green Lane (Welland), Smeeton Westerby (Langton) and Glebe Farm (Eye). Here all species were used as opposed to those just comprising over 5% abundance, as in Figures 5.3 to 5.5, since diversity is a measure of absolute species number and abundance. Average TRP concentrations for these sites were 2640, 322 and 4 $\mu g\ l^{-1}$ respectively. Diatom diversity at the three sites shows no distinct pattern through the summer months (Figure 5.6).

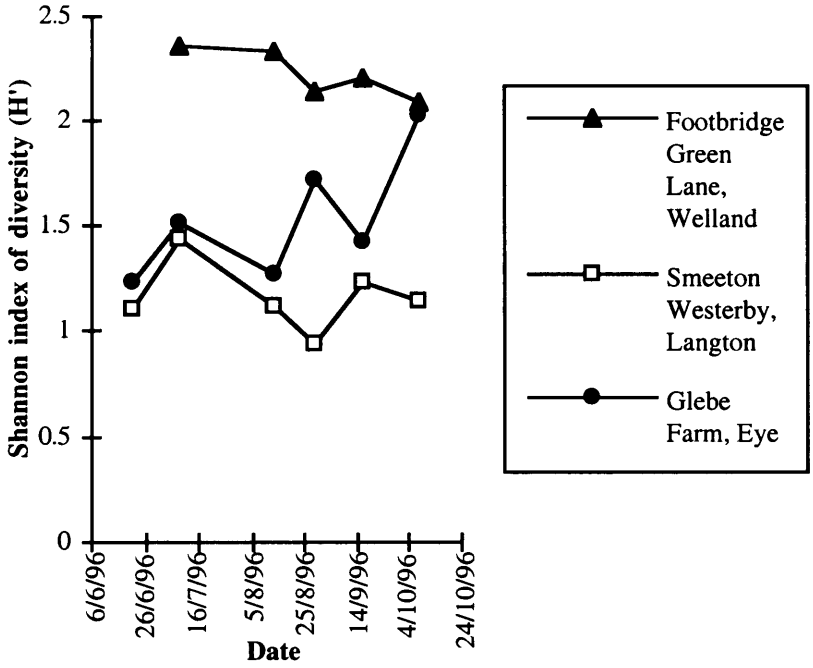


Figure 5.6 Change in species diversity at three sites during the summer months of 1996

Epilithic diatoms respond to seasonal change through shifts in species dominance and variation in biomass (Hynes, 1970; Blum, 1956). *Amphora pediculus* was noted to have a specific maxima but for the most part seasonality played a minor part in influencing assemblage. Species richness remained little altered between the months June to October although this time period did not encompass the main biomass peak in early spring and the shift to winter diatoms in the autumn.

Diversity indices for all 1996 epilithic samples were compared to SRP given that seasonal changes in diversity were not apparent. Increasing phosphorus concentrations had no impact on species diversity (Figure 5.7).

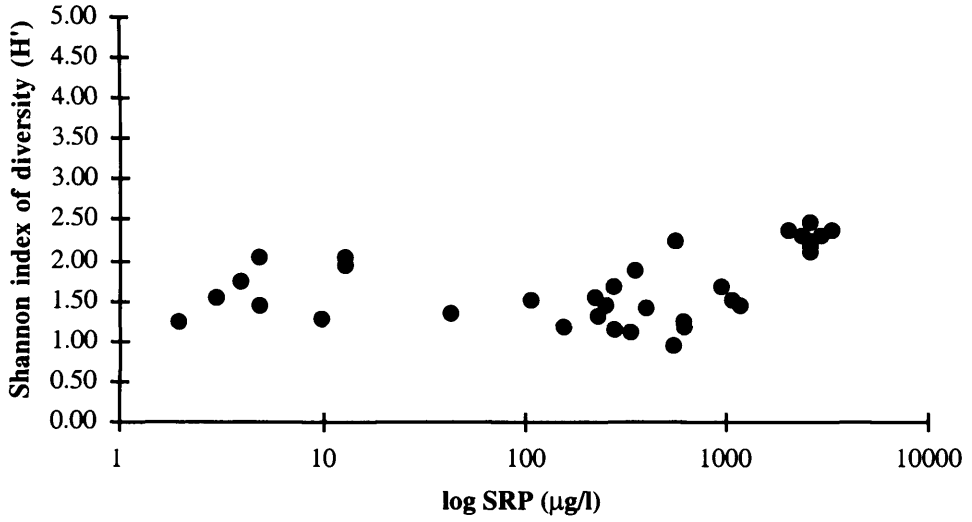


Figure 5.7 Species diversity correlated with log SRP (µg l^{-1})

5.3.3 Comparison of diatom assemblage found on stone and *Cladophora*

A difference was observed in the Shannon diversity (H') of diatom species found on stone and *Cladophora* using 1995 diatom data (Table 5.3). Statistical significance between H' values for the two substrate was found in eight of the nine pairs using Student's *t* test. Diatom floras sampled from stone were more diverse than samples taken from *Cladophora* for seven of the pairs. The opposite applied for one sample taken at Thorpe Langton on 17/10/95. The samples taken at Tugby on Eye Brook were of similar diversity.

Table 5.3 Comparison of diatom diversity on stone and *Cladophora* using the Shannon index of diversity (Magurran, 1988)

Date	Stream	Site	Substrate	Diatom count (N)	species no.(S)	H' (1)	*t* (2)	df
31/7/95	Medbourne	Green Lane	Stone	213	12	1.902	3.243	380.544
31/7/95	Medbourne	Green Lane	*Cladophora*	199	14	1.571		
31/7/95	Eye	Stockerston	Stone	197	18	2.15	4.928	393.927
31/7/95	Eye	Stockerston	*Cladophora*	223	13	1.617		
31/7/95	Eye	Skeffington	Stone	216	12	1.789	9.866	432.991
31/7/95	Eye	Skeffington	*Cladophora*	218	6	0.833		
1/8/95	Stonton	Thorpe Langton	Stone	203	20	2.066	4.389	399.901
1/8/95	Stonton	Thorpe Langton	*Cladophora*	214	14	1.525		
1/8/95	Welland	Lubenham, E. Farndon	Stone	201	18	2.34	4.328	387.985
1/8/95	Welland	Lubenham, E. Farndon	*Cladophora*	224	18	1.842		
1/8/95	Langton	Gauging Station	Stone	204	15	2.25	11.27	472.724
1/8/95	Langton	Gauging Station	*Cladophora*	278	8	1.333		
5/9/95	Welland	Lubenham, E. Farndon	Stone	232	18	2.191	8.561	443.422
5/9/95	Welland	Lubenham, E. Farndon	*Cladophora*	214	8	1.398		
25/9/95	Eye	Tugby	Stone	208	15	1.538	-0.559	411.691
25/9/96	Eye	Tugby	*Cladophora*	204	13	1.607		
17/10/95	Stonton	Thorpe Langton	Stone	218	15	1.38	-8.201	389.800
17/10/95	Stonton	Thorpe Langton	*Cladophora*	201	19	2.269		

(1) $H' = -\sum pi \ln pi$ where pi, the proportional abundance of the ith species=(n_i / N)
(2) $t_{0.05,2,\infty}$= 1.960

Substrate-associated differences in diatom assemblage were not reflected in DQI values for this paired dataset (Table 5.4). A paired *t* test demonstrated no statistical difference between the values obtained for diatom samples taken from the two substrate types.

Table 5.4 DQI values for paired 1996 diatom samples

Date	Stream	Site	DQI value for stone sample	DQI value for *Cladophora* sample
31/7/95	Medbourne	Green Lane	71.5	77.5
31/7/95	Eye	Stockerston	94.0	82.7
31/7/95	Eye	Skeffington	66.8	85.6
1/8/95	Stonton	Thorpe Langton	91.9	84.2
1/8/95	Welland	Lubenham, E. Farndon	85.7	75.3
1/8/95	Langton	Gauging Station	83.0	72.5
5/9/95	Welland	Lubenham, E. Farndon	82.7	78.1
25/9/95	Eye	Tugby	85.8	76.5
17/10/95	Stonton	Thorpe Langton	94.8	82.3

5.3.4 Comparison of Diatom Quality Index with SRP

The Diatom Quality Index for each of the diatom samples was compared against concentration of SRP μg l^{-1} (Figures 5.8 to 5.10). The 1995 DQI values alone showed no correlation (Figure 5.8). The addition of 1996 Welland catchment values in Figure 5.9 however increased the range of SRP data and the lower results (<50 μg l^{-1}) corresponded to slightly higher DQI values. A significant relationship was only found for 1996 results at sites below 1000 μg l^{-1} (r^2=0.596, p<0.001)(Figure 5.10).

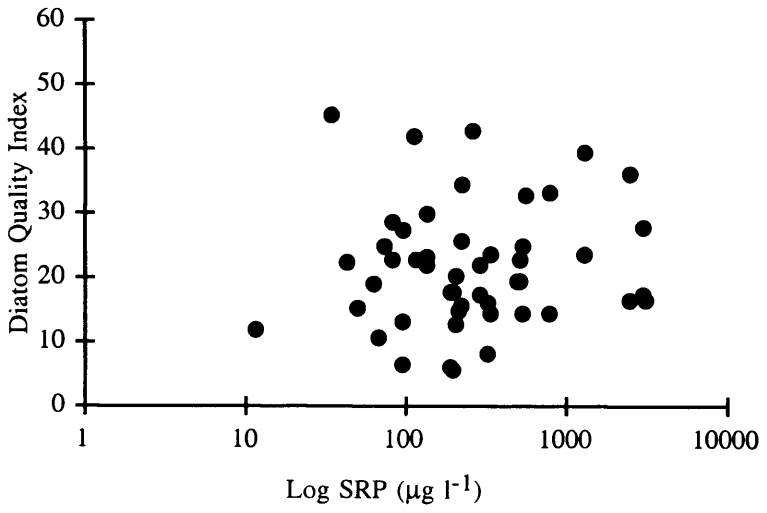


Figure 5.8 Diatom Quality Index values against log SRP for all rivers sampled in 1995

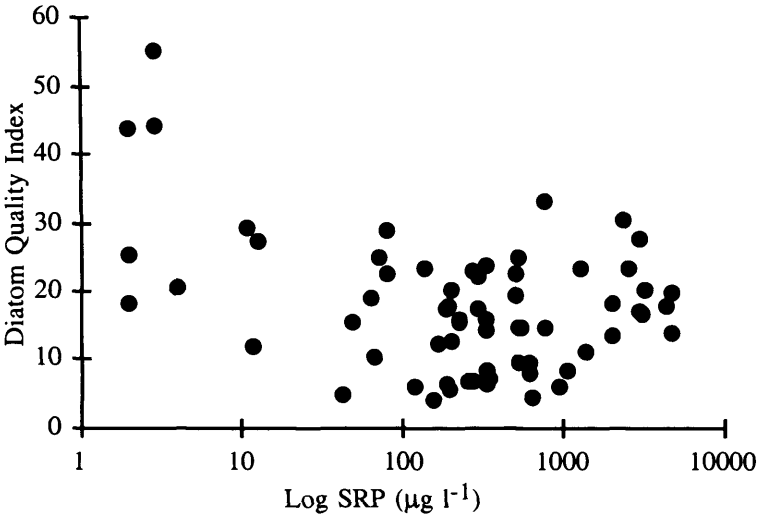


Figure 5.9 Diatom Quality Index values against log SRP for Welland catchment samples (1995-96)

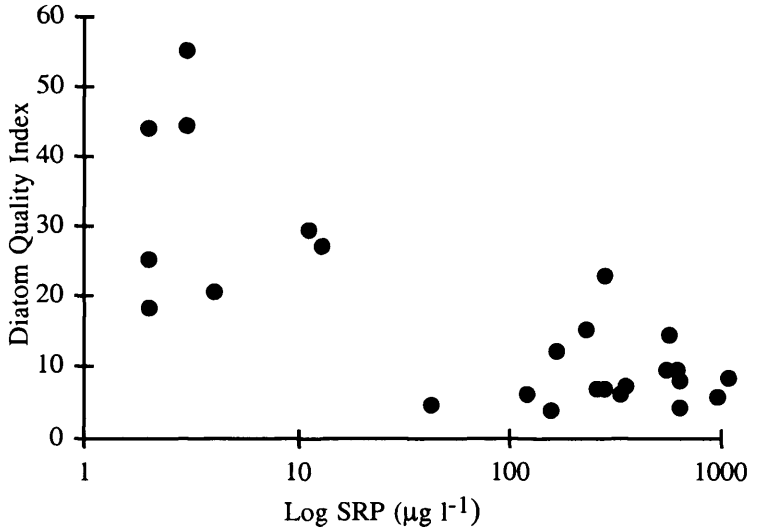


Figure 5.10 Diatom Quality Index values against log SRP up to 1000 μg l^{-1} for Welland catchment samples (1996)

All except five of the eighty three slides examined indicated that the sites were 'free of significant organic pollution'. Table 5.5 details the five sites that indicated varying degrees of contamination by organic pollution. None of the 1996 samples contained high enough abundances of designated pollution tolerant species to be included in Table 5.5.

Table 5.5 Extent of organic pollution at five sites as indicated by proportions of tolerant taxa

Site	River/stream	% of valves belonging to tolerant taxa	Indication of degree of organic pollution
Thorpe Langton Ford (10.5.95)	Stonton	64.73%	heavy contamination
Thorpe Langton Ford (23.5.95)	Stonton	40.66%	some evidence of organic pollution
Gauging Station (1.8.95)	Langton	31.37%	some evidence of organic pollution
Skeffington (18.9.95)	Eye	26.36%	some evidence of organic pollution
North Pickenham (9.8.95)	Wissey	51.10%	organic pollution likely to contribute to eutrophication

Diatom Quality Index values ranged from 3.8 to 55.2 for all samples collected in 1995 and 1996 indicating streams that are highly eutrophic to those of intermediate trophic status. The paucity of organic pollution tolerant species throughout the samples suggests that the vast majority of diatom communities sampled were subject to eutrophication and not organic pollution. This was anticipated given that none of the sites were directly downstream of a sewage effluent outfall.

5.4 Discussion

Sixty five diatom taxa were identified in samples taken in 1995 and 1996. Taxa were not evenly represented within the samples with on average 82% of all samples dominated by four species. This is consistent with the findings of other workers (Cox, 1995; Round, 1993). The domination of samples by so few species was not an artefact of phosphorus concentration which is evident from the comparison of diversity with SRP concentration (Figure 5.10).

The most decisive response of diatom species assemblage to phosphorus was above and below 50 $\mu g\ l^{-1}$ SRP concentration (assuming the sample taken at Hothorpe on 13.8.96 was aberrant). Below this concentration, species assemblage shifted in composition. The main indicators of this shift were *Achnanthidium minutissima* which dominated over 51% of low SRP sites, *Navicula tripunctata* (over 26%) and *Diploneis* sp. (0-4%). *A. minutissima* and *N. tripunctata* occurred in some samples over 50 $\mu g\ l^{-1}$ SRP but were not nearly as numerous. *Diploneis* sp. was only present at the site with the very lowest SRP concentrations. Sites with more than 50 $\mu g\ l^{-1}$ SRP concentration were characterised by the presence and abundance of five species; *Rhoicosphenia abbreviata*, *Achnanthidium lanceolata*, *Nitzschia dissipata*, *Melosira varians* and *Amphora pediculus*.

Species assemblage differences above 50 μg l^{-1} were poorly differentiated and possibly related to stream velocity as opposed to phosphorus concentration. These results would therefore suggest that above this concentration, phosphorus is saturating and no longer a limiting resource. Vigorous species that would not be able to compete as efficiently below this concentration are able to flourish.

The number of species that actually responded to differences in SRP concentration were few, evidenced by the percentage compositions of assemblages seen in Figures 5.3 to 5.5. There were in total fourteen species that were consistently above 5% abundance in all of the samples. Besides the seven that were indicator species (as discerned by multivariate analysis) these other seven species showed no pattern of variation with phosphorus. Three of these species (*Navicula lanceolata*, *Nitzschia amphibia* and *Synedra ulna*) are recognised as having broad ecological ranges (Cox, 1996).

The correlation between indicator species and phosphorus concentration found in this research is consistent with the findings of other workers. *Diploneis* sp. was the most pollution sensitive species found. *Diploneis* species favour very low nutrients and are found in oligotrophic waters (Cox, 1996; Kelly, 1995). *Achnanthidium minutissimum* and *A. lanceolatum* provided the most clearly contrasted response to phosphorus concentration of all diatom species. *A. minutissimum* is more sensitive towards pollution than *A. lanceolatum* and is associated with waters that are well oxygenated with low SRP concentrations (Steinberg and Scheifele, 1988; Round, 1993; Cox, 1996). Guzkowska and Gasse (1990) found *A. minutissimum* to dominate in English urban lakes between 10 to 80 μg l^{-1} phosphate (precise determinand unknown). *A. minutissimum* var. *saprophila* is a variety of this species that is tolerant to organic pollution and has been found in continental Europe but not in the UK (Kelly and Whitton, 1994). *A. minutissimum* var. *minutissimum* was most probably the variety found in this study. *A. lanceolatum,* in constrast, is commonly found in nutrient-rich rivers and streams (Kelly, 1995) and is pollution tolerant (Cox, 1996). *Navicula tripunctata* is tolerant of only moderate pollution (Cox, 1996).

Rhoicosphenia abbreviata (also referred to as *R. curvata* in the literature), *Amphora pediculus, Nitzschia dissipata* and *Melosira varians* are pollution tolerant and abundant in eutrophic waters (Kelly and Whitton, 1994; Cox, 1996). *R. abbreviata* is more commonly epiphytic than epilithic (Round, 1993). Steinberg and Scheifele (1988) describe *R. abbreviata* and *N. dissipata* as 'eutrophent' which means that their development is promoted under nutrient rich conditions. They classify *Amphora pediculus* however as being sensitive to pollution which contrasts with the opinions of Kelly and Cox.

Overall, the species assemblages across the Welland catchment streams indicated that most sites were eutrophic based on the autecological ranges described above. TWINSPAN analysis did not divide sites above 50 $\mu g\ l^{-1}$ according to SRP concentration. These sites were characterised by the presence and abundance of species that are tolerant of/flourish under eutrophic conditions. These results could not find evidence for species differentiation above this SRP concentration.

Application of Kelly's Diatom Quality Index showed a relationship between SRP and diatom assemblage below 100 $\mu g\ l^{-1}$. Between the 100 $\mu g\ l^{-1}$ and 1000 $\mu g\ l^{-1}$ the data were scattered. The original work from which the DQI was developed was based on studies of rivers with SRP concentrations ranging from 0.1 to 1000 $\mu g\ l^{-1}$ (Kelly and Whitton, 1995); over half of these rivers were in the north of England and Scotland. The lower end of this range is rarely found in lowland enriched waters. In practical terms, these data suggest that useful information concerning a streams phosphorus levels or trophic status is limited to those streams below 100 $\mu g\ l^{-1}$ SRP when using this Index to assess diatom composition.

These results imply that gradation of eutrophy using differences in diatom assemblage is problematic given that no such groupings were discernible above 50 $\mu g\ l^{-1}$. In terms of river monitoring, indices are a useful tool for interpreting and conveying the results of diatom surveys. However, these results suggest that indices of eutrophication (as opposed to indices of organic pollution) are limited to classifying oligotrophy, mesotrophy and eutrophy. Since many rivers and streams in lowland regions are eutrophic, comparison of diatom community between sites using indices would be very limited.

CHAPTER 6 BIOLOGICAL RELATIONSHIP BETWEEN PHOSPHORUS AND BIOMASS OF ATTACHED ALGAE

6.1 Introduction

Chapter 5 examined the extent to which phosphorus concentrations found in lowland streams influence diatom community. The aim of this chapter is to examine algal response in terms of biomass.

The attached algal community as a whole is considered in this chapter but as three distinct biomass compartments; filamentous *Cladophora*, the whole biofilm and diatoms within the biofilm. It was observed that these particular compartments dominated most surfaces within Welland catchment streams. The biomass of *Cladophora* and biofilm was quantified by measuring chlorophyll 'a' content. Diatom was measured by analysis of biovolume. These measurements were compared to the ambient phosphorus concentrations found at each sites sampled.

Laboratory channels and streamside troughs have been used to examine the effect of phosphorus on algal communities (Horner *et al.*, 1990) and the interactive effect of this nutrient with nitrogen (Stockner and Shortreed, 1978), light and herbivory (Rosemond, 1993), temperature (Bothwell, 1988) and velocity (Horner *et al.*, 1983; Horner and Welch, 1981). Artificial stream channels are commonly used in such experimental work to guarantee that all but the variable under investigation are controlled. Field studies concerning the algal-phosphorus relationship have produced more variable conclusions (Pitcairn and Hawkes, 1973; Henley, 1980; Marsden *et al.*, 1997; Moore, 1977) which is partly attributable to the influence of other growth controlling factors and also the higher phosphorus concentrations encountered in natural rivers.

The effect of phosphorus concentration on algal biomass was investigated within streams of the upper Welland catchment. Experimental work was conducted in streams as opposed to using laboratory stream channels. Instream abiotic variables were controlled for by introducing clay channels to house stones collected from the streams. This allowed for the positioning of clay channels in a number of stream reaches that differed in ambient phosphorus concentration but were homogenous in terms of stream environment.

6.2 Methodology

6.2.1 Sample collection

Table 6.1 details the sites within the Welland catchment that were chosen and the mean summer phosphorus concentrations at each reach.

Table 6.1 Sampling sites and mean summer SRP ($\mu g\ l^{-1}$)

Stream	Site	mean SRP ($\mu g\ l^{-1}$) May to August 1996
Eye	Glebe Farm	4
Medbourne	Green Lane	13
Welland	Great Bowden	152
Welland	Hothorpe	237
Langton	Smeeton Westerby	322
Stonton	Noseley	414
Eye	Skeffington	809
Welland	Foot Bridge, Green Lane	2640
Langton	Gauging Station	2990

All nine sites were sampled on four dates for chlorophyll 'a', biovolume and species determination (no biovolume samples were prepared on the first date)(Table 6.2). Eight stones were collected from each site; four were used for chlorophyll 'a' analysis and four for biovolume and species determination. Three of the nine sites were sampled on four other occasions and the sample stones subject to chlorophyll 'a' analysis and species determination (no biovolume samples were taken). These were Glebe (Eye Brook), Smeeton Westerby (Langton Brook) and Footbridge on Green Lane (Welland). Extra samples were taken from these three sites to provide increased temporal data. Stones were chosen at random and transported to the laboratory in sealed polystyrene boxes where they were stored in a cold room at 4°C.

Table 6.2 Timetable for sample collection, May to October 1996

	Collection of stones for chlorophyll 'a', biovolume and species determination from **nine sites**	Collection of stones for chlorophyll 'a' and species determination from **three sites**	Collection of glass slides for *Cladophora* plant counts from **four sites**
22.5.96	√		
21.6.96		√	√
9.7.96	√		
25.7.96		√	√
12.8.96	√		
29.8.96		√	√
16.9.96	√		
7.10.96		√	√

Homogeneity of stream site was achieved by introducing artificial 'riffles'. These consisted of clay channels (1 metre by 0.15 metre) into which stones from the stream reach were placed (Figure 6.2). The stones were confined to clay channels to enable alteration of the depth of the riffle with fluctuations in stream flow. Depth was

maintained at approximately 5cm below the surface. Stones were introduced into the troughs at the beginning of March 1996 to enable acclimatization prior to the first collection date (22.5.96). The channels were introduced to open stream reaches with no riparian shading. The stream sites, although chosen for comparability of size and flow, were subject to differing stream velocities. Flow was altered to reduce disparity in stream velocity between sites. This was achieved by building instream dams up- and downstream of the channels. Stream velocity was monitored at fortnightly intervals using either an A.OTT. Type C2 Kempton flow meter (impeller type) or a Marsh-McBirney Model 2000 Flow Mate ™ (electromagnetic type). Chicken wire was secured to the top of the upstream dam to intercept debris and invertebrates.

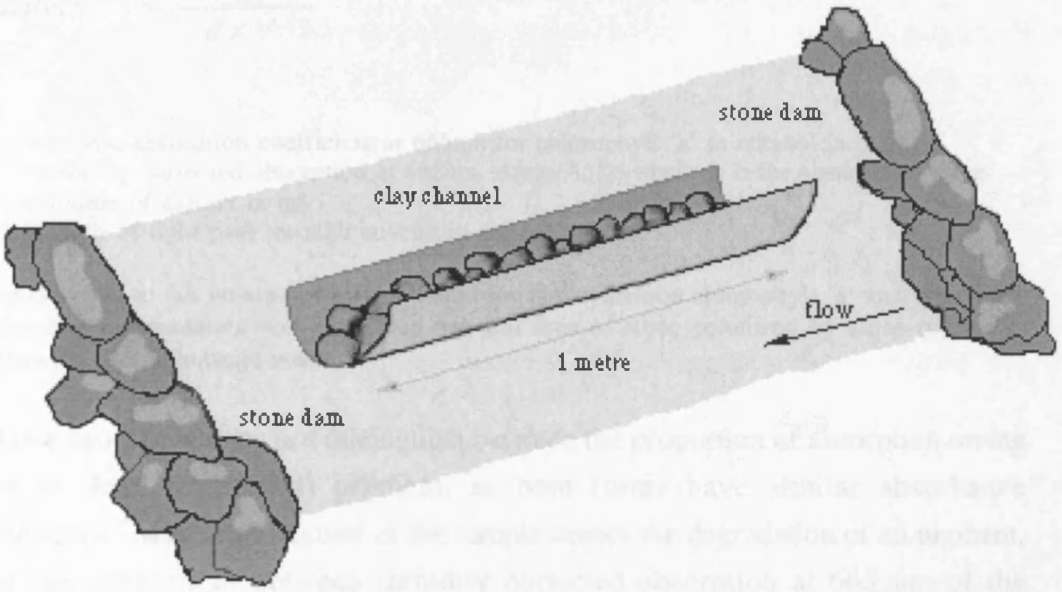


Figure 6.1 *In situ* clay channel

6.2.2 Analysis of chlorophyll 'a'

The majority of filamentous algae on sample stones was identified as *Cladophora*. Identification was confirmed microscopically. Other types of filamentous algae and moss species were found during this study and were recorded.

Prior to removal of any algae from stones, a blue wax crayon was used to outline the area colonised by algae. This provided a guideline for later determination of area using aluminium foil. Visible *Cladophora* growth was then removed from four stones from each site and the longest filament per stone measured and recorded. *Cladophora* plants were vigorously agitated in a beaker of water using a magnetic stirrer. This was done to remove silt and epiphytic growth. *Cladophora* filaments were then moved from the beaker to Whatman GF/C glass fibre filter. This filter was placed on a slide heater at low temperature to remove excess water. Care was taken to avoid allowing the *Cladophora* to dry out as this would damage chloroplasts.

After removal of all visible *Cladophora* plants, the biofilm remaining on the stone was removed using a stiff brush and swilled into a measuring cylinder. This suspension was then filtered through a Whatman GF/C glass fibre filter.

Biofilm and *Cladophora* samples (including the filter paper with the *Cladophora* filaments) were analysed separately for chlorophyll 'a' using the cold ethanol extraction procedure (MEWAM, 1980). Absorbance of the extracts was measured using a Hewlett Packard B452 Diode Array Spectrophotometer at 665 nm and 750 nm, in order to compensate for background turbidity. Total pigment concentration was calculated from the following equation:

$$CHla(\mu g cm^{-2}) = \frac{k \times E_{665} \times v}{d \times V}$$

where

k	=specific absorption coefficient at 665nm for chlorophyll 'a' in ethanol (=12.0)
E_{665}	=turbidity corrected absorption at 665nm. A_{665}-A_{750} where A is the absorbance value.
v	=volume of extract in ml
d	=length of light path through cuvette in cm (=1cm)

'V' usually refers to the volume of water filtered for phytoplankton chlorophyll 'a' analysis. Here the absorption measurement was expressed per unit area of stone colonized by algae ($\mu g\ cm^{-2}$) rather than per unit volume of water.

The above procedure does not distinguish between the proportion of absorption owing to live or dead (degraded) pigment, as both forms have similar absorbance characteristics. Mild acidification of the sample causes the degradation of all pigment, so that the differences between turbidity corrected absorption at 665 nm of the unacidified and acidified samples can be used to estimate the amount of undegraded pigment initially present. Samples were acidified by adding 0.01 ml of 0.3M hydrochloric acid to the cuvette. The samples were then left for approximately 10 minutes prior to spectrophotometry to allow complete degradation of pigment. The phaeopigment (degraded) content of samples was calculated from:

$$CHla(\mu g cm^{-2}) = \frac{k \times R(E_{665} - (F(E_{665} - E_{665a}))) \times v}{d \times V}$$

where

R	= maximum ratio of E_{665}:E_{665a} in the absence of phaeopigments (=1.7)
F	= factor derived from the difference in absorbency of chlorophyll 'a' at 665 nm before and after acidification (=2.43)
E_{665a}	= turbidity-corrected absorption at 665 nm after acidification

The surface area of stone colonised by algae was estimated using aluminium foil. The stone was covered as precisely as possible with foil up to the crayon line and excess

folds cut away. The surface area was then calculated from the weight of this foil covering divided by the weight of a one square cm piece of foil. The final chlorophyll 'a' result was expressed as mg m^{-2} of stone surface to allow for comparison with chlorophyll 'a' results from the literature.

Total chlorophyll 'a' values were used for analysis of results. This effectively gives an estimate of algal abundance irrespective of the state of the cells. This measurement was used since it represented the potential biomass present at the time of sampling assuming that dead cells were once part of the living assemblage and not imported.

6.2.3 Analysis of biovolume

The biovolume of the diatom community was measured from the remaining four stones from each site. This was achieved using a 'template' area which in this instance was a 1.5 cm diameter plastic tube. The tube was sealed to the surface of the stone using plasticine and petroleum jelly. Once a watertight seal had been made, water was placed inside the tube and the biofilm scrubbed from the stone area using a stiff paint brush. The biofilm suspension was then removed using a suction pipe and decanted into a beaker. This was then made up into a known volume by addition of water and transferred to a sealed container. Two to four areas per stone were sampled using this procedure. Lugol's iodine solution was added to the sample to kill and weigh the diatoms. Sample bottles were placed in a cold room at 4°C for storage.

Diatom cells were enumerated using a counting chamber and *Zeiss Axiovert 100* inverted microscope (Figures 6.2 and 6.3). A count consisted of the number of cells within a single transect of the chamber floor. The area for counting within the field of view was delineated by a box graticule. Subsamples of 1ml were taken from sample bottles and left for 1 hour to allow cells to settle to the floor of the chamber. Counting was performed using a x10 eyepiece and x20 objective and the cells identified broadly to genus.

It was necessary to statistically validate the counting regime. Preliminary counts of 50 transects were therefore made for statistical analysis. A viable counting method would require that cells are randomly distributed on the floor of the chamber for a one transect count to be representative. It was also necessary to prove that sequential 1ml subsamples withdrawn from the same sample bottle were equitable in terms of cell number and composition.

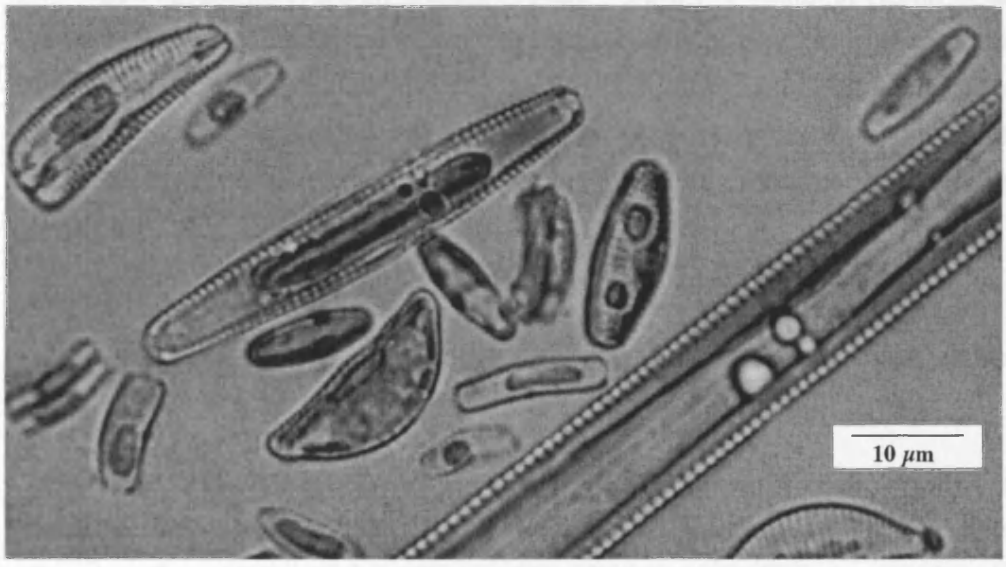


Figure 6.2 Live epilithic diatoms (Rhoicosphenia abbreviata, Amphora pediculus, Synedra ulna, Achnanthidium minutissimum and Navicula sp.)
(photograph: G.L. Evans)

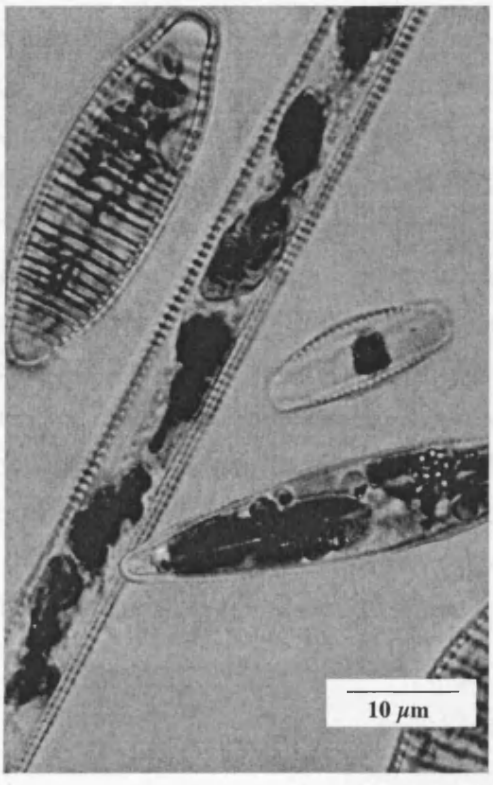


Figure 6.3 Live epilithic diatoms (Diatoma vulgare, Synedra ulna, Amphora sp. and Navicula sp.)
(photograph: G.L. Evans)

The extent to which settled diatom cells were randomly distributed was assessed using a chi-squared test on replicate transect counts within one chamber (Lund *et al.*, 1958). This test suggested that there was some significant variation in the numbers of genera counted in each transect count. It was therefore not reasonable to count only one transect per sample as this may not have been representative of the numbers and composition of the whole sample. An average of three transect counts was therefore used.

The cell counts from different subsamples were found not to vary significantly. This was tested using ANOVA having established that the count data approximated normality.

Cell volume was calculated by applying cellular dimensions to formulae for solid geometric shapes most closely matching the shape of the cells. A selection of examples of each recorded genus were therefore measured. Average cell volumes for genera were used to calculate biovolume for samples collected on the same dates. Mean dimensions were calculated for each collection date assuming that diatom size varies throughout the year. Cell dimensions were measured using a x10 eyepiece and x1000 oil immersion objective.

Attributing a geometric shape to some diatoms was simple given that *Melosira* and *Cocconeis* constitute a cylinder ($\pi lw^2/4$). There was very little mention in the literature regarding the more irregularly shaped epilithic diatoms; authors for the most part focused on planktonic genera (Wetzel and Likens, 1991; MEWAM, 1990; Vollenweider, 1974; Bellinger, 1974; Nalewajko, 1966). In simplest terms, the valve area of a Naviculoid diatom could be estimated from the formulae for two triangles. This value then multiplied by the depth of the girdle would estimate volume. An alternative method was used. This method made use of Image Analysis software to obtain an accurate measure of valve area for several examples of a particular genus. The images used for this were traced outlines of photographed diatoms that had then been scanned into a computer. A function relating the length and width of each genus to area was obtained. Table 6.3 details the functions used to measure the valve area of several genera of diatom and the formulae finally used to calculate biovolume:

Table 6.3 Formulae for calculating biovolume of diatom genera

Diatom	function	formula
Diatoma	0.80	0.80lwb
Encyonema/Cymbella	0.83	0.83lwb
Fragilaria	0.86	0.86lwb
Gyrosigma	0.72	0.72lwb
Large *Navicula*	0.80	0.80lwb
Large *Nitzschia*	0.82	0.82lwb
Reimeria	0.78	0.78lwb
Small *Navicula*	0.74	0.74lwb
Small *Nitzschia*	0.79	0.79lwb
Synedra	0.82	0.82lwb
*Gomphonema**	0.71	$0.71lw((b_1+b_2)/2)$
*Rhoicosphenia**	0.77	$0.77lw((b_1+b_2)/2)$
*Surirella**	0.83	$0.83lw((b_1+b_2)/2)$

For example, the volume of a *Diatoma* cell is 0.80xlxwxb where 'l' is the length of the valve, 'w' is valve width at the widest point and 'b' the breadth of the girdle. This formula relates to those diatoms in the table that are linear/rectangular in girdle view (all except those that are asterisked).

Diatoms whose valves are asymmetrical about the transapical plane include *Rhiocosphenia*, *Surirella* and *Gomphonema*. The formula used to describe these diatoms was $fxlxw(b_1+b_2/2)$ where b_1 refers to the smallest girdle breadth and the b_2 the largest (Figure 6.4). The girdle shape of these diatoms approximates a trapezium.

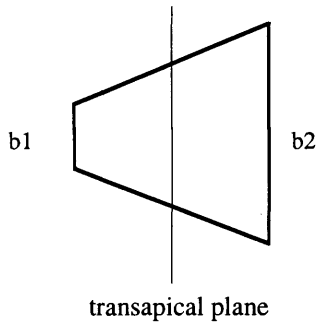


Figure 6.4 Approximate girdle view of *Rhiocosphenia*, *Surirella* and *Gomphonema*

The formulae used to calculate the volume of an *Achnanthidium* cell was $wb(l-w+\pi/4w)$(Wetzel, 1991).

Table 6.4 details the average size of diatom genera for each month within the samples. The measurements were averaged for at least ten individuals of each genus. Those at the bottom of the table were averaged over three months since they occurred infrequently within the samples.

Table 6.4 Average size of diatom in samples

Diatom Genus	Average size (μm^3)	Month
Achnanthidium	74.20	July
Amphora	23.43	July
large *Navicula*	3238.40	July
Melosira	5079.56	July
Rhoicosphenia	798.72	July
Small *Navicula*	327.52	July
Small *Nitzschia*	303.83	July
Achnanthidium	309.28	August
Amphora	37.67	August
Large *Navicula*	3243.49	August
Melosira	3772.11	August
Rhoicosphenia	834.42	August
Small *Navicula*	314.50	August
Small *Nitzschia*	424.76	August
Achnanthidium	170.40	September
Amphora	21.92	September
Large *Navicula*	2181.28	September
Melosira	8117.80	September
Rhoicosphenia	688.80	September
Small *Navicula*	487.73	September
Small *Nitzschia*	380.87	September
Gomphonema	483.51	July-Sept
Reimeria	867.36	July-Sept
Small *Encyonema/Cymbella*	936.24	July-Sept
Cocconeis	939.78	July-Sept
Surirella	1633.44	July-Sept
Fragilaria	1650.34	July-Sept
Cyclotella	2977.05	July-Sept
Synedra	6971.20	July-Sept
Diatoma	7253.92	July-Sept
Large *Nitzschia*	13342.63	July-Sept
Large *Encyonema/Cymbella*	19485.91	July-Sept
Gyrosigma	62208.00	July-Sept

6.2.4 Analysis of slide colonisation

The effect of phosphorus concentration on colonisation and growth of *Cladophora* spores was investigated at four sites. Four replicate mounted glass slides were introduced into Glebe (Eye Brook), Footbridge at Green Lane (Welland), Hothorpe (Welland) and Skeffington (Eye Brook). Slides were mounted in pairs and attached to bricks with a strong adhesive. Autoclave tape was used to bind paired slides together. The bricks were buried in the stream bottom to prevent turbulent flow over the slides from influencing colonization. On collection, one of the paired slides was detached using a scalpel and transported to the laboratory in a sealed box. These were then

viewed under a light microscope and the number of micro- and macroscopic *Cladophora* plants counted over the whole slide. Sample dates for collection of slides are detailed in Table 6.1. These artificial substrates were introduced to streams in March to allow for colonization prior to collection.

6.2.5 Methods for analysis

The four replicate results for chlorophyll 'a' content of *Cladophora* or biofilm were averaged to give one value per site per date for each algal component. This number of replicates was chosen as the maximum possible within the analytical time frame. *Cladophora* filaments were averaged for the four stones collected at each site per collection date. The number of each genus for biovolume analysis were averaged for the two to four templates sampled per rock (template number depended on the size of colonized area available on each stone). This number was converted to a biovolume value per stone. Biovolume values for each stone were then averaged for the four stones collected at each site per date.

Statistical comparison between biomass (as chlorophyll 'a' or biovolume measurement) and stream phosphorus and water velocity was made using linear regression analysis.

6.3 Results

6.3.1 Analysis of biofilm and *Cladophora* biomass

Periphyton chlorophyll 'a' relates to the whole attached algal community and is the summation of biofilm and *Cladophora* chlorophyll 'a'. These values were plotted against log SRP and stream velocity and showed considerable scatter (Figures 6.5 and 6.6). SRP results were those taken on the algal sampling date (or nearest value to this date), stream velocity values were averaged across the period of sample collection. Most scatter was observed above the 100 μg l^{-1} mark in the comparison of periphyton with stream phosphorus. The two high chlorophyll 'a' values below 10 μg l^{-1} represent nearly all biofilm.

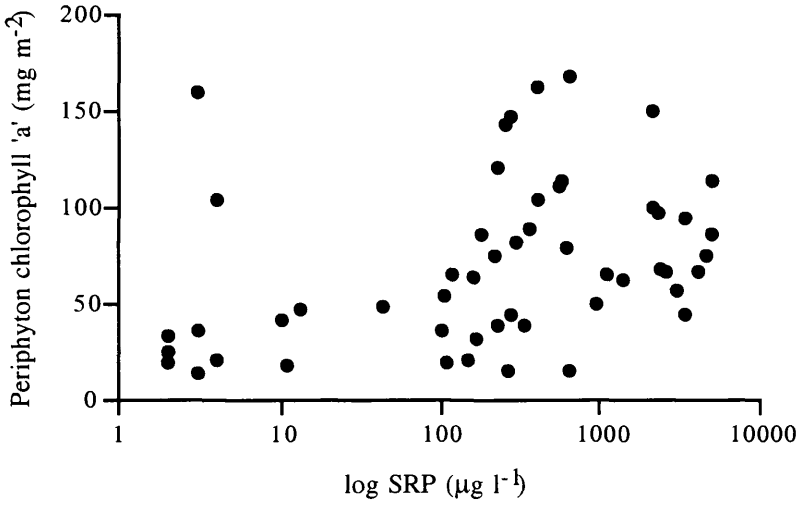


Figure 6.5 Periphyton chlorophyll 'a' against log SRP

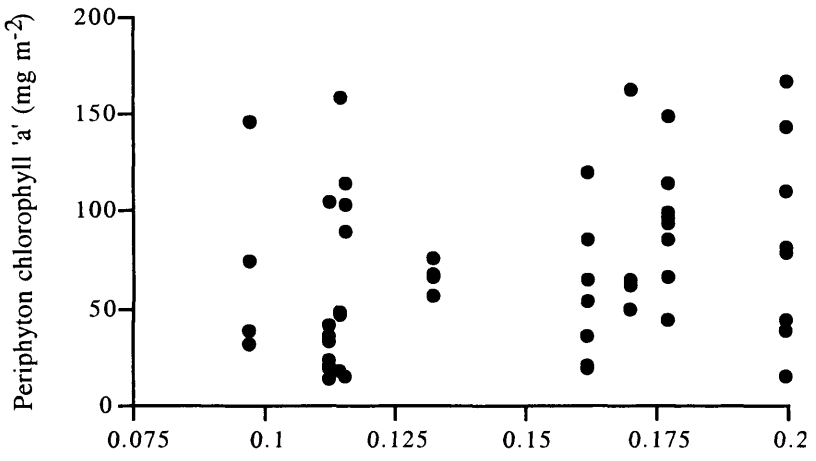


Figure 6.6 Periphyton chlorophyll 'a' against mean summer stream velocity

The same comparison was made between these stream variables and *Cladophora* chlorophyll 'a'(Figures 6.7 and 6.8). The comparison with SRP suggests that below 100 μg l^{-1}, biomass was restricted. The comparison with stream velocity indicates a positive trend towards increasing biomass with higher stream velocity. This latter relationship was significant when the chlorophyll 'a' results were averaged for all dates at each site (r^2=0.791, $p<0.05$) (Figure 6.9).

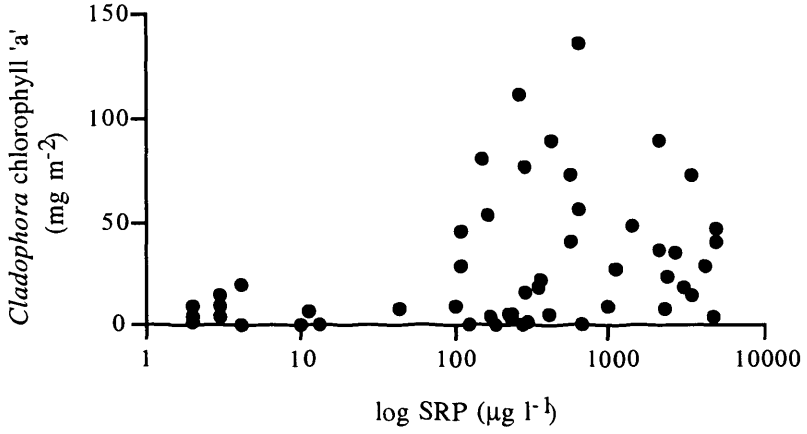


Figure 6.7 Chlorophyll 'a' of *Cladophora* against log SRP

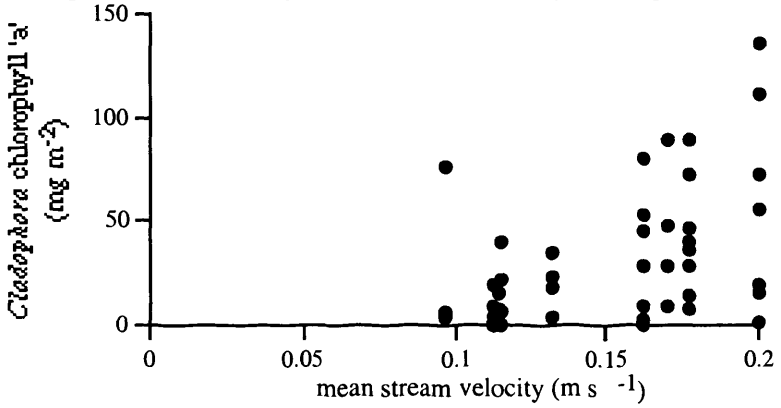


Figure 6.8 Chlorophyll 'a' of *Cladophora* against mean summer stream velocity

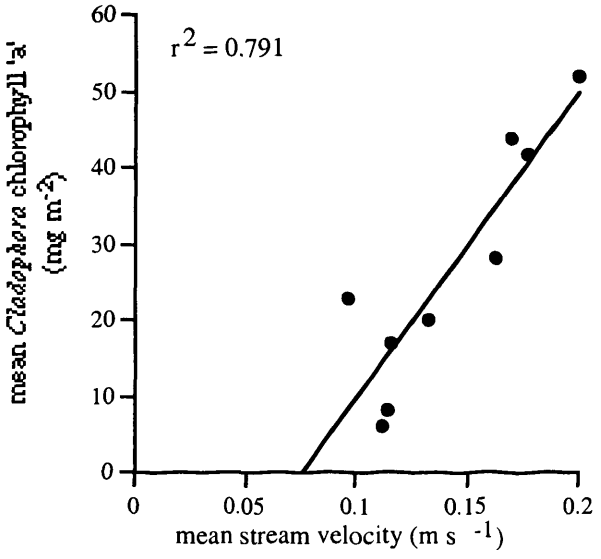


Figure 6.9 Mean chlorophyll 'a' of *Cladophora* against mean summer stream velocity

These data were further examined to assess whether stream velocity and SRP act in conjunction with each other to increase chlorophyll 'a' levels. The results of a stepwise multiple regression suggested that stream flow was by far the main controlling factor and SRP only improved this relationship very slightly (r^2=0.859, $p<0.05$).

The comparison of SRP with the longest *Cladophora* filament (Figure 6.10) showed a similar scatter of data points as Figure 6.7. As a different measure of plant yield, this confirmed the likely limiting affect of low concentrations of SRP (below 100 μg l^{-1}). Similarly, the better correlation was with mean summer stream velocity as opposed to SRP (Figure 6.11). Figure 6.12 shows the mean of all filament length results per site against velocity. This relationship is statistically significant (r^2=0.776, $p<0.05$).

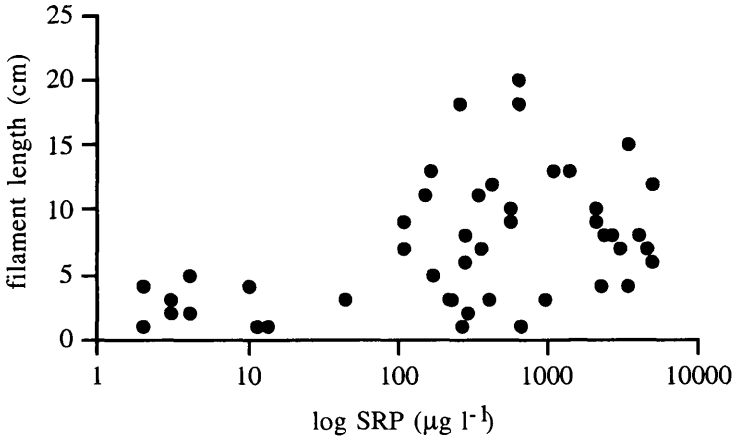


Figure 6.10 Longest *Cladophora* filament length against log SRP

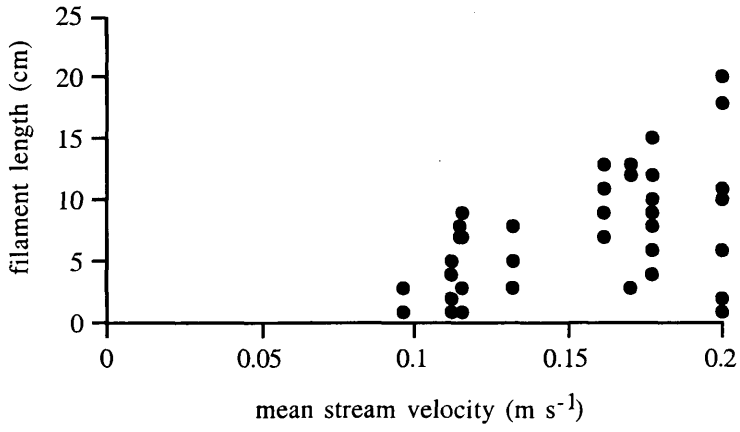


Figure 6.11 Longest *Cladophora* filament length against mean summer stream velocity

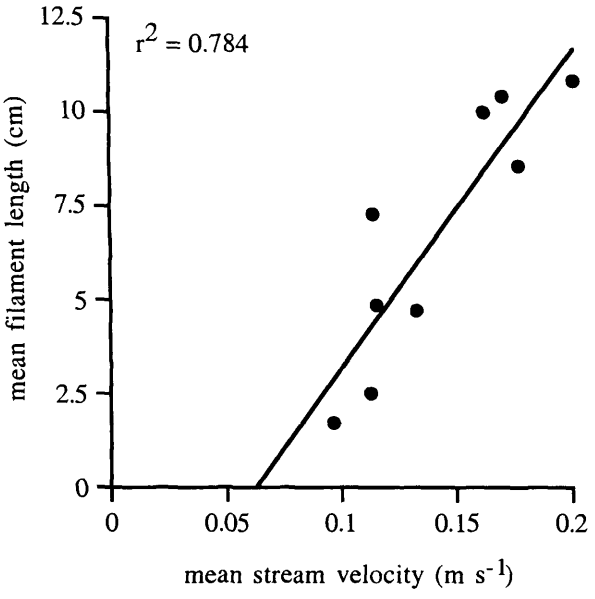


Figure 6.12 Longest *Cladophora* filament length against mean summer stream velocity

Values for filament length and chlorophyll 'a' against phosphorus and velocity were equivalent given the similarity in distribution of data points in the above graphs. The relationship between the longest filament length and chlorophyll 'a' content of *Cladophora* (r^2=0.681, $p<0.05$) was a significant one and appeared not to be linear (Figure 6.13). This has practical implications given the ease with which one method can be performed compared to the other. Filament measuring is non-destructive and can be performed *in situ* without the need for lengthy laboratory procedure.

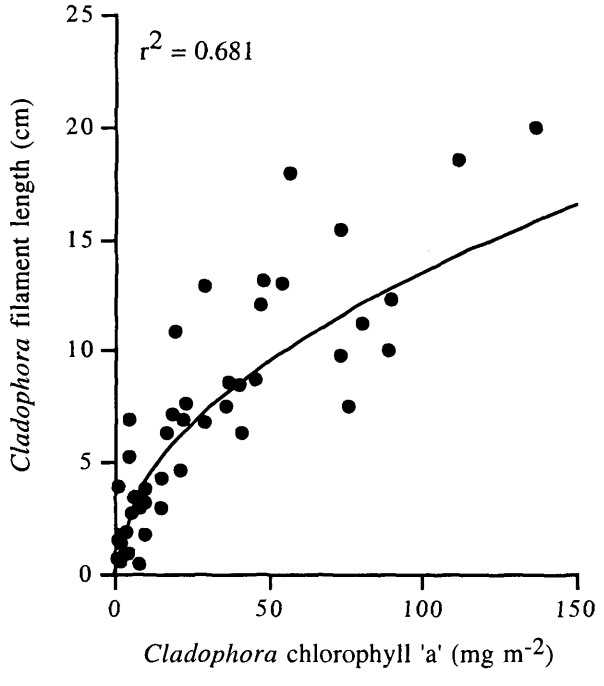


Figure 6.13 Comparison of longest *Cladophora* filament length with *Cladophora* chlorophyll 'a'

The relationship between biofilm chlorophyll 'a' and SRP (Figure 6.14) indicated growth limitation below 100 μg l^{-1} similar to the comparison of SRP with *Cladophora* biomass. The outlying points below 10 μg l^{-1} are those mentioned in relation to Figure 6.5. These two data points were samples taken on the 22.5.96 at Glebe (Eye Brook) and Hallaton (Medbourne). Notes taken at the time of analysis of these sample stones refer to loosely attached biofilm and a fine down covering of *Cladophora* and *Microspora.* Inadvertent inclusion of filamentous strands into the biofilm sample may have artificially augmented the chlorophyll 'a' measurement for these sites. Reference to a loosely attached biofilm may be a consequence of the these two sites having two of the lowest summer flows. Low flow would encourage the settling of imported organic and mineral particles. The phaeopigment content of these chlorophyll 'a' samples however was less than 10% of the live proportion suggesting that if organic imports were a source of additional pigment then such imports would have been living as opposed to degraded.

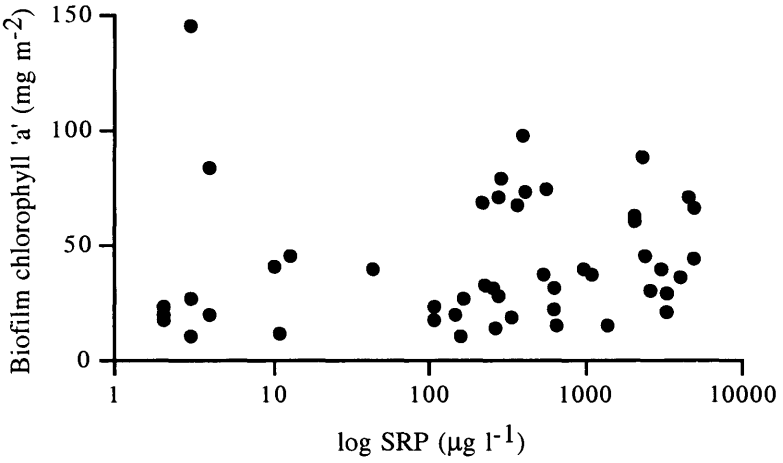


Figure 6.14 Chlorophyll 'a' of biofilm against log SRP

Figure 6.15 illustrates the combined response of *Cladophora* and biofilm to stream velocity. *Cladophora* biomass was found to increase whereas biofilm biomass decreased. The question therefore arises whether the negative response of biofilm is independent of *Cladophora* growth or not. Intuitively, macroalgae will cause shading of biofilm as it grows. The relationship in Figure 6.16 between diatom biovolume and stream velocity provides evidence that biofilm negatively responds to flow by a reduction in biomass *per se*. This would suggest that variation in biofilm biomass was independent of *Cladophora* growth.

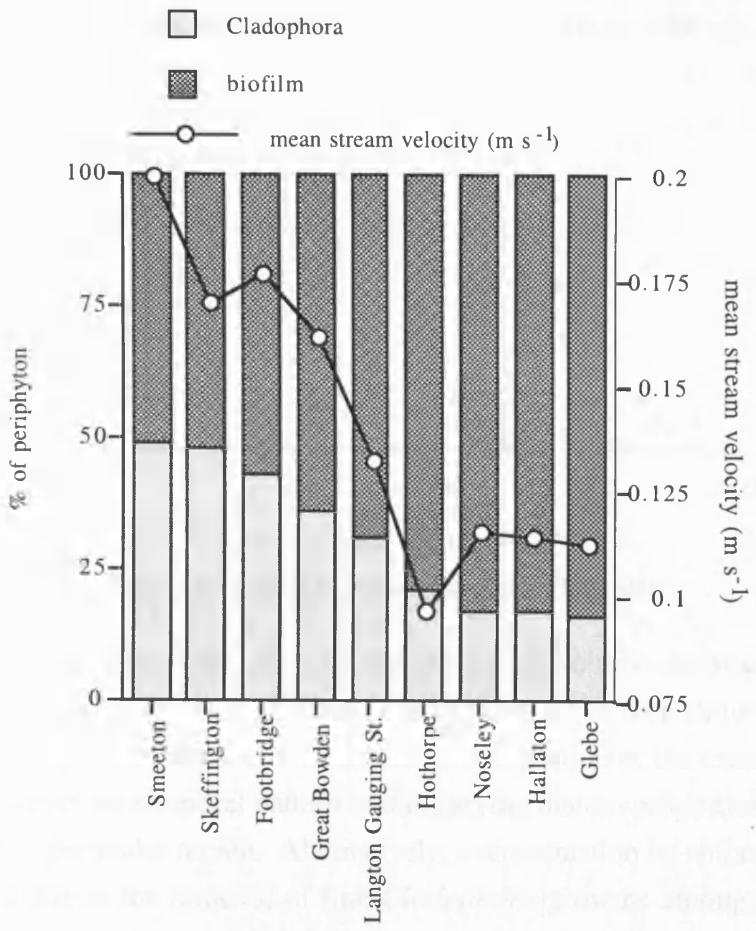


Figure 6.15 Percentage of periphyton composed of biofilm or *Cladophora* against average stream velocity at each Welland site

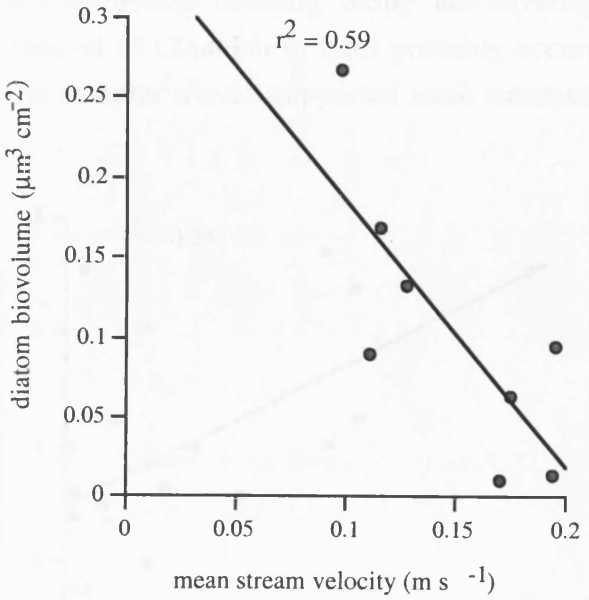


Figure 6.16 Diatom biovolume against mean stream velocity

The relationship between diatom biovolume and SRP was similar to that between biofilm and SRP (Figure 6.17). Below 100 µg l^{-1}, biovolume was low although admittedly there are only three data points here. The two outlying data points below 10

$\mu g\ l^{-1}$ mentioned previously are absent because biovolume was not measured on 22.5.98.

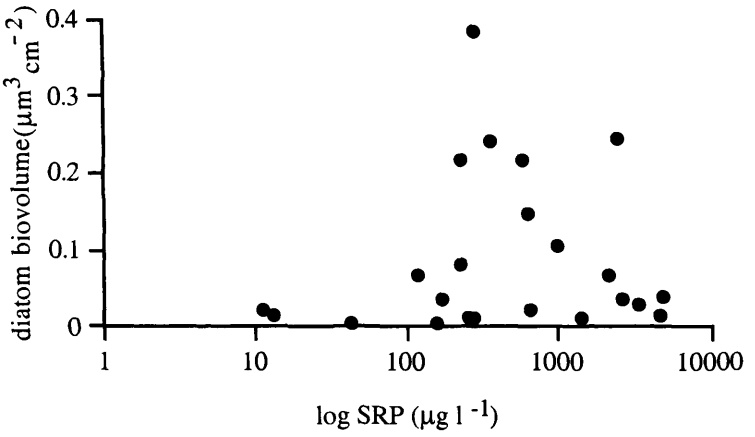


Figure 6.17 Diatom biovolume against log SRP

A comparison of biofilm chlorophyll 'a' and diatom biovolume showed a statistically significant relationship (r^2=0.35, $p<0.05$)(Figure 6.18). The data shows some scatter though, indicating that diatoms are not a constant proportion of the measured biofilm. There was however no temporal pattern to this varying diatom proportion which might have related to a particular month. Alternatively, overestimation by chlorophyll 'a' may have occurred due to the removal of fine *Cladophora* growths amongst the biofilm. There were four sets of sample stones in particular that had high chlorophyll 'a' and low biovolume results. Descriptive notes taken at the time of analysis for these four samples described the *Cladophora* as being 'wispy' and covering the stone in a fine down. Incomplete removal of *Cladophora* most probably occurred prior to biofilm extraction. Most stone samples overall supported more substantial, easily removed *Cladophora.*

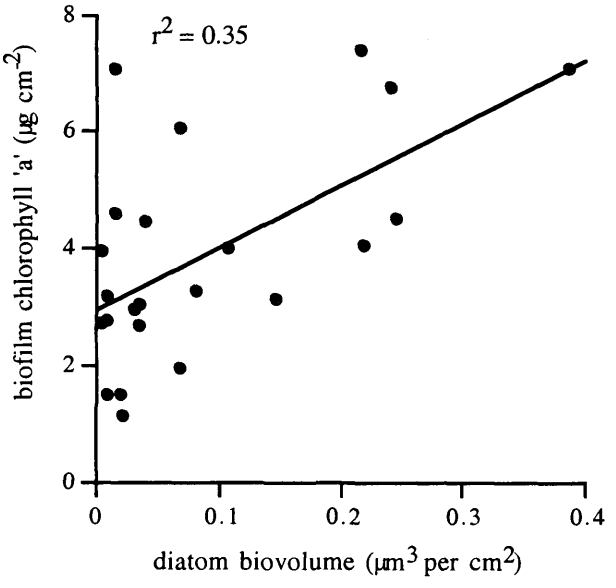


Figure 6.18 Biofilm chlorophyll 'a' against diatom biovolume

Seasonal variations in algal biomass are examined in Figures 6.19 to 6.22. Results from the three sites sampled most frequently are used. The annual spring peak in diatom biomass was observed during May 1996. The decrease in June and July was followed by some recovery in biomass during August to September but the same high biomass peak was not achieved. Phaeopigment or degraded pigment content of the biofilm increased with the slumps in biofilm biomass that occurred mainly in June and July but also towards September and October. This suggests that the photosynthetic cells of the biofilm were dying back as opposed to being exported. *Cladophora* chlorophyll 'a' for these three sites showed less consistency across sites (Figure 6.21). Nevertheless, there is a general trend of increasing biomass after the point of biofilm biomass decline in the spring. Longest filament length (Figure 6.22) reflects variations in *Cladophora* chlorophyll 'a' suggesting that increase in biomass was due to lengthwise growth as opposed to lateral growth over the stone.

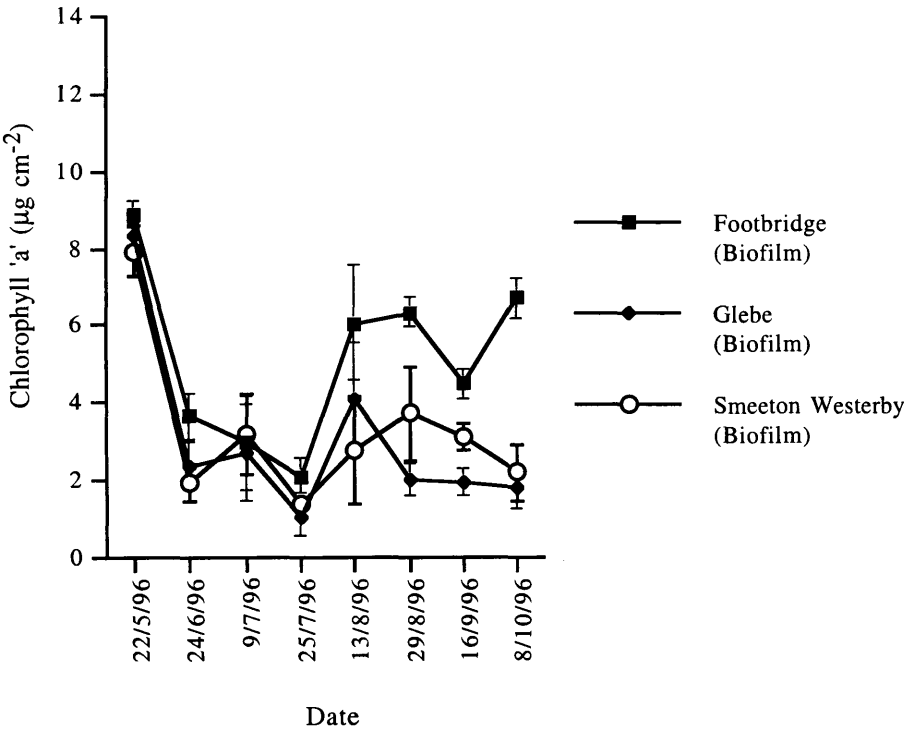


Figure 6.19 Temporal variation in biofilm chlorophyll 'a' at three sites

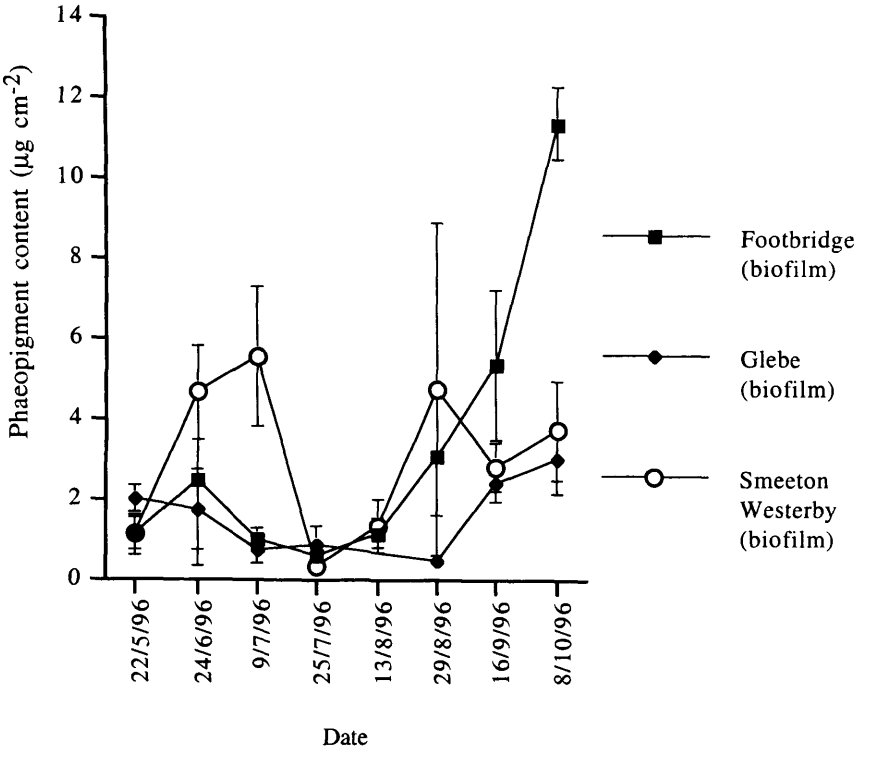


Figure 6.20 Temporal variation in biofilm phaeopigment at three sites

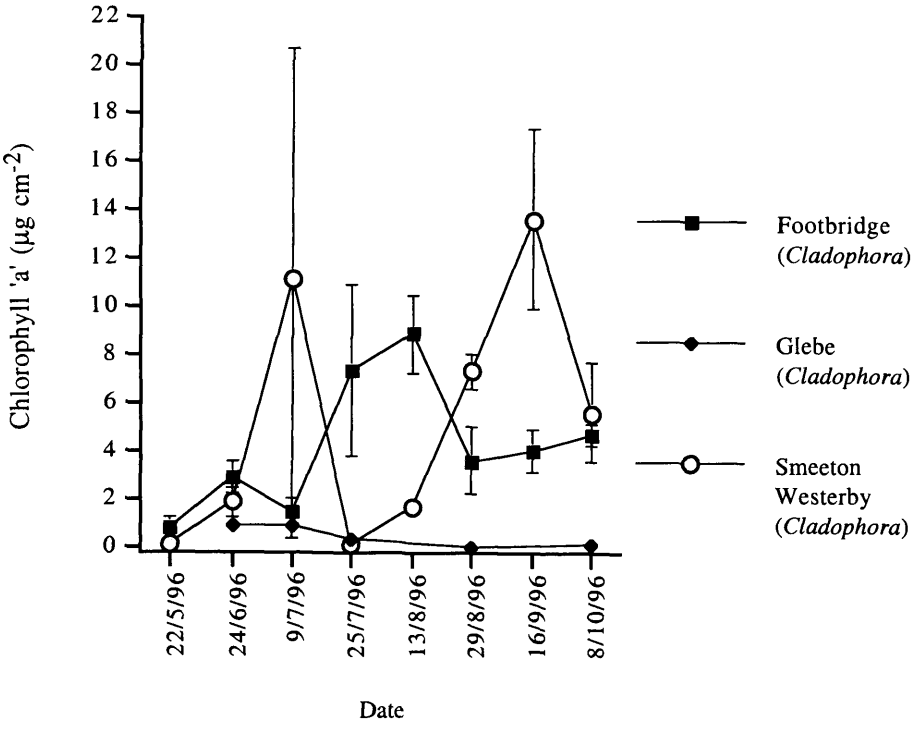


Figure 6.21 Temporal variation in *Cladophora* chlorophyll 'a' at three sites

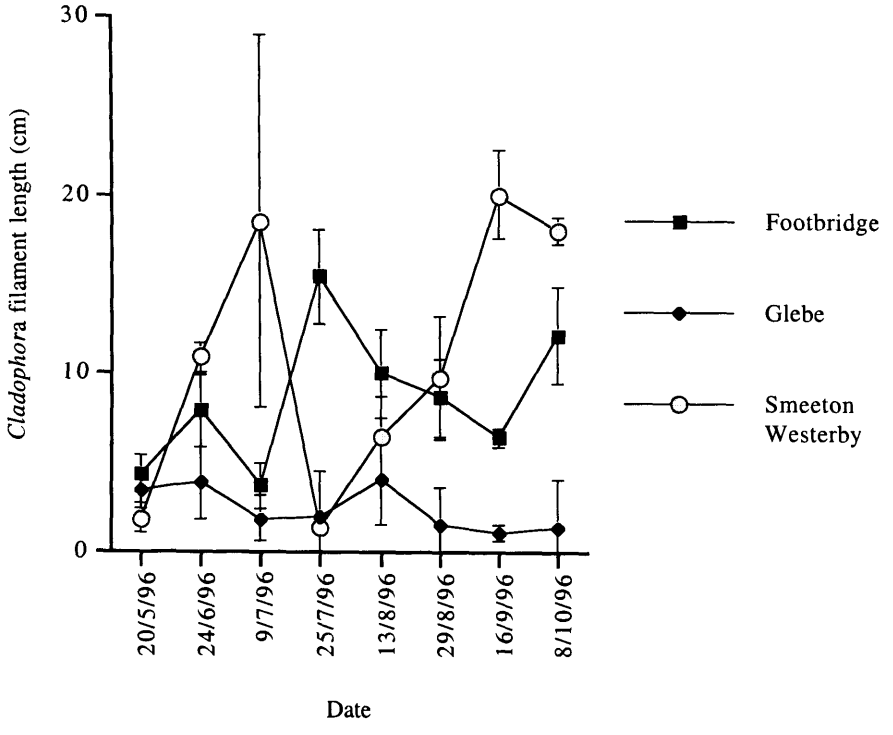


Figure 6.22 Temporal variation in longest *Cladophora* filament length at three sites

6.3.2 Analysis of slide colonisation

Figures 6.23 to 6.26 show the results of counting the number of *Cladophora* plantlets on glass slides introduced into selected stream reaches. The entire growth on each slide was counted into three size categories; <3mm, 3-10mm and >10mm. Each bar of a graph represents the mean size value for four replicate slides. The colonization and growth of this species was then compared across the sites. The stream reach conditions at each site are summarised in Table 6.5.

Table 6.5 Mean SRP and stream velocity at four sites

Welland Site	Mean summer SRP	Mean summer stream velocity
Footbridge (Welland)	2640 $\mu g\ l^{-1}$	0.18 $m\ s^{-1}$
Glebe (Eye)	4 $\mu g\ l^{-1}$	0.11 $m\ s^{-1}$
Skeffington (Eye)	809 $\mu g\ l^{-1}$	0.17 $m\ s^{-1}$
Hothorpe (Welland)	237 $\mu g\ l^{-1}$	0.097 $m\ s^{-1}$

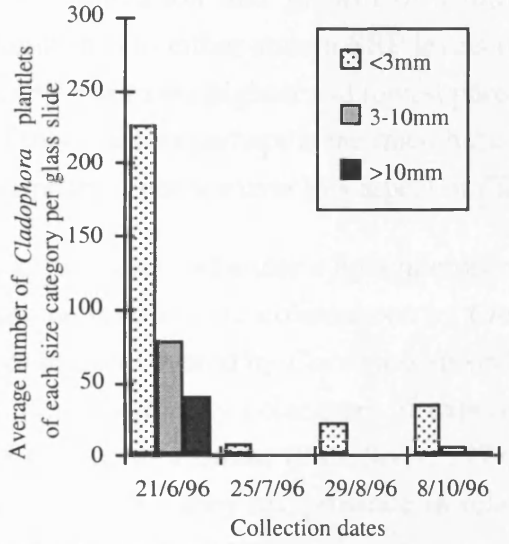


Figure 6.23 *Cladophora* growth on glass slides (Footbridge, Welland)

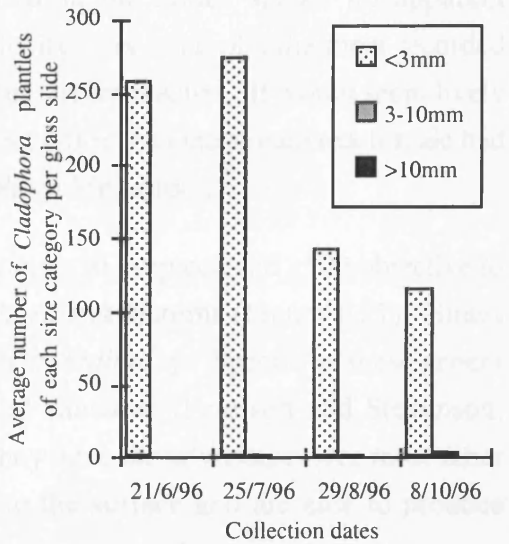


Figure 6.24 *Cladophora* growth on glass slides (Glebe, Eye Brook)

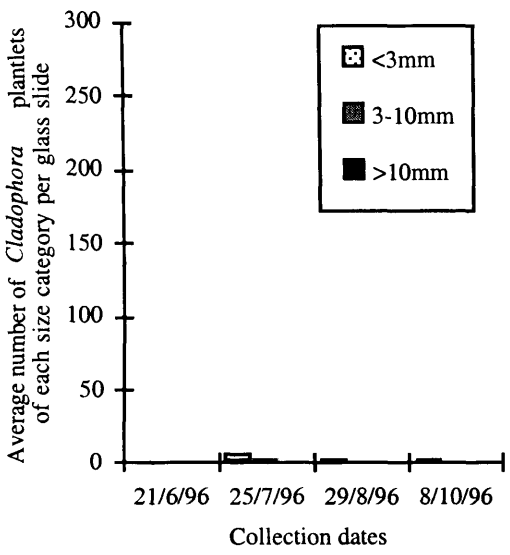


Figure 6.25 *Cladophora* growth on glass slides (Skeffington, Eye Brook)

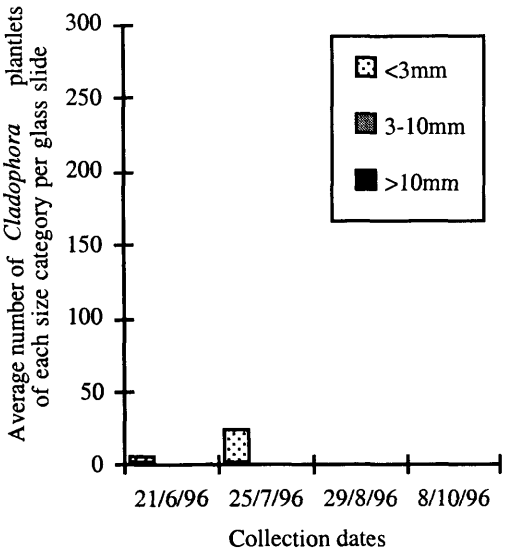


Figure 6.26 *Cladophora* growth on glass slides (Hothorpe, Welland)

The colonization and growth of *Cladophora* on the slides shows no apparent relationship to either stream SRP levels or velocity. For example, the most recorded growth was in the highest and lowest phosphorus stream reaches. It would seem likely that other factors perhaps at the microhabitat scale rather than the stream reach scale had paramount influence over this aspect of *Cladophora* life history.

Slides were viewed under a light microscope using x10 eyepiece and x100 objective to examine microscopic colonisation by *Cladophora* and diatoms (Figure 6.27). Slides were mostly covered by *Cocconeis* sp. and *Achnanthidium* sp. Species of these genera are renowned early colonizers of experimental substrate (Peterson and Stevenson, 1989; Korte and Blinn, 1983; Siver, 1977). They have the advantage over most other diatoms in that they lay prostrate in relation to the surface and are able to produce mucilaginous exudates.

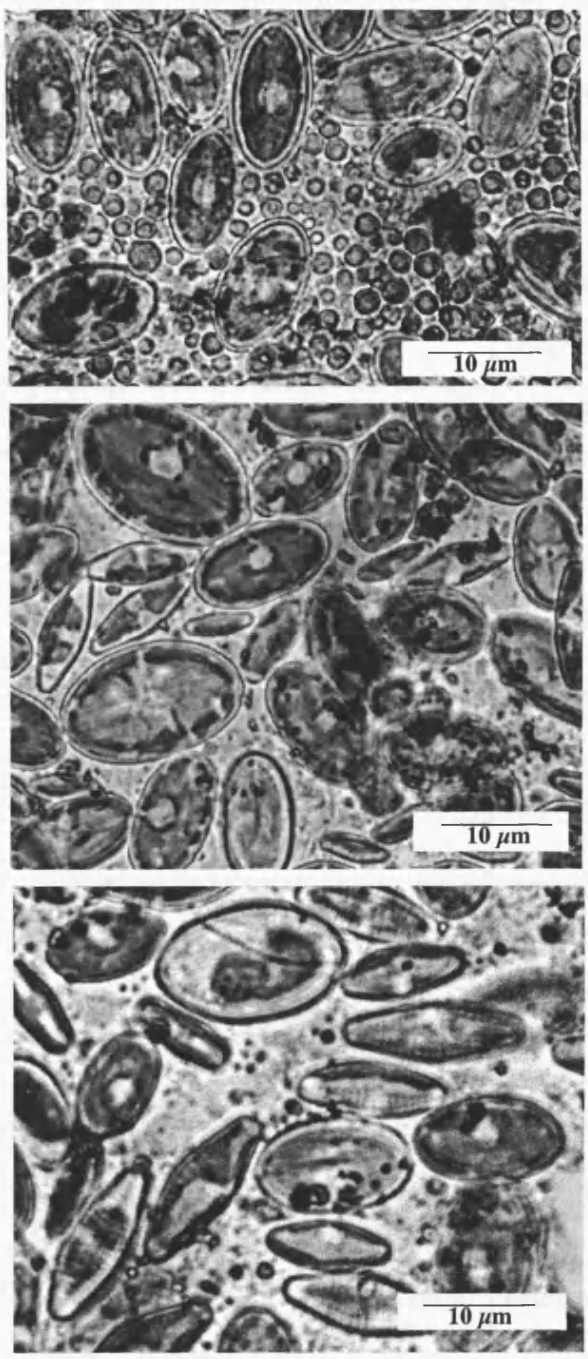


Figure 6.27 Slide colonisation by Cocconeis sp. and Achnanthidium sp. (photograph: G.L. Evans)

6.3.3 Other recorded taxa

Algal genera other than *Cladophora* were recorded where found. These taxa were localized and of comparatively very low density. Table 6.6 details these algae in addition to moss species and invertebrates found on the sample stones. Taxa are included in the table if they occurred on any of the four sample stones per site.

Table 6.6 Other algal taxa and invertebrates recorded on sampled stones.

Site	Stream	Date	Algal genus	Moss species	Invertebrate
Glebe	Eye	22.5.96	*Microspora*		
Smeeton Westerby	Langton	22.5.96	*Microspora*		Hydropsychidae
Foot Bridge	Welland	22.5.96			chironomid larvae
Great Bowden	Welland	22.5.96	*Oedogonium*		
Noseley	Stonton	22.5.96		*Amblystegium serpens*	
Glebe	Eye	9.7.96	*Chaetophora*		
Foot Bridge	Welland	9.7.96			cased caddis, chironomid pupae
Noseley	Stonton	9.7.96			chironomid larvae and pupae
Skeffington	Eye	9.7.96			cased caddis
Hothorpe	Welland	9.7.96	*Microspora*	*Amblystegium serpens*	
Glebe	Eye	13.8.96	*Ulothrix*		cased caddis, chironomid larvae
Smeeton Westerby	Langton	13.8.96	*Enteromorpha*		
Foot Bridge	Welland	13.8.96	*Enteromorpha*		cased caddis snails, leeches,*Asellus*
Great Bowden	Welland	13.8.96			snails, leeches, *Asellus*
Noseley	Stonton	13.8.96		*Leptodictyum riparium*	cased caddis
Skeffington	Eye	13.8.96			cased caddis
Hallaton	Medbourne	13.8.96			cased caddis
Smeeton Westerby	Welland	16.9.96	*Enteromorpha*		snails
Foot Bridge	Welland	16.9.96	*Enteromorpha*		freshwater sponge
Great Bowden	Welland	16.9.96		*Leptodictyum riparium*	
Noseley	Stonton	16.9.96		*Leptodictyum riparium*	cased caddis
Hallaton	Medbourne	16.9.96	*Draparnaldia*		
Gauging Station	Langton	16.9.96			cased caddis
Smeeton Westerby	Langton	8.10.96			snails
Foot Bridge	Welland	8.10.96	*Enterophora*		snails

6.4 Discussion

Phosphorus was potentially the limiting nutrient in the upper Welland during 1995 to 1996. This was indicated from the ratio of total oxidised nitrogen and soluble reactive phosphorus which was mostly above a ratio of 10:1 during the summer of 1995 (refer to Figure 4.15). Nitrogen was therefore in excess of photosynthetic requirements which given the lower phosphorus concentrations in 1996 would suggest that this held true for the following year. This in-stream ratio provides an indication of nutrient limitation. Confirmation of this ratio would require analysis of the content of either nutrient in photosynthetic cells.

Periphyton biomass was restricted in these streams below 100 $\mu g\ l^{-1}$ SRP. This boundary level applied to both *Cladophora* and biofilm biomass when considered separately. Critical growth controlling levels for *Cladophora* are cited in the literature between <10 $\mu g\ l^{-1}$ to <1000 $\mu g\ l^{-1}$ (Pitcairn and Hawkes, 1973; Marsden *et al.*, 1997; Wong and Clark, 1976; Freeman, 1986). The chlorophyll 'a' of other filamentous species increased up to 75 $\mu g\ l^{-1}$ SRP (Horner *et al.*, 1983) and diatom accumulation to be a function of SRP up to 40-50 $\mu g\ l^{-1}$. The critical level of 100 $\mu g\ l^{-1}$ indicated by restricted growth of periphyton in this research corresponds to the findings of Marsden *et al.* (1997). He found that beyond this level of SRP *Cladophora* was overall more abundant in the Forth Catchment, Scotland.

It would be anticipated that so-called nuisance levels of periphyton should not occur below the established critical SRP level of 100 $\mu g\ l^{-1}$. Nuisance levels of filamentous periphyton chlorophyll 'a' in streams and rivers were estimated to occur above 100-150 mg m^{-2} (Welch *et al.*, 1988). They defined nuisance in terms of 'aesthetic displeasure'. Two samples had in excess of 100 mg chlorophyll 'a' m^{-2} of *Cladophora* in this study. These samples were from Smeeton Westerby (Langton) taken on the 9.6.98 and 16.9.96. Ambient summer SRP concentration was 322 $\mu g\ l^{-1}$. This was certainly not the highest SRP measurement when compared to the other eight sites, however, Smeeton Westerby did have the highest summer stream velocity. This concurs with the results suggesting that the influence of stream velocity is paramount in determining levels of biomass when concentrations of SRP exceed 100 $\mu g\ l^{-1}$.

Chlorophyll 'a' measurements were not high owing to the range of velocities involved and the shallow depth at which the experimental channels were maintained. Welch, Quinn and Hickey (1992), for example, measured chlorophyll 'a' at levels of 1200 mg m^{-2} for periphyton sampled from New Zealand streams. Substrate in these streams lay at depths of between 0.14 to 0.45 m unlike the 0.05 to 0.1 m in this experiment; velocities ranged from 0.17 to 0.8 m s^{-1} compared to less than 0.2 m s^{-1} in the Welland catchment. *Cladophora* is known to morphologically respond to stream velocity and

turbulence becoming more stream lined with increasing flow and more branched with increasing turbulence (Dodds and Gudder, 1992). Lengthy *Cladophora* streamers and therefore high biomass is favoured by deeper water (which lessens turbulence) and fast stream velocity. Shallow depths and low flow in the upper reaches of lowland streams are unlikely to support 'nuisance' levels of *Cladophora* according to the guidelines proposed by Welch *et al.* At Noseley (Stonton), Foot Bridge (Welland) and Gauging Station (Langton), *Cladophora* was visually very apparent and seemingly covered most of the stream area. If standards for nuisance levels are used to describe river habitats then some measure of cover is required and not just a measurement for chlorophyll 'a'.

Cladophora growth took off in June 1996 after the diatom peak in May. The pattern of seasonal growth in *Cladophora* is well documented and the growth spurt in late spring is triggered by increasing temperature (Dodds and Gudder, 1992; Whitton, 1970). Growth is bimodal and a second peak in biomass occurs in late autumn. Abundance of diatoms is greatest in spring although a secondary peak can occur in autumn (Allan, 1995). The results suggested that the consecutive peaks in diatom and *Cladophora* biomass were unrelated. The rationale for thinking otherwise relates to potential shading out of diatoms with filament growth. Increases in *Cladophora* chlorophyll 'a' measurements consistently coincided with measurements of longest filament length suggesting filament growth was longitudinal as opposed to lateral. Lateral growth describes increasing biomass due to the growth of more plants on the stone surface causing impingement of surface area otherwise inhabited by biofilm.

New *Cladophora* plants are propagated through the release of akinetes and zoospores (Bellis and McLarty, 1967). The thick-walled thallus of previous years' growth are able to overwinter and it has been suggested that new growth from these are the source for the following years' peak in abundance in May and June (Mason, 1996). Colonisation of glass slides by *Cladophora* zoospores/akinetes supported this theory since the plants that established on the slides did not grow over 5cm in length and were fragile. New growths such as these were unlikely to contribute significantly to overall biomass.

Poor relationships between biomass and nutrient variables have been attributed to harvesting by invertebrate grazers and some fish (Welsh *et al.*, 1992; Hynes 1970). Filamentous green algae are generally considered unpalatable (Lamberti and Resh, 1985) although such algae have been found to be a good food source for snails of the genus *Lymnaea* (Calow, 1970 in Allan, 1995). Chicken wire was initially used to encase the clay channels but interference with flow and snagging of instream debris proved counter productive. The wire was instead secured on top of the upstream dam to intercept debris and also invertebrates. A number of grazing genera were however

noted but these were mostly at low density except on one occasion. Large numbers of small snails were observed on the Welland at the Footbridge site on 13.8.96. Moderation of biomass by invertebrate grazers was therefore a possibility especially at the Footbridge site on the Welland where invertebrates were recorded overall on five of the eight collection dates. Grazing was however unlikely to have a significant impact on biomass. *Cladophora* and diatom biomass were both higher on average at the Footbridge site than at the Gauging Station site on Langton Brook. Both of these sites have average summer SRP concentrations in excess of 2500 $\mu g\ l^{-1}$. Furthermore, each rock was examined closely during analysis and no evidence of harvesting such as a cleared patch of stone surface was observed.

CHAPTER 7 DISCUSSION

The health and economic impact of conspicuous cyanobacterial blooms in lakes has driven much of the research into freshwater eutrophication. The seminal work of Vollenweider in 1968 established that phosphorus and nitrogen were likely causes of eutrophication. Subsequent research to determine the route taken by nutrients into lakes have examined nutrient budgets and sediment transport within the rivers that flow into eutrophic lakes. Research into eutrophication of Lough Neagh (Foy *et al.*, 1982) is one such notable example. Rivers in such catchments are perceived as conduits or delivery routes and the impact of eutrophication on rivers themselves has been of latent importance.

The aim of this research was to identify the concentration of phosphorus in lowland streams above which no further increase in algal biomass or changes in diatom species will occur. Investigation of the relationship between algae and phosphorus is justified in terms of the need to quantify and monitor the impact of anthropogenic eutrophication on stream organisms. Research for this thesis addressed two aspects of river ecology; lotic phosphorus processes and their effect on attached algae. These areas of research have previously been considered separately within the literature; where they have been investigated together greater emphasis is placed on one or the other. The intention here was to bridge this divide and examine, in the context of the lowland river environment, both aspects in relation to each other.

The occurrence of high and low phosphorus rivers throughout the Anglian Region was initially examined alongside likely factors determining levels of this nutrient. Meybeck (1982) considered that, on a global scale, phosphorus is naturally present in concentrations of approximately 10 $\mu g\ l^{-1}$ SRP or 25 $\mu g\ l^{-1}$ total dissolved phosphorus (TDP). Phosphorus concentrations in the Anglian Region ranged between $<10\ \mu g\ l^{-1}$ to over 10,000 $\mu g\ l^{-1}$ SRP; a clear departure from their intended concentration even if global variability is taken into account. However, the frequency of averaged SRP data for 997 EA sites was significantly skewed towards the lower end of this range with 21% of these data falling below 100 $\mu g\ l^{-1}$ SRP (section 4.2). Investigation of potential factors that may determine in-stream phosphorus concentrations (section 3.2) gave some indication of the likely nature of these low phosphorus (below 100 $\mu g\ l^{-1}$ SRP) streams. Sewage effluent was seen to have a distinct influence over in-stream concentrations as indicated by the trend observed in Figure 3.2. Furthermore, first and second order streams made up 67% of the total number receiving no effluent input. In view of the fact that 52% of the 997 sites in the dataset were sampling sites on first and second order streams, this would suggest that there is a sizeable group of non-effluent polluted streams in the Region. A significant difference was also found between the

phosphorus concentrations found in chalk and clay rivers. The dataset used for this comparison had only those river data with less than 500 DWF m^3 day^{-1} effluent to remove the influence of sewage effluent derived phosphorus. It was therefore concluded that low phosphorus streams are most likely to be small headwater tributaries possibly on chalk geologies.

Phosphorus was used in this research as a yardstick for eutrophication but its use necessitates that its role as a primary limiting nutrient is established. That is not to say plant and algal growth across all study streams is constrained for want of phosphorus. Rather, within the existing range of phosphorus concentrations, a level of this nutrient can be determined below which there is a reduction in photosynthesiser biomass due to lack of phosphorus. Nitrogen is also required in quantities that may exceed supply in the water column and is a contender as a primary limiting nutrient. The relative amounts of nitrogen and phosphorus is therefore important as a means of establishing which is the most likely controlling nutrient. Other macro- and micro nutrients are also needed by photosynthesising cells but are usually present in excess of demand. The possibility of limitation, however, by one such nutrient should not be entirely discounted.

The critical ratio above which phosphorus was considered limiting in this research was 10:1 (total oxidised nitrogen to soluble reactive phosphorus). 83% of samples taken from the upper reaches of eight major rivers had nutrient ratios in excess of this ratio and were therefore potentially phosphorus limited during summer months (section 4.2). For the upper Welland streams this value was 75% during the same period of the year (section 4.3.2). Eight of the twelve potentially nitrogen limited samples in the upper Welland had in fact over 1000 $\mu g\ l^{-1}$ SRP. Forsberg and Ryding (1980) refer to nitrogen limitation occurring in the 'upper eutrophic state'.

These values give only a potential picture of nutrient status. The use of algal bioassay (Ram and Plotkin, 1983) or fertilisation experimentation (Chessman, 1992; Peterson *et al.*, 1985; Peterson *et al.*, 1993; Harvey *et al.*, 1998) would provide a more precise method for determining nutrient limitation.

The frequency of routine collection of water samples for phosphorus analysis by the Environment Agency was augmented for three sites on nine selected major rivers. This was achieved by inserting an extra sample in between their sample collection to give at least fortnightly sample intervals. These data were used to evaluate in greater detail the amount of effluent derived phosphorus making up in-stream concentrations of phosphorus. This was achieved by comparison of in-stream load against effluent load (section 4.3). The fluctuation in phosphorus through weeks and even days can be

considerable. This is evident from the time series graphs shown in section 4.4.2 which show the annual fluctuation of SRP concentration at two sites; only one of which receives upstream effluent. Since load calculation uses the mean monthly average for SRP, the more samples taken the more precise the estimate of the mean value and therefore the load. The frequency of sample collection is therefore of importance perhaps more so for high phosphorus river sites that have a considerable range of phosphorus concentration (Figure 4.31). The frequency of sample collection for phosphorus analysis in this research was ultimately constrained by the logistics of sampling nine rivers spread over a large geographical area.

The comparison of in-stream load to effluent load for the inter-catchment rivers (Waithe Beck, Great Eau, Wensum, Bure, Little Ouse, Wissey, Deben and Alde) showed a positive linear relationship. Although statistically significant, this relationship was supported by a few data points from high phosphorus river sites; below 10 kg SRP per day the data showed considerable scatter providing no evidence for the impact of effluent on rivers with low loads or those receiving minor STW effluent (Figure 4.6). Intra-catchment data from the upper Welland was used to ascertain whether effluent had a significant impact below 10 kg SRP per day. The graph (Figure 4.23) shows a positive trend which indicates that small headwater streams can be as readily impacted by effluent from small village sewage works as major rivers on situated near large towns and cities.

The percentage contribution of each potential source of phosphorus was estimated for the most intensively sampled catchment (upper Welland) as 43% point source, 27% diffuse and 30% background. The point source percentage value is comparable to the estimate of 41% made by Morse *et al.* (1993). The background estimate was derived from the extrapolation of the value of 3.26 kg km^{-2} ann^{-1} SRP to catchment areas upstream of each site. This value was obtained from the average load values per upstream area for four sites. These sites were the only ones to receive no effluent from upstream STWs and furthermore lie downstream of pasture rather than arable fields. The distinction between the extent of diffuse and background contribution of phosphorus to watercourses was therefore not exacting being constrained by the widespread number of effluent impacted watercourses and uniform pattern of agricultural land use. A more accurate picture of diffuse sources of phosphorus to the Welland catchment was undertaken and MAFF census data for agricultural land use was used for this purpose. The resolution of the data was however insufficient to draw any firm conclusions. Such conclusions would perhaps require the extensive gathering of ground data as well as comprehensive land use information from each farmer and riparian land owner.

Phosphorus going into rivers via a sewage works outfall is mostly in a soluble form. This was observed in the data comparing percentage of total phosphorus comprising SRP for a non-impacted site and an effluent enriched site (Figures 4.35 and 4.36). Since SRP is considered the most bioavailable form of phosphorus (section 2.1.1), STW effluent is not only increasing the absolute amount of phosphorus entering rivers but supplying it in a highly utilizable form.

River sediments are, broadly speaking, phosphorus sinks in dry months and phosphorus sources in wet months. Increased phosphorus concentration through sediment entrainment or run off will inevitably have a lesser impact on in-stream algal growth since periods of high discharge and rainfall occur mostly outside of the growing season. Diffuse sources of phosphorus have therefore less of an impact on in-stream algae. Mid-summer storm events however do occur and sediment entrainment can release phosphorus over a short time period. Utilisation of this phosphorus by algae depends on discharge rates such that increased flow may enhance nutrient uptake but may also cause scouring particularly if accompanied by high amounts of suspended solids. Storm events are therefore important in terms of catchment transfer of phosphorus but are of limited significance to a river algal community continually impacted throughout the year by effluent from an upstream STW. This interpretation is however river-orientated and takes no account of the importance of transport of phosphorus by river to lake. This is of particular relevance here given that the Anglian Region encompasses the Norfolk Broads. All sources of phosphorus whether diffuse or point will be transferred to lakes. Ephemeral storm events may not have a significant impact on river algae but may be the source of phosphorus loaded stream water to a downstream waterbody and thus impact lake algae.

Chapter five examined the relationship between diatom community and phosphorus concentration. A species within a natural system occupies a niche; a set of chemical and physical conditions within which an organism thrives. Phosphorus is usually scarce under natural conditions and an important resource used by all stream autotrophs. Competition for a given limited resource dictates that species become adapted to different levels of this resource. Within a resource continuum, certain species are adapted to utilising the resource when it is at low concentrations and others are adapted to utilisation at higher levels. In this context, changes in diatom assemblage have been related to water quality. Assemblage changes have mostly been used to assess levels of organic pollution (Descy, 1979; Rumeau and Coste, 1988; Prygiel and Coste, 1993). More recently, they have been used as a means of assessing eutrophication (Steinberg and Schiefele,1988; Round, 1993; Kelly, 1996a)(section 5.1).

Diatom communities in the rivers studied for this thesis were examined within stretches of streams within the same upper catchment area. Thus the objective was to investigate community differences within a specific geographical area under elevated phosphorus concentrations typically found in lowland rivers in this region. Previous studies in the UK have, for example, compared assemblages over a broad geographical area to SRP concentrations in excess of 1000 $\mu g\ l^{-1}$ (Kelly, 1995); correlated diatom communities within a discrete river system to phosphorus indices but not absolute concentration (Cox,1995); and examined diatom assemblage up- and downstream of STWs (Rose and Balbi, 1997).

The results of multivariate analysis showed that diatom assemblage changes occurred below 50 $\mu g\ l^{-1}$ SRP (section 5.3.1). The lack of diatom groupings in relation to phosphorus and the autecological knowledge regarding the species found above 50 $\mu g\ l^{-1}$ SRP suggested that this phosphorus level approximated the onset of eutrophication. Stream velocity was considered to influence diatom assemblage above 50 $\mu g\ l^{-1}$ SRP.

Diatom species diversity was examined in respect to temporal variation, phosphorus concentration and the difference in assemblage found on two different substrate (sections 5.3.2 and 5.3.3). No pattern of diversity change was observed throughout the period June to October 1996. Similarly, increasing SRP concentration showed no correlation with diatom diversity. Differences in diversity were found, however, in diatom samples collected contemporaneously from stone and *Cladophora*; epilithic samples were significantly more species rich than epiphytic samples. This suggests that diatom samples from *Cladophora* can not be used as a substitute for stone samples where stone substrate is not available at a river site. Furthermore, these data suggest that diatom diversity is perhaps not directly impacted by increasing phosphorus concentrations. Rather, a decrease in diatom diversity may occur as a result of the effective reduction of in-stream stone surface area by *Cladophora*.

Studies examining the relationship between *Cladophora* growth and phosphorus have previously been performed in the field. Levels of *Cladophora* growth have been assessed using mean annual dry weight (Pitcairn and Hawkes, 1973) and percentage cover (Wharfe and Taylor, 1984; Marsden *et al.*, 1997). These studies all observed that the correlation between *Cladophora* growth and phosphorus concentration was poor, which was most probably related to the effects of physical factors on *Cladophora* growth and also the methods used to assess biomass. Welch *et al.* (1988) also conducted an *in situ* study comparing whole periphyton biomass in this instance to phosphorus and nitrogen levels. No relationship was observed with either of these nutrients which was considered the result of grazing pressure, shading and possible nitrogen limitation. Similarly, physical variables were attributed to the poor

performance of a model put forward to predict periphytic biomass as a function of phosphorus concentration, nutrient uptake, velocity, light and temperature (Welch *et al.*, 1992). Other factors such as invertebrate grazing in combination with shading, unstable substrata and very high turbulence were thought to be the problem. Laboratory streams have also been used and provide the means of observing the effect of phosphorus concentration in isolation from other stream variables (Horner *et al.*, 1983). However, results obtained under such conditions may not be readily transferable to a natural stream environment.

In light of previous studies, algal biomass experiments were conducted in the field under manipulated conditions to avoid the effects of physical factors (section 6.2.1). Field experimentation as opposed to laboratory based artificial streams were used so that results would inherently reflect the processes occurring in the streams under study. Chlorophyll 'a' analysis was used as a more precise way of measuring biomass although the use of percentage cover (Wharfe and Taylor, 1984; Marsden *et al.*, 1997) does allow for assessment of a greater number of stream sites (section 6.2.2).

Homogeneity of stream variables across sites was achieved with limited success given that stream velocity could only be controlled within a limited range. This factor was therefore incorporated into analysis of biomass. Grazing by invertebrates was deterred by placing a chicken wire barrier upstream of the experimental clay channels. This method undoubtedly had its limitations. However few grazers were observed and attempts to incorporate any physical barrier around the channels interfered with flow and caused shading when snagged with upstream debris.

Results showed that growth of *Cladophora* and biofilm was restricted under 100 $\mu g\ l^{-1}$ within the range of 4 to 2990 $\mu g\ l^{-1}$ SRP (mean summer values)(section 6.3.1). Water velocity had a profound effect on biomass. Biofilm biomass decreased linearly with increasing stream velocity and *Cladophora* growth increased linearly with increasing velocity within the range of 0.1 to 0.2 $m\ s^{-1}$ mean summer stream velocity. Biomass response to increasing phosphorus levels was neither linear above or below 100 $\mu g\ l^{-1}$. A linear relationship might certainly have been expected below 100 $\mu g\ l^{-1}$. The fact that there were few data points and stream velocity was strongly influential may have confounded any such linear relationship.

The critical level of 100 $\mu g\ l^{-1}$ indicated by restricted growth of *Cladophora* and biofilm biomass in this research corresponds to the findings of Marsden *et al.* (1997). This value is in excess of the phosphorus level proposed by Horner *et al.* (1983) who found that periphytic biomass increase occurred up to a maximum of 75 $\mu g\ l^{-1}$ SRP.

Similarly, it is higher than the 75 μg l^{-1} total phosphorus proposed by Dodds *et al.* (1998) as the boundary between mesotrophy and eutrophy.

Cladophora biomass was assessed by measurement of the longest filament present on each sample stone alongside chlorophyll 'a' extraction of the whole algal crop. Comparison of these two methods of biomass estimate showed a significant correlation (Figure 6.11). This method may have some potential as a simple, effective means of assessing biomass of this algae which is not as time consuming as pigment analysis and more precise than percentage cover estimation. It also suggests that increases in biomass are due to lengthways growth as opposed to lateral growth over the surface of the substrate to which it is attached.

Diversity is generally acknowledged to be reduced by increasing pollution (Patrick *et al.*, 1954; Magurran, 1988). The result of eutrophication in many rivers is an in-stream monoculture of *Cladophora*. Comparison of diatom species diversity with phosphorus concentration however showed that there was no decline in diversity. If a range of taxa including macrophytes and bryophytes had been examined the outcome may have supported what is often anecdotally observed.

Lobo *et al.* (1995) noted that diversity indices, in particular species richness, tended to be highest at intermediate levels of organic pollution. Intuitively, this would also apply to eutrophication. A narrow range of phosphorus concentration must theoretically exist in which species richness and evenness is optimum. This phosphorus range is otherwise termed mesotrophy. Within this trophic zone, *Cladophora* biomass would be sub optimal due to limitation by phosphorus concentration. In relation to this study, restricted *Cladophora* growth would occur below approximately 100 μg l^{-1} SRP.

Twenty one per cent of streams and rivers in the Anglian Region have 100 μg l^{-1} or less SRP. Algal response to phosphorus concentration suggests that approximately eighty per cent therefore have soluble reactive phosphorus concentrations in excess of biological requirement and as such are eutrophic. Phosphorus concentrations in eighty per cent of Anglian streams and rivers are therefore conducive to the potential development of high biomass or 'nuisance' levels of *Cladophora* . Such development will however be highly dependent on in-stream physical conditions and the incidence of grazing invertebrates.

In terms of river management, restoration of rivers to a pre-eutrophication state is geographically dependent. Background levels of phosphorus are such in these study rivers to exclude the possibility of oligotrophy. Mesotrophy would however be an achievable goal. It is therefore important to understand the relationship between in-

stream organisms (particularly the photosynthesising community) and phosphorus to be able to establish the levels of phosphorus that preclude excessive growth of a few species to the detriment of overall diversity.

SUMMARY

1. Literature is reviewed examining the extent to which phosphorus controls autotrophic growth alongside nitrogen and the mechanisms for catchment export and and transport downstream. Previous research on the relationship between attached riverine algae and phosphorus is reviewed and the role of this nutrient placed into context with other growth controlling factors.

2. Preliminary comparison of factors influencing river phosphorus concentrations (measured as soluble reactive phosphorus) on a regional scale suggested that STW effluent and solid geology were important. First and second order streams made up 67% of those without effluent input. A significant difference was found between the phosphorus concentrations in chalk and clay rivers. It was therefore concluded that low phosphorus streams are most likely to be small headwater tributaries on chalk geologies.

3. Phosphorus concentrations in the Anglian Region ranged between less than 10 $\mu g\ l^{-1}$ to over 10,000 $\mu g\ l^{-1}$ SRP. However, the frequency of five years' averaged SRP data for 997 EA sites was skewed towards the lower end of this range with 21% of these data falling below 100 $\mu g\ l^{-1}$ SRP. Phosphorus concentrations in approximately half of sampling sites was between 100 and 500 $\mu g\ l^{-1}$ SRP.

4. The ratio of nitrogen to phosphorus (total oxidised nitrogen to soluble reactive phosphorus) for the upper reaches of eight rivers indicated that 83 % of sites on these rivers during summer months were potentially phosphorus limited when the boundary ratio of 10:1 was applied. For the upper Welland tributaries in Leicestershire this value was 75% during the same period of the year. [This is the ratio of nutrients within the water column; a precise indication of nutrient limitation would require laboratory bioassay or in-stream fertilisation experiments]

5. Phosphorus loadings were calculated in order to assess the contribution to in-stream phosphorus made by effluent from STWs. A significant relationship between effluent load and instream load was found in the small headwater streams of the River Welland (receiving below 5 kg SRP per day)($r^2=0.58$, $p<0.05$) and eight other rivers within the Region ($r^2=0.83$, $p<0.05$). Background load for the upper Welland catchment was 3.26 $kg\ km^{-2}\ ann^{-1}$ SRP (n=4). The amount of phosphorus from diffuse origins was assumed to be the in-stream load not accounted for by point source and background load. The percentage contribution of each was estimated for the most intensively sampled catchment (upper Welland) as 43% point source, 27% diffuse and 30% background.

6. Effluent-impacted streams showed a distinct pattern of seasonal phosphorus concentration. An increase in phosphorus concentration in the summer months followed declining flows in contrast to the opposite pattern in streams without point source. A higher percentage of total phosphorus was made up of soluble reactive phosphorus.

7. Differences in diatom species assemblage were found by multivariate analysis in phosphorus concentrations below and above 50 $\mu g\ l^{-1}$ SRP. Evidence from the species in each group and spatial ordination of groups suggested that stream velocity was responsible for diatom associations above this phosphorus concentration.

8. The Shannon index of diversity did not relate to differences in phosphorus concentration or time of year of sampling (June to October 1996). A statistical difference in diversity was observed between diatoms removed from stone and those extracted from *Cladophora*.

9. Application of the Diatom Quality Index showed differences in diatom assemblage in relation to phosphorus concentration below approximately 100 $\mu g\ l^{-1}$ SRP, but no difference above this.

10. Chlorophyll 'a' values for *Cladophora* and biofilm did not show a significant relationship with soluble reactive phosphorus. However, below 100 $\mu g\ l^{-1}$ SRP, chlorophyll 'a' values were lower than those above 100 $\mu g\ l^{-1}$ SRP.

11. A linear relationship was observed between *Cladophora* and biofilm biomass (measured as chlorophyll 'a') and stream velocity regardless of phosphorus concentration. *Cladophora* growth increased linearly with increasing velocity within the range of 0.1 to 0.2 $m\ s^{-1}$ mean summer stream velocity . Biofilm biomass decreased linearly with increasing stream velocity within this range. These relationships were observed only when values of stream velocity and biomass were averaged for the period May to October for each site.

12. Measurements of longest *Cladophora* filament length on each stone compared against chlorophyll 'a' content of *Cladophora* for the same sample stone showed a significant relationship ($r^2=0.681$, $p<0.05$). This suggests that increases in biomass were due to lengthwise growth as opposed to lateral growth over the stone.

13. Phosphorus concentrations in approximately eighty per cent of Anglian streams and rivers were above 100 $\mu g\ l^{-1}$ SRP which, in the light of the algal results, would suggest that there is potential for development of high biomass or 'nuisance' levels of *Cladophora*. Only two samples out of 52 had nuisance levels (over 100 mg chlorophyll 'a' m^{-2}) of *Cladophora*. Chlorophyll 'a' measurements were perhaps not as high as anticipated owing to the range of velocities and the shallow depth within the tributaries chosen for study.

14. This research showed the confounding influence of water velocity when comparing stream biomass of algae with phosphorus concentration. Incidence of high standing crops of these algae will depend on this and other instream physical variables and the incidence of grazing invertebrates.

APPENDIX 1.
RIVER MAPS AND CATCHMENT INFORMATION

Rivers Little Ouse, Sapiston and Wissey

The Little Ouse and Sapiston rivers lie to the south east of the Ely Ouse catchment and the Wissey to the north east. This eastern area of the catchment is more elevated than the lower lying fenland basin to the west. The Little Ouse and Sapiston rivers flow through a surface geology of boulder clay overlying chalk bedrock for most of their length. The Wissey rises on boulder clay over chalk. Surface geology then changes in the midde and lower reaches to chalk. The sampling points used in this research lay within the 'boulder clay' influenced upper reaches. Water quality along the length of the Little Ouse is designated as good (1b) to fair (2) (National Water Classification) and along the Sapiston as poor in the upper reaches to good in the lower reaches above its confluence with the Little Ouse. The Wissey is considered good (1b) to fair (2) in the upper and lower reaches and is the longest Class 1A (very good) stretch of river in the catchment in its middle section.

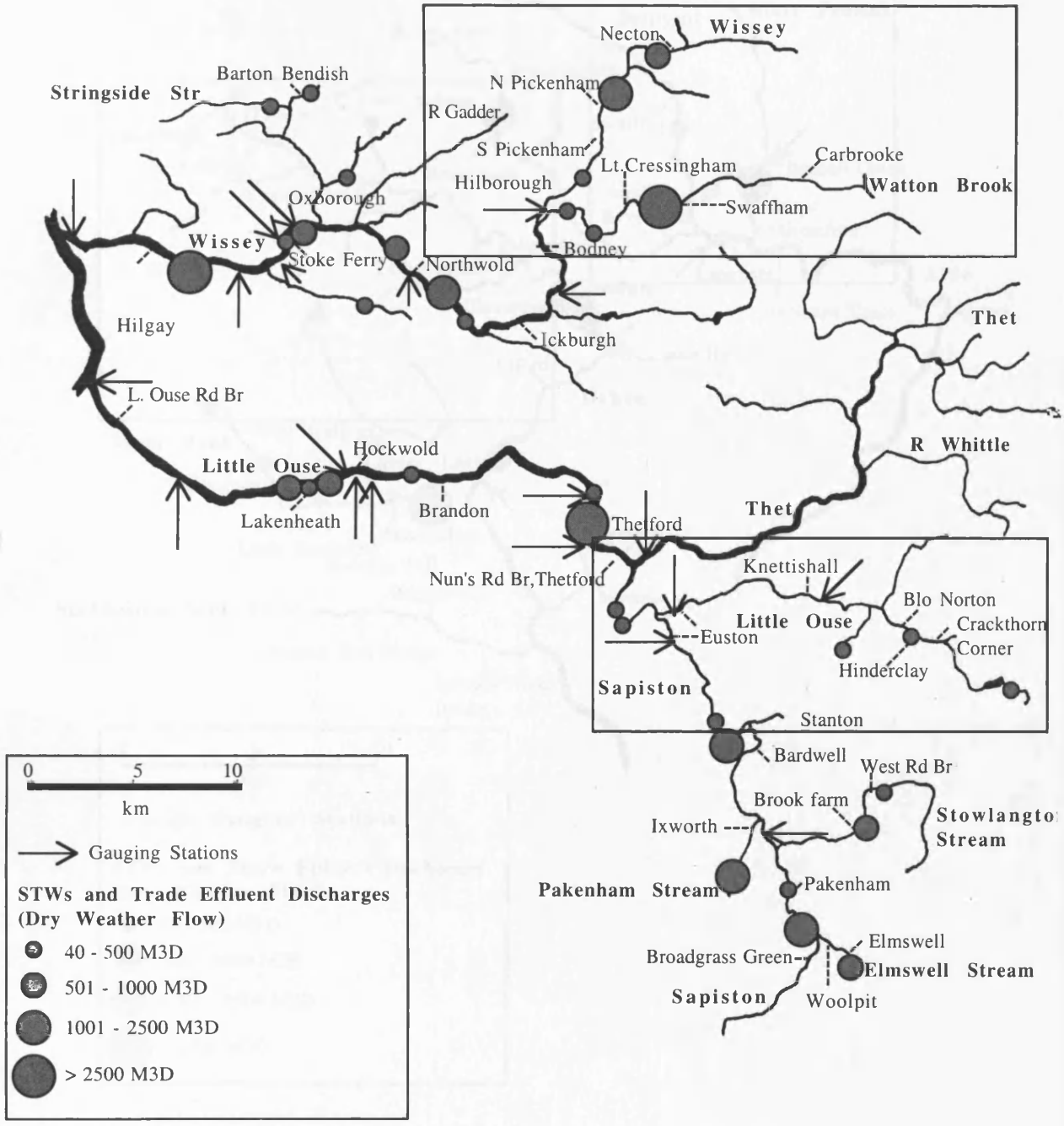


Figure 1. Sampling sites on Rivers Little Ouse, Sapiston and Wissey

Rivers Deben and Alde

The Deben, for most of its length, cuts through boulder clay and flows over a bed of sand and gravel. The heavy boulder clay surface geology within the catchment though means that there is very little interaction with the sand and gravel aquifers below ground and flows are relatively flashy. The result is a river that has naturally low flows during dry conditions. Water quality from Debenham to Cretingham is classed as fair (2). Below Cretingham to the tidal limit at Ufford the river is designated as good (1b).

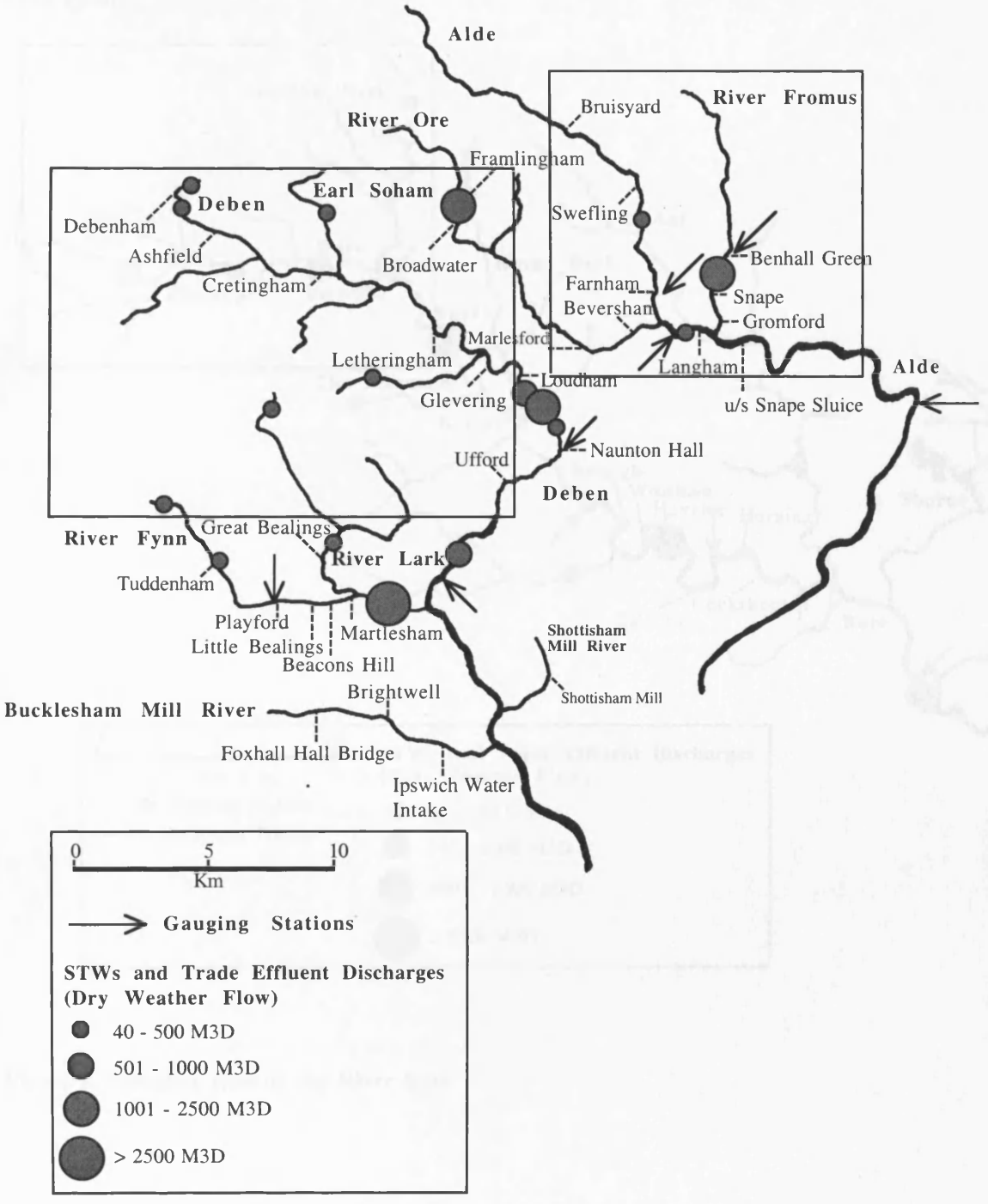


Figure 2. Sampling sites on Rivers Deben and Alde

River Bure

The Bure is located to the north of the Yare catchment. The upper reaches flow through a region of sand and gravel over chalk and Norwich crag (sand and gravel). The surface geology of the southern area of the Yare catchment is principally boulder clay. The northern areas, including the upper reaches of the Bure, Wensum and Ant, are on comparatively more permeable geologies and as a results have relatively higher and more stable baseflows. Water quality along the upper Bure is classed according to the NWC as 1a (very good).

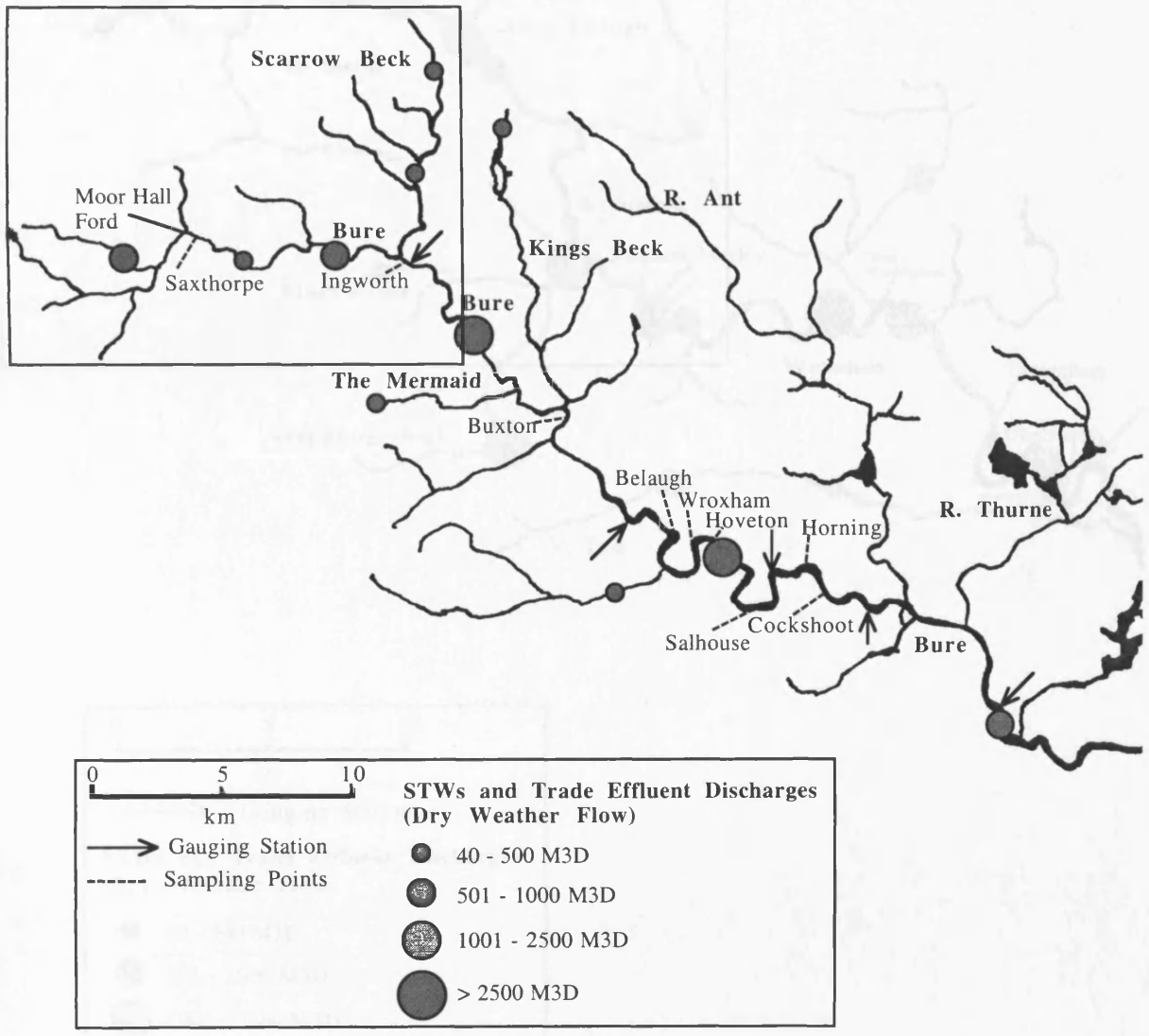


Figure 3. Sampling sites on the River Bure

River Wensum

The River Wensum is located in the north west of the Yare catchment. Outcrops of sands and gravels overlay either boulder clay or brickearth in the upper reaches. Water quality is designated 1a (very good).

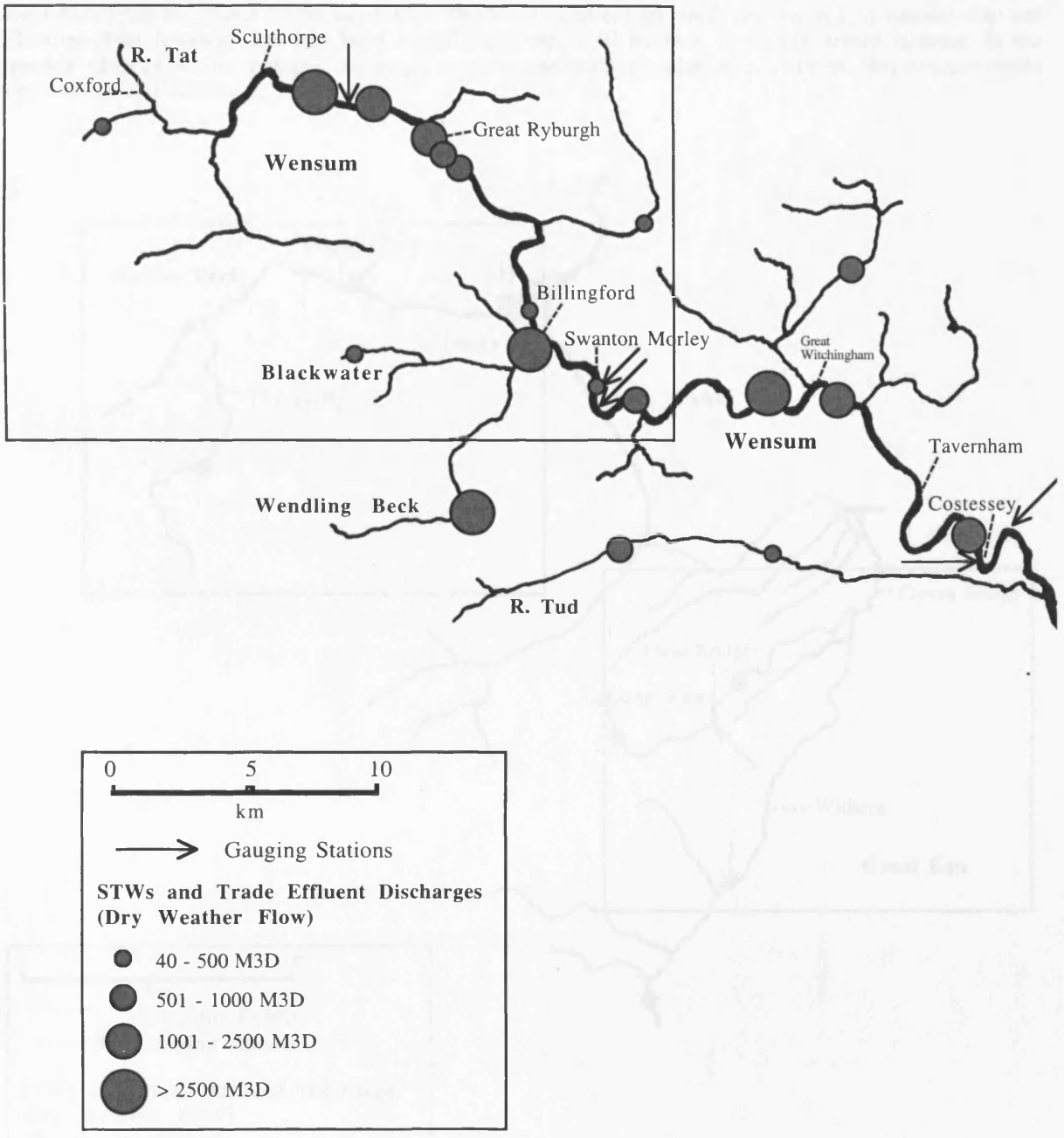


Figure 4. Sampling sites on the River Wensum

Waithe Beck and Great Eau

Waithe Beck and the Great Eau are located on the Lincolnshire coast within the Louth Coastal catchment. These rivers have very different upland and lowland areas. There is a chalk outcrop over much of the western part of the Louth Coastal catchment which extends as far as Brigsley on the Waithe Beck and about 4km from the source of the Great Eau. The central and eastern areas are covered by boulder clay and alluvium. This lowland area has been significantly modified by man to enable arable farming. In the absence of gravity and pumped drainage systems and tidal inundation protection, this region would otherwise be tidal marsh .

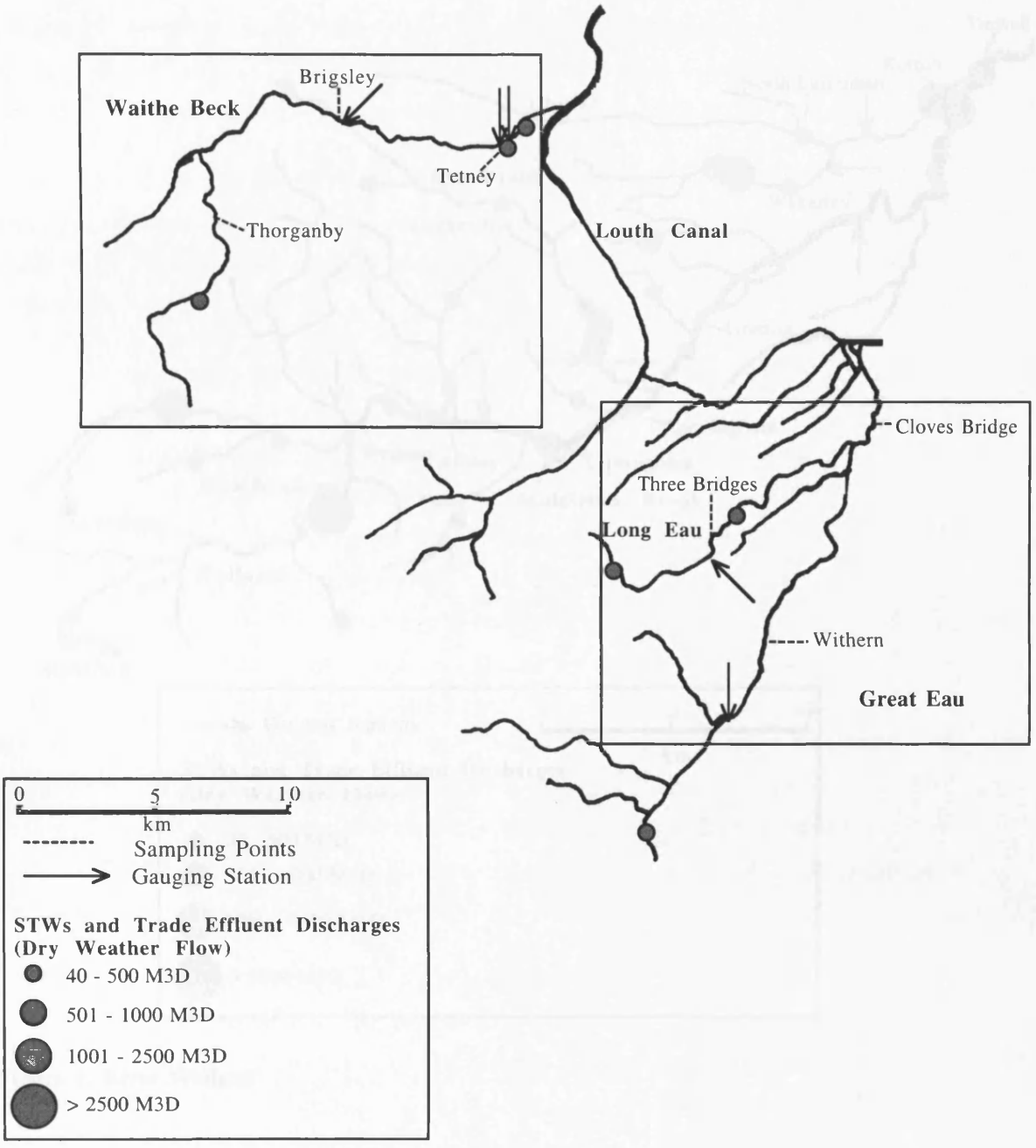


Figure 5. Sampling sites on the Great Eau and Waithe Beck

River Welland

The River Welland is a lowland clay river extending from the headwaters near Market Harborough, Leicestershire to the Wash Estuary along the Lincolnshire coast. In the upper reaches, the Welland runs in a bed of alluvium overlying lower lias clay. This surface geology of alluvium is replaced in the midde and lower reaches by middle and upper lias. From the headwaters to Tinwell just below the confluence with the River Chater, water quality is generally good (1b) to fair (2) (NWC). The poorer section designated as only fair (2) is a short stretch that lies just downstream of Market Harborough.

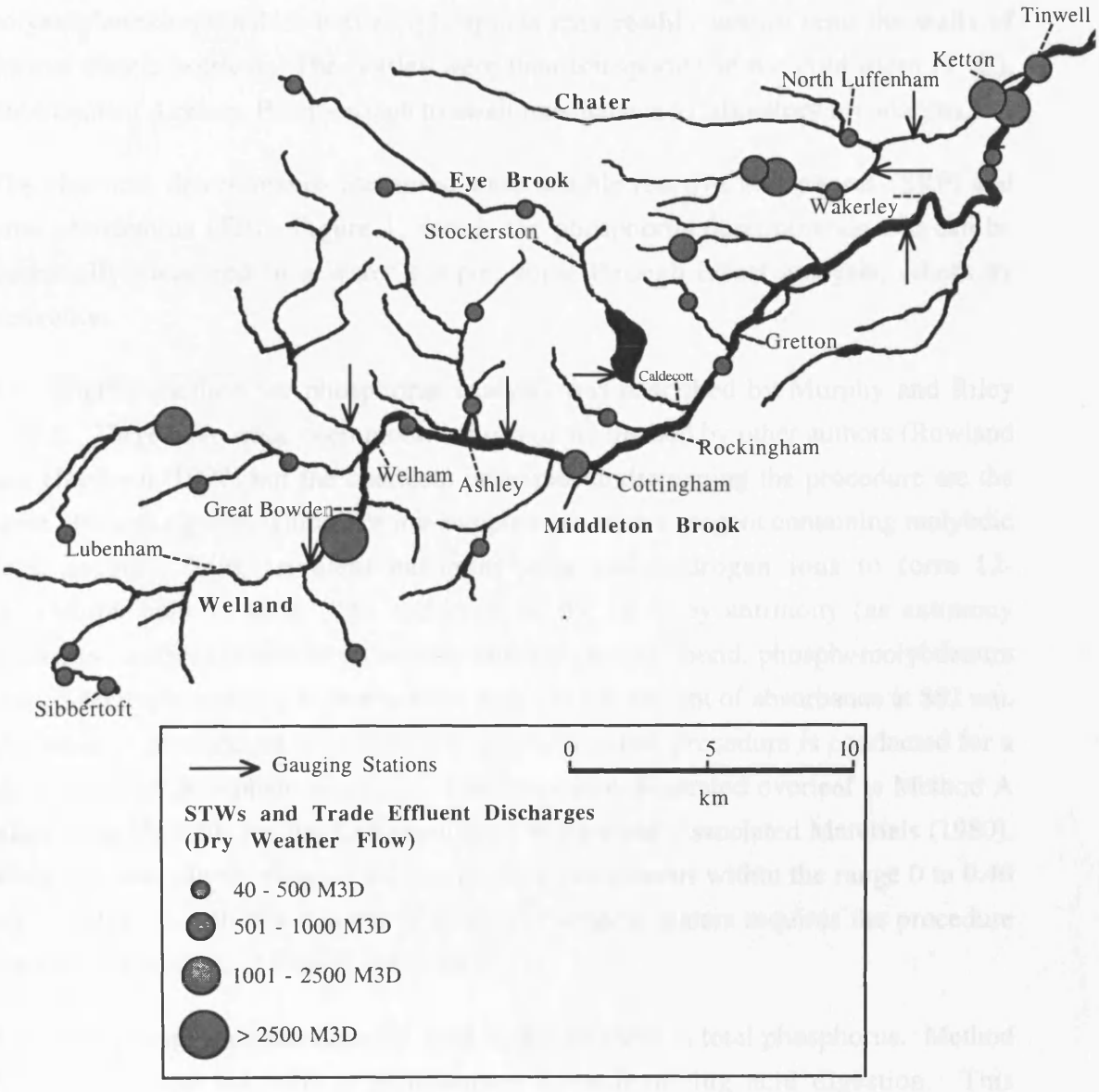


Figure 6. River Welland

APPENDIX 2.

CHEMICAL DETERMINATION OF SOLUBLE REACTIVE PHOSPHORUS AND TOTAL PHOSPHORUS

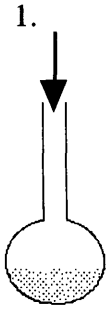
Water samples for chemical analysis were taken from stream or river sites directly where access could be gained or else with the aid of a bucket and rope. Care was taken to avoid disturbing bottom sediments which may contaminate the sample and alter the level of phosphorus. Water was removed as far as possible from the middle of the channel, mid-water column. Samples were decanted or taken directly into polyethyleneterephthalate bottles (phosphate may readily absorb onto the walls of normal plastic bottles). The bottles were then transported to the cold room (4 °C), Environment Agency, Peterborough to await transference to laboratory for analysis.

The chemical determinands measured were soluble reactive phosphorus (SRP) and total phosphorus (TP). Figure 1. details the phosphorus determinands that can be potentially measured in a water sample; some through direct analysis, others by derivation.

The original method for phosphorus analysis was described by Murphy and Riley (1962). There have since been modifications to the method by other authors (Rowland and Haygarth, 1997) but the chemical principles underpinning the procedure are the same. Phosphate ions within a water sample react with a reagent containing molybdic acid, ascorbic acid, trivalent antimony ions and hydrogen ions to form 12-molybdophosphoric acid. The reduction of the latter by antimony (as antimony potassium tartrate) produces an intense blue/purple compound, phosphomolybdenum blue. Spectrophotometry is then used to measure the amount of absorbance at 882 nm. The mass of phosphorus is extrapolated given the same procedure is conducted for a set of standard phosphate solutions. The procedure illustrated overleaf is Method A taken from Methods for the Examination of Waters and Associated Materials (1980). Method A sets out to measure soluble reactive phosphorus within the range 0 to 0.40 mg l^{-1}. The phosphorus content of more oligotrophic waters requires the procedure described in Method B (MEWAM, 1980).

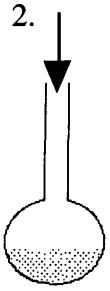
The other phosphorus determinand used in this research is total phosphorus. Method A is again used but after a pretreatment stage involving acid digestion. This pretreatment converts all forms of phosphorus to reactive phosphorus which can then be analysed as described above.

The following describes the procedure for determining soluble reactive phosphorus (SRP) and is taken from Method A, Methods for the Examination of Waters and Associated Materials (MEWAM, 1980). The sample is filtered through a 0.45 μm membrane filter prior to the following analysis for measurement of soluble reactive phosphorus; without filtration the phosphorus fraction measured would be total reactive phosphorus (TRP)(Figure 1).

1. 

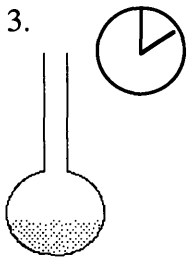
Transfer a volume of sample not exceeding 40 ml to a 50 ml calibrated flask adding sufficient deionised or distilled water to make up the volume to 40 ml. The volume of sample to be used can be estimated from the following table:

Expected concentration (mg P/l)	Aliquot to be used (ml)
<0.2	40.0
0.2-0.5	20.0
0.5-1	10.0
1-2	5.0
2-5	2.0
5-15	1.0

2. 

Add 8 ml of mixed reagent using an automated pipette.

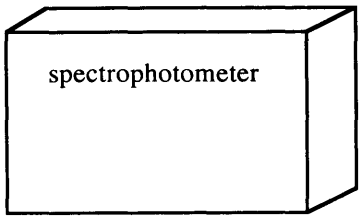
Mixed reagent is composed of 14% sulphuric acid, 4% ammonium molybdate, 0.28% antimony potassium tartrate and 1.76% ascorbic acid. These chemicals are mixed in the ratio 10:3:1:6 respectively. Reagent should be prepared fresh as required and kept refrigerated when not in use.

3. 

Leave for 10 minutes

4. 

Measure absorbance of solution at 882 nm using 40 mm cells with a reference cell of distilled or deionised water



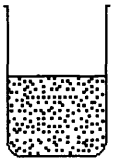
Stages 1 to 4 are then repeated using 40 ml of deionised or distilled water in place of the water sample. This measurement provides a blank.

Stages 1 to 4 may also be repeated using the water sample but replacing the mixed reagent with 14 % sulphuric acid. In this way, the extent to which the colour and turbidity of the water sample contribute towards absorbance can be assessed. This procedure may be foregone once it has been established that the contribution is not significant.

The absorbance due to phosphorus in the water sample is given by $A_p=A_s-A_b$, where A_s is the absorbance of the water sample and A_b the absorbance of the blank. Where correction is required for significant coloration by the water sample the equation becomes $A_p=A_s-A_b-A_c$; A_c being the absorbance when sulphuric acid is used in place of the mixed reagent.

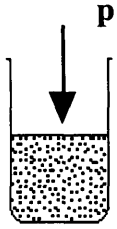
The mass of phosphorus (in μg P) is determined by comparison of the value derived for A_p with a calibration curve. Such a calibration curve is compiled from the results of performing stages 1 to 4 with known phosphate solutions. Standard phosphate solution is made up by dissolving 0.4394±0.0005 g of anhydrous potassium dihydrogen orthophosphate in water and diluting to 1 litre in a calibrated flask (1 ml of this solution is therefore equivalent to 100 μg P). Further dilution of 10±0.02 ml of this with 1 litre of water yields a final solution where 1 ml is equivalent to 1 μg P. To a series of 50 ml calibrated flasks, this standard solution is added in volumes of 0.00, 1.00, 2.00, 5.00, 10.00 and 15.00 ml. These volumes translate to masses of 0.0, 1.0, 2.00, 5.0, 10.0 and 15.0 μg P. The results of absorbance measurements for each (A_s-A_b) can then be plotted against μg P. Comparison of this curve with the absorbance measurement for the water sample gives a value for mass M (in μg P). The phosphorus concentration (C, measured in mg l^{-1}) in the original sample is calculated from C=M/V, where V is the volume of water sample analysed at the outset.

The following describes the digestion method for determining Total Phosphorus (MEWAM, 1980). This method precedes Method A as described above. The sample is not filtered.

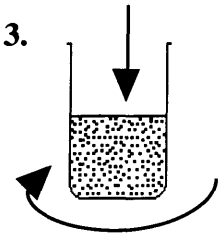
1. 

Transfer a volume of sample not exceeding 40 ml to a 150 ml graduated beaker adding sufficient deionised or distilled water to make up the volume to 40 ml. The volume of sample to be used can be estimated from the following table:

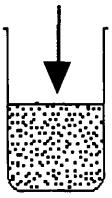
Expected concentration (mg P/l)	Aliquot to be used (ml)
<0.2	40.0
0.2-0.5	20.0
0.5-1	10.0
1-2	5.0
2-5	2.0
5-15	1.0

2. pH 

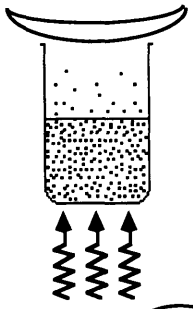
Neutralize with sodium hydroxide or sulphuric acid if necessary, the quantity of either should be determined on a separate portion of the sample

3. 

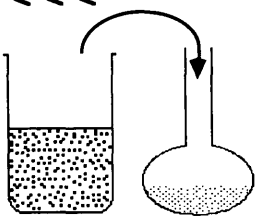
Add 0.2±0.01 g ammonium persulphate and swirl to dissolve

4. 

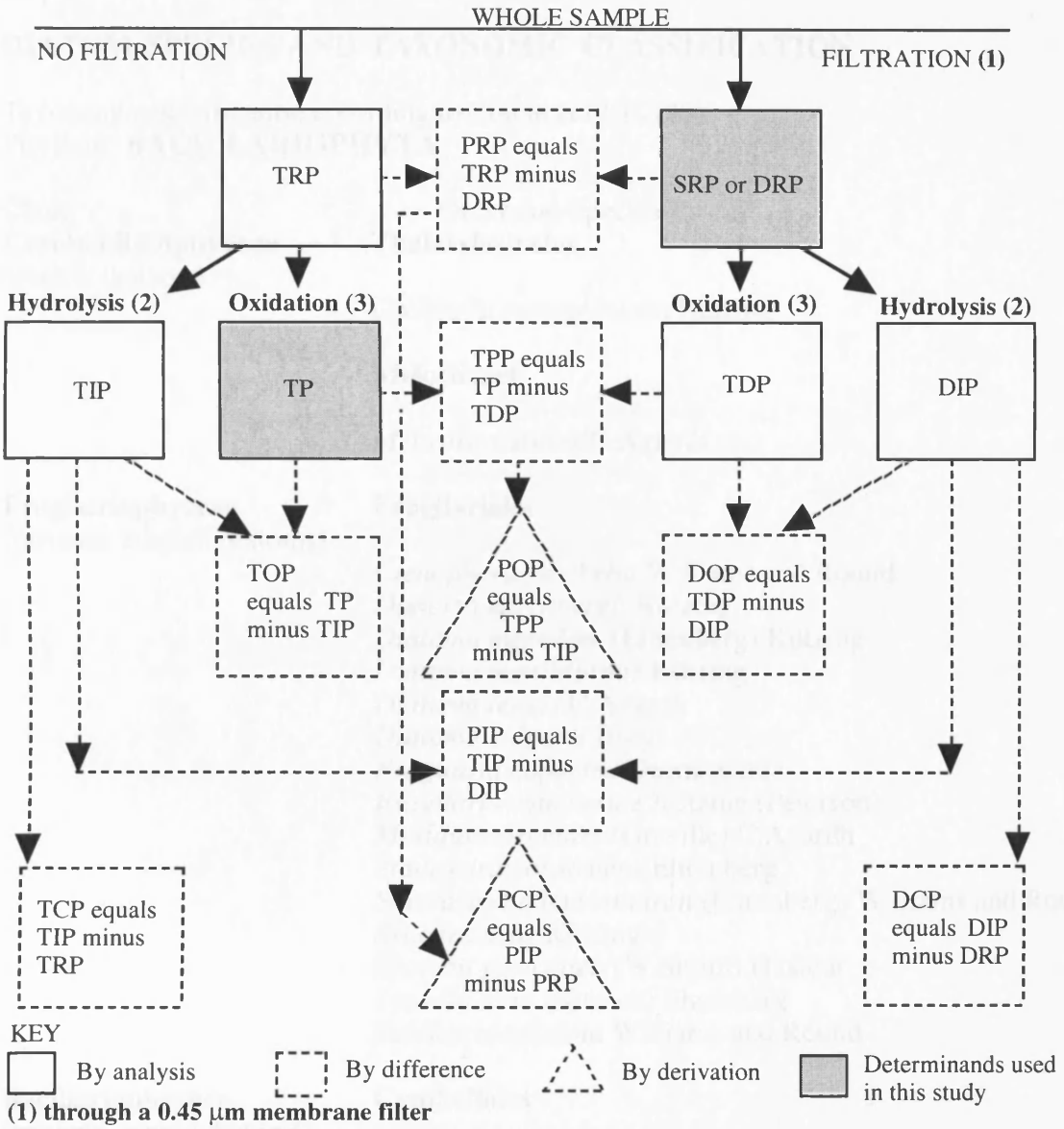
Add 10±0.2 g sulphuric acid and mix well

5. 

Cover beaker with watch glass and heat to boil the solution gently for 25±5 minutes. The solution should not be allowed to evaporate to less than 15 ml and may be maintained at 20±5 ml by addition of water. Allow solution to cool and then neutralize with sodium hydroxide.

6. 

Transfer solution to a 50 ml calibrated flask and continue with the procedure for determination of total reactive phosphorus (see Method A).



(1) through a 0.45 μm membrane filter
(2) acid hydrolysis for determination of inorganic component only
(3) acid oxidation/digestion for inorganic and organic components (see above)

Determinands obtained by direct analysis		Determinands derived by calculation	
TRP	Total Reactive Phosphorus	PRP	Particulate Reactive Phosphorus
SRP (DRP)	**Soluble Reactive Phosphorus =Dissolved Reactive Phosphorus**	PIP	Particulate Inorganic Phosphorus
TIP	Total Inorganic Phosphorus	TCP	Total Condensed Phosphorus
DIP	Dissolved Inorganic Phosphorus	DCP	Dissolved Condensed Phosphorus
TP	**Total Phosphorus**	PCP	Particulate Condensed Phosphorus
TDP	Total Dissolved Phosphorus	TPP	Total Particulate Phosphorus
		TOP	Total Organic Phosphorus
		DOP	Dissolved Organic Phosphorus
		POP	Particulate Organic Phosphorus

Figure 1. Relationship between phosphorus determinands (adapted from MEWAM, 1980 and Mainstone *et al.* 1996)

APPENDIX 3.

DIATOM SPECIES AND TAXONOMIC CLASSIFICATION

Taxonomic classification according to Round *et al.* (1990)
Phyllum: **BACILLARIOPHYTA**

Class	Order and Species
Coscinodiscophyceae (centric diatoms)	**Thalassiosirales**
	Cyclotella meneghiniana Kützing
	Melosirales
	Melosira varians C.Agardh
Fragilariophyceae (pennate, araphid diatoms)	**Fragilariales**
	Ctenophora pulchella Williams and Round
	Diatoma ehrenbergii Kützing
	Diatoma mesodon (Ehrenberg) Kützing
	Diatoma moniliformis Kützing
	Diatoma tenuis C.Agardh
	Diatoma vulgaris Bory
	Fragilaria capucina Desmazières
	Fragilaria vaucheriae Kützing (Peterson)
	Meridion circulare (Greville) C.Agardh
	Staurosira construens Ehrenberg
	Staurosirella leptostauron (Ehrenberg) Williams and Round
	Synedra acus Kützing
	Synedra parasitica (W.Smith) Hustedt
	Synedra ulna (Nitzsch) Ehrenberg
	Tabularia tabulata Williams and Round
Bacillariophyceae (pennate, raphid diatoms)	**Cymbellales**
	Cymbella aspera (Ehrenberg) Cleve
	Cymbella lanceolata (Ehrenberg) Van Heurck
	Encyonema caespitosa Kützing
	Encyonema minutum (Hilse in Rabenhorst) D.G.Mann
	Encyonema prostratum (Berkeley) Kützing
	Encyonema silesiacum (Bleisch in Rabenhorst) D.G.Mann
	Gomphonema augur Ehrenberg
	Gomphonema augustatum (Kützing) Rabenhorst
	Gomphonema clavatum Ehrenberg
	Gomphonema olivaceum (Hornemann) Brébisson
	Gomphonema parvulum Kützing
	Gomphonema truncatum Ehrenberg
	Reimeria sinuata (Gregory) Kociolek and Stoermer
	Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot

Achnanthales

Achnanthidium delicatulum Kützing
Achnanthidium lanceolatum Brébisson
Achnanthidium minutissimum Kützing
Cocconeis pediculus Ehrenberg
Cocconeis placentula Ehrenberg

Naviculales

Diploneis sp.
*Gyrosigma attenuatum (*Kützing) Rabenhorst
Navicula capitoradiata Germain
Navicula capitata Ehrenberg
Navicula cari Ehrenberg
Navicula cryptocephala Kützing
Navicula cryptotenella Lange-Bertalot
Navicula gregaria Donkin
Navicula lanceolata (C.Agardh) Ehrenberg
Navicula menisculus Schumann
Navicula radiosa Kützing
Navicula tripunctata (O.F.Müller)
Navicula veneta Kützing

Thalassiophysales

Amphora libyca Ehrenberg
Amphora ovalis Kützing
Amphora pediculus (Kützing) Grunow

Bacillariales

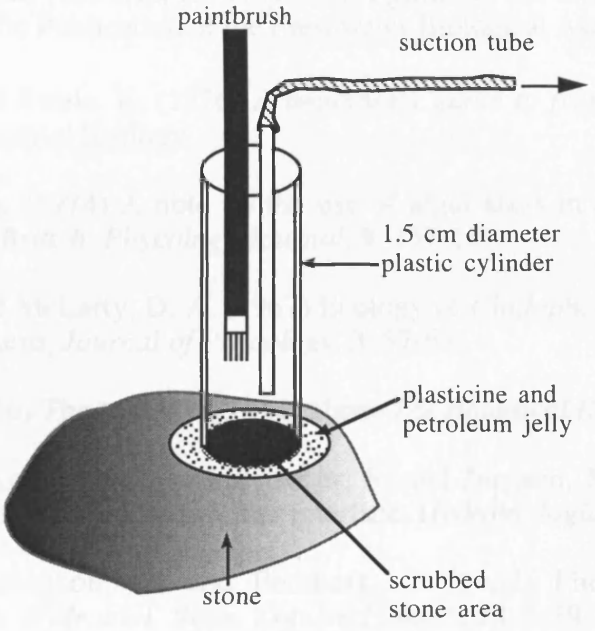
Hantzschia amphioxys (Kützing) Grunow
Nitzschia acicularis (Kützing) W.Smith
Nitzschia amphibia Grunow
Nitzschia dissipata (Kützing) Grunow
Nitzschia heufleriana Grunow
Nitzschia linearis (C.Agardh) W.Smith
Nitzschia palea (Kützing) W.Smith
Nitzschia recta Hantzsch ex Rabenhorst
Nitzschia sigmoidea (Nitzsch) Ehrenberg
Nitzschia vermicularis (Kützing) Hantzsch

Surirellales

Cymatopleura elliptica (Brébisson) W.Smith
Cymatopleura solea (Brébisson) W.Smith
Surirella brebissonii Krammer and Lange-Bertalot

APPENDIX 4.

APPARATUS USED FOR REMOVAL OF BIOFILM FROM A STONE SURFACE



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