Phytoplankton dynamics of the river nene, England

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PHYTOPLANKTON DYNAMICS OF THE RIVER NENE, ENGLAND

by

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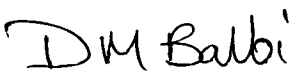
This thesis results entirely from my own work and has not been offered in candidature for any other degree or diploma.



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ABSTRACT

The distribution of suspended algae was investigated in a 69-km length of a small lowland river in the UK, the Nene (annual median discharge at km 91.7 = 6 $m^3 s^{-1}$). Variations in chlorophyll *a* data collected between 1975 and 1998 by water management organisations at km 91.7 were evaluated against a range of physical and chemical variables. Interpretation was aided by additional sampling between 1993 and 1997.

The latter half of the 24-year period had significantly higher temperatures and sunshine-hours and significantly lower ammonium concentrations. Discharge, temperature and sunshine-hours were significant predictors of chlorophyll concentration, particularly between January and June, and spring chlorophyll maxima ranged from 106 to 276 $\mu g L^{-1}$.

Centric diatoms were the most abundant taxa in the main-river and, in the absence of other limiting factors, appeared to be restricted by the availability of silica. There was also evidence that the centric diatoms suffered from severe parasitism.

Inter-year phytoplankton abundance was most variable in the summer, and years with abundant submerged macrophytes had particularly low phytoplankton numbers.

Spring phytoplankton peaks occurred earlier and had smaller amplitude at downstream sites than those further upstream. Average spring chlorophyll concentrations (April - June) increased significantly between km 22.4 and km 43.9, thereafter remaining high to km 91.7. Spatial trends were attributed to changes in channel morphology, retention time, dead zones, longitudinal variations in current velocity, temperature and silica limitation.

An appraisal of the Utermöhl method of counting phytoplankton was made and a new technique proposed, called 'spaced fields'. The spaced fields method accurately identified small changes in phytoplankton abundance and was used to identify short-term temporal and small-scale spatial trends in the Nene.

ABBREVIATIONS AND ACRONYMS

$\bar{x}$	Arithmetic mean
∅	Diameter
χ^2	Chi square statistic
[chl]	Chlorophyll concentration ($\mu g\ L^{-1}$)
ANOVA	Analysis of variance
BFI	Base flow index
BOD	Biochemical oxygen demand
CCA	Canonical correspondence analysis
CANOCO	Canonical community ordination
CCAP	Culture Collection of Algae and Protozoa. FBA, Ambleside, Cumbria
CuSum	Cumulative sum
d	Day
DIC	Dissolved inorganic carbon
df	Degrees of freedom
DO	Dissolved oxygen
D/S	Downstream
EC	European Community
EA	Environment Agency
FBA	Freshwater Biological Association
FRP	Filterable reactive phosphorus
f_e	Frequency expected
f_o	Frequency observed
fo	Form (taxonomic classification)
GALD	Greatest axial linear dimension of algal cell or colony (excluding flagella, spines or fibres)
h	Hour
I	Irradiance
ind	Individual
ha	Hectare
HPLC	High performance liquid chromatography
l	Length
LCL	Lower confidence limit (95% level unless otherwise stated)
LIMNDAT	Limnological database system
LOI	Loss on ignition
LOIS	Land ocean interaction study (see Leeks et al., 1997)
MS	Mean square
n	Total number of sampling units in sample
N:P	N:P ratio
NGR	National grid reference
NLS	EA's National Laboratory Service
NRA	National Rivers Authority
NS	Not significant
p	Probability
PEEP	Phytoplankton ecology evaluation program
PET	Polyethylene terephthalate
PR	Pseudo-random fields
P:Si	P:Si ratio

Q	Discharge ($m^3 s^{-1}$)
R&D	Research and development
r^2	Square of correlation between observed and fitted *y* values. Fraction of the variation in *y* that is explained by the fitted equation
RF	Random fields
SEM	Scanning electron microscope
SF	Spaced fields
SIMCOUNT	Computer simulation of counting algae in sedimentation chambers
Sp	Species (plural, spp.)
SS	Sum of squares
ST	Spaced transects
SD	Standard deviation
STW	Sewage treatment works
Syn	Synonym
S^2	Variance
t	Time
T	Temperature (°C)
TN	Total nitrogen
TON	Total oxidised nitrogen (NO_2-N + NO_3-N)
TP	Total phosphorus
TRP	Total reactive phosphorus
UCL	Upper confidence limit (95% level unless otherwise stated)
U/S	Upstream
UWWT	Urban waste water treatment
var	Variety (taxonomic classification)
w	Watts
z_{eu}	Euphotic depth (m)
z_s	Secchi disc depth (m)

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1 INTRODUCTION

1.1 Prelude

This thesis came about as part of a larger National Rivers Authority (NRA) and later Environment Agency (EA) project to investigate the impact of P removal from major sewage treatment works (STW) discharging to the River Nene. P removal was originally a water company initiative and was undertaken as a prelude to the implementation of the Urban Waste Water Treatment (UWWT) Directive, which requires P concentrations to remain below prescribed limits at major discharges. At that time I was employed by the NRA and was asked to investigate phytoplankton dynamics in the river system, as a part of the wider project with which I was also involved. Historical data for suspended chlorophyll at a downstream site on the Nene indicated the periodic occurrence of high concentrations of phytoplankton (relative to lakes). I had previously worked on lake and reservoir phytoplankton, but had little experience of suspended algae in rivers so this project presented an ideal opportunity to expand my knowledge and experience. There was insufficient time to undertake a detailed evaluation of river phytoplankton within the constraints of the wider project and it was therefore decided to augment the routine work with this part-time research (with the financial and practical support of the NRA, and later EA).

Shortly before commencing this research I was involved in another NRA project which aimed to implement standardised phytoplankton methodologies to the Anglian Region. During this time I made progress in this area, but there was insufficient time to undertake a full appraisal of counting in sedimentation chambers, which would have benefited from computer simulation and detailed analysis. As counting phytoplankton was fundamental to the success of the Nene investigation it was decided to combine these two complimentary pieces of work, which resulted in this thesis.

1.2 Introduction

Interest in river phytoplankton has increased in recent years, but attention has focused on larger rivers. Systems of fourth order or greater normally support distinctive phytoplankton communities (Vannote et al., 1980; Reynolds and Descy, 1996), with their distribution and periodicity regulated by physical (Descy and Gosselain, 1994) , chemical (Swale, 1969) and/or biological factors (Gosselain et al., 1998b).

The introductory material that follows contains a review of key factors that influence the abundance of river phytoplankton. This is succeeded by consideration of the timing, amplitude and species composition of river phytoplankton populations. The review commences with an appraisal of quantitative methods, as these are fundamental to investigations of phytoplankton dynamics.

1.3 Quantitative methods

Introduction

Accurate estimates of phytoplankton abundance are essential to quantitative investigations of phytoplankton dynamics and such estimates generally involve a measure of quantity or biomass. Quantitative investigations often involve counting the taxa present in a subsample and, if required, the results can be converted to biomass, following further measurements. However, photosynthetic pigment content or cellular carbon are more rapidly attained and are widely used as measures of phytoplankton biomass.

Biomass

All planktonic autotrophs contain pigments, which become excited in the presence of light and, along with a complex of biochemical activity, form the basis of photosynthesis and carbon assimilation. The principal photosynthetic pigments are chlorophyll *a*, *b* and *c* and several forms of carotenoids (Lewin, 1974). Of all the pigments chlorophyll *a* is the most widespread, being found in all phytoplankton phyla (Meeks, 1974). Cellular carbon is another widely used measure of phytoplankton biomass and the conversion ratio of 50:80 (carbon:chlorophyll) is often used (Harris, 1986). Gosselain et al. (1994) compared two methods of estimating cellular carbon, using estimations from measured cell volumes and chlorophyll conversion, and found poor agreement between the two approaches.

Using chlorophyll concentration as a surrogate for phytoplankton biomass has numerous limitations. Different species contain different photosynthetic pigments (Boney, 1974) and the same taxa under varying physiological conditions can contain differing quantities of chlorophyll per unit volume (Meeks, 1974). Additionally, green algae and cryptophytes contain proportionally more chlorophyll per unit volume than diatoms or blue-green algae (Reynolds, 1993). Another important consideration is the quantity of degradation products, collectively referred to as phaeopigment, which can

introduce error into chlorophyll results. Phaeopigment is a degraded form of chlorophyll and can be present in appreciable amounts (Marker, 1992). Kowalczewski and Lack (1971) examined the quantities of total and degraded chlorophyll in the Kennet and Thames at Reading and found the Kennet to have proportionally more phaeopigment (average 50%) than the Thames (average 32%). Recent developments in pigment analysis using HPLC provide a mechanism of identifying the abundance of the main taxonomic groups in mixed samples (Descy et al., 2000).

Fluorometric estimations of chlorophyll concentrations are widely used, also. This sensitive method (Butterwick et al., 1982) can be applied directly to water samples, and has become known as '*in vivo*' fluorecence (Jones, 1979).

Quantity and identification

Direct counting has several advantages over other methods of assessing algal abundance, such as pigment analysis. Firstly, the algae are actually seen thus allowing changes in appearance to be observed, such as dead or dying cells, parasitism, presence of spores or heterocysts. Thus providing the opportunity to collect ecological information which would be otherwise unattainable. Direct counting also allows small numbers of specific algae to be distinguished from others or detritus (Lund and Talling, 1957; Butterwick et al., 1982).

A prerequisite to cell counts is accurate identification and live examination of specimens is by far the best initial approach (Belcher and Swale, 1979). Cell colour, size, shape and movement are all key taxonomic features (Fritsch, 1948) that can be lost or severely altered following fixation. However, few phytoplankton can satisfactory be counted in the live state and examination of live material followed by a count of preserved specimens is probably the best compromise.

Methods of direct counting were reviewed by Jones (1979), which included various counting devices such as sedimentation chambers. Utermöhl (1958) standardised the method of concentrating and counting phytoplankton in sedimentation chambers using an inverted microscope, and this technique was given statistical consideration by Lund et al. (1958).

1.4 Key factors controlling river phytoplankton

Introduction

This section contains a review of key factors that control river phytoplankton abundance and periodicity. This section also contains background information that is particularly pertinent to this thesis.

1.41 Physical environment

Velocity and discharge

River velocity and discharge are intrinsically linked, and are both often referred to as 'flow'. Velocity is the current speed (m s^{-1}) whereas discharge refers to volume per unit time (m^3 s^{-1}) and is often calculated as the product of average velocity and the cross-sectional area of the wetted channel. Velocity varies both laterally and vertically within a river and average velocity is often calculated as the product of several current readings across a channel divided by their sum, with the mean vertical velocity being found at 0.6 of the total depth, from the surface (Shaw, 1998). A significant positive relationship exists between average velocity and discharge, both increasing in a downstream direction (Round et al., 1998). Therefore both velocity and discharge can be used as a correlative for evaluating the impact of flow on phytoplankton, although if an approximation of retention time or distance travelled by suspended algae is needed then velocity must be used.

Water Management Bodies continuously record discharge at permanent gauging stations, which usually consist of a concrete weir and a water level recorder. Discharge is measured as level or 'stage height' at these sites, following the calibration of the stage-discharge relationship (Shaw, 1998). These kinds of gauging stations are not suitable for navigable river systems and measuring flow here is often less accurate than in smaller rivers, where weirs can be constructed. In recent years ultrasonic gauging equipment has been used in larger rivers, although its accuracy is often inhibited by an accumulation of silt or submerged plant growth (D. Glenn, pers. comm.). If discharge measurements are required for ungauged sites then they can be calculated by catchment scaling from gauged sites, preferably including the influence of discharges and abstractions and supported by spot measurements. Water Management Bodies rarely record velocity *per se* but use measurements as part of discharge calculations.

However, estimates of average velocity can to be made from discharge if the discharge-level and level-wetted area relationships are known or attainable, or by using the method described by Round et al. (1998).

Very high discharge inhibits or suppresses phytoplankton development by continuously removing cells before they have time to attain a high density. High discharge can also result in elevated levels of suspended solids, which reduce light availability and suppress growth at depth (Reynolds, 1994). Low discharge may also inhibit phytoplankton development through insufficient turbulence to maintain algae (particularly diatoms) in suspension (Gosselain et al., 1994). The relationship between turbulence and velocity is of great significance to the structure of phytoplankton communities in rivers and these factors are intrinsically linked to depth and channel roughness.

Depth, turbulence and channel roughness

Turbulent flow is necessary to maintain most phytoplankton in suspension (Reynolds, 1993). In fact, particles will remain in suspension for over four times longer in a continuously mixed system compared to a static column (Smith, 1982). At low velocity, viscosity can overcome turbulence and flow can become laminar (Smith, 1975) producing unfavourable conditions for all but the most buoyant species. The extent of turbulence in rivers is determined by depth and velocity, with shallow slow velocity systems tending towards laminar flow and deeper rivers being more likely to produce turbulence (Reynolds, 1994a). Indeed, Reynolds et al. (1990) demonstrated that the loss of suspended particles from flowing water was influenced more by depth than velocity.

Channel roughness impacts directly on velocity by creating frictional resistance. A 'laminar sub-layer' of fluid exists next to solid boundaries, such as the riverbed in deep, slow flowing rivers (Carling, 1992). If objects on the riverbed project above this sub-layer then the surface is considered as 'dynamically rough' and can impede velocity. For example, a river with a depth of 3 m and a slope of 1:10000 would have its average velocity reduced from approximately 175 to 75 cm s^{-1} when bed roughness of a height of 10 cm is introduced (Smith, 1975). Seasonal variation in velocity, depth and submerged macrophyte growth will have significant implications on the amount of turbulence produced and therefore the environment of phytoplankton.

Greater turbulence will be necessary to maintain those algae of greatest density (such as diatoms) in suspension, and too much turbulence has been shown to be detrimental to the green alga *Scenedesmus quadricauda* (syn. *S. communis*) (Hondzo and Lyn, 1999), which is common in British rivers (Belcher and Swale, 1979).

Sedimentation and entrainment

Most algae, with the exception of gas-vacuolate blue-green algae, have a density greater than water and in the absence of turbulent motion or motile organs are destined to sink (Round, 1984). Sinking velocity varies considerably between the groups, with the heavier diatoms sinking at least twice as quickly as non-siliceous algae with similar geometric properties (Sommer, 1988).

Phytoplankton have evolved several mechanisms to depress their settlement velocity, which relate to size, density and form resistance. Reynolds (1993) compared the settling velocity of live and freshly killed *Stephanodiscus astraea* cells. A positive relationship was found between the size of dead diatoms and settling velocity, with larger algae sinking more quickly. However, this relationship did not hold true for live specimens, with relatively low settlement velocities throughout the size classes. These experiments suggest that some properties of the living cells are instrumental in reducing sinking velocity, and could relate to extra-cellular projections or an intra-cellular reduction in density. Reduced density can be achieved through the storage of relatively 'light' lipids and the secretion of mucilage. Lipids generally account for between 2% and 20% dry weight of algae (Reynolds, 1993) and as these are often lighter than water they can contribute to an overall reduction in density. Several types of planktonic algae, particularly the blue-greens and the greens secrete mucilage. Mucilage increases the size of an alga with a minimal increase in density, which up to a point will reduce sinking rate (Reynolds, 1993).

Variations in form resistance are utilised by many algae as a mechanism to reduce sinking rate, without altering density. Spherical cells will sink more quickly than a flattened cell of equal density, providing the latter can maintain its greatest linear dimension perpendicular to the direction of sinking. Many diatoms, such as *Stephanodiscus hantzschii* which are common in European rivers, increase their form resistance through chain formation and the production of fibrous projections. Walsby and Xypolyta (1977) compared the sinking velocity of the centric diatom *Thalassiosira*

fluviatilis before and after the removal of its chitin fibres, with removal resulting in a doubling of the sinking rate.

Colony formation occurs in many groups and will inevitably result in an increase in overall sinking rate. However, if an increase in size has other advantages, such as resistance to grazing, then an increased size could be offset by an increase in form resistance (Reynolds, 1993). Examples of colonies that increase form resistance are filaments (e.g. *Melosira varians*), discs (e.g. *Pediastrum duplex*), ribbons (e.g. *Fragilaria crotonensis*) and stellate groups (e.g. *Actinastrum hantzschii*).

Dead zones

Fritsch (1903) and later Margalef (1960) recognised the importance of eddies and backwaters as areas where phytoplankton populations could develop and contribute to those in the main flow. These areas of retention exist throughout river systems and are often referred to as dead zones. Wallis et al. (1998) investigated the influence of dead zones on solute transport in rivers in north-west England. They considered dead zones to be areas of dynamic storage where some mixing takes place. These areas include the periphery of open channels, within turbulent eddies, wakes around roughness elements and reverse flow associated with pools and bends. Solutes that entered these areas are temporarily trapped and mixed with the contents of the dead zone, to some degree, before being released back into the main flow. Their work involved the evaluation and timing of tracer concentrations at fixed points along a river reach. Wallis et al., (1989) found that solute retention was reach specific and greatest in channels with most structural heterogeneity.

If dead zones could retain solutes then it is likely that they could also provide areas for phytoplankton development. Reynolds and Glaister (1992) evaluated *in situ* growth at fixed points in many UK rivers in April, June and September. They concluded the downstream increase in phytoplankton biomass could not be possible without a contribution of algae from dead zones. This work followed detailed analysis of the phytoplankton of a single dead zone in the Severn (Reynolds, 1991). The low fluid exchange of dead zones is also likely to result in them warming and retaining heat more readily than the main river, as indicated by Reynolds and Gaister (1992). Other studies indicate that high phytoplankton abundance is attainable in rivers through *in situ* growth alone and can occur without the contribution of dead zones (Skidmore et al., 1998).

Temperature and solar radiation

Algae utilise solar radiation to fix carbon during photosynthesis and the rate at which these biochemical processes occur is temperature-dependent. Therefore, temperature and solar radiation have a great influence on phytoplankton growth, and as they are intrinsically linked it is often difficult to distinguish the contribution of each factor (Soeder and Stengel, 1974). In many species the relationship between light and algal growth is a rectangular hyperbolic function, with growth inhibition occurring at supersaturating levels, possibly resulting from damage caused by ultraviolet radiation. Temperature usually has a second-degree polynomial relationship with algal growth, indicating an optimal temperature range.

Phytoplankton populations can exhibit locally variable temperature optima (Round, 1984), which also occur between phyla. Sosnowska (1985) found that the dominant planktonic alga changed from diatoms to greens to blue-greens concurrently with increasing temperature. Garnier et al. (1995) modelled phytoplankton dynamics in the Seine and used temperature optima drawn from the literature of 20°C and >30°C, for diatoms and greens, respectively.

Photosynthesis can also be limited by the availability of carbon during periods of high phytoplankton density, as dissolved carbon dioxide is removed from the water faster than it can be replenished by diffusion from the atmosphere (Hein, 1997).

1.42 Chemical environment

Carbon and pH

Carbon dioxide is very soluble in water and forms an association with the carbonate-bicarbonate system to be the principal source of carbon for photosynthesis (Round, 1984). The major chemical pathway in photosynthesis is the conversion of carbon dioxide and water to carbohydrates and oxygen, using light energy that is harvested by a pigment such as chlorophyll (Hall and Rao, 1972). As carbon dioxide is removed from the water faster than it is replenished the equilibrium between carbon dioxide, bicarbonate and carbonate ions shifts towards the latter and results in an increase in pH (Harris, 1986). Photosynthesis, and therefore phytoplankton growth, can be limited by the lack of carbon dioxide and the detrimental effects of high pH, and the latter can impact on numerous physiological and biochemical processes (Stewart, 1974).

However, carbon limitation and the detrimental affects of high pH are less likely to impact on river phytoplankton than in other less dynamic environments.

Nitrogen and phosphorus

The productivity potential of open water phytoplankton is generally limited by available P (Moss, 1988), as demonstrated in Alton Water, Suffolk by Perkins and Underwood (2000). The optimum P concentration for phytoplankton varies considerably, although laboratory studies indicate that for diatoms and green algae it lies between 100 $\mu g\ L^{-1}$ and 2 $mg\ L^{-1}$ (Kuhl, 1974). Many algae have the ability to incorporate P rapidly and in some cases to an extent that far exceeds need (Ketchum, 1939), the so called 'luxury uptake'. Many algae have also been shown to produce 'surface' phosphatase activity during periods of P deficiency, which may enable them to utilise organic P (Kuhl, 1974; Whitton, 1992).

The most common sources of N used by algae are NH_4^+ and NO_3^- with the former being preferentially utilised (Morris, 1974). The ratio of N:P is often used as an indicator of a particular nutrient's importance and a molecular N:P ratio that exceed 16:1 is thought to indicate possible P limitation (Redfield, 1958).

A competitive advantage for nutrients has been demonstrated amongst phytoplankton. Diatoms have been shown to be good competitors for nutrients, especially phosphorus. In P-limited steady-state competition experiments diatoms outcompeted blue-green and green algae, except when silica was limiting (Sommer, 1983). Shafik et al. (1997) found that the centric diatom *Cyclotella meneghiniana* had a higher growth rate when P was limiting compared to when either N or Si were the limiting factor. An explanation for the competitive advantage of diatoms for P utilisation was proposed by Werner (1977) who suggested that the silica frustules of diatoms could act as an effective absorbing agent for dissolved substances at low concentrations. Competitive advantage for nutrients is also thought to result in species succession in diatoms. A successional sequence of diatoms has been shown, with the dominant taxa changing concurrently with decreasing silica and increasing phosphorus concentrations (Tilman et al., 1982).

The relationship between the concentrations of P (usually TP) and phytoplankton biomass (total chlorophyll) has been extensively explored in standing waters (OECD, 1992), but little work of this type exists for rivers. Steinberg and Hartmann (1988) thought that the development of blue-green algae could be described

by physical factors, above a TP concentration of 10μg L^{-1}. As lowland rivers often contain abundant inorganic N and P (Dokulil, 1995) it is unlikely that these nutrients are limiting phytoplankton here (Kelly and Whitton, 1998; Wehr and Descy, 1998). In urbanised regions P concentrations in rivers correlate negatively with flow, as the point sources are diluted. Whereas, in agricultural catchments N shows the opposite response as increased leaching occurs with elevated precipitation (Whitton and Kelly, 1998).

The fate of nutrients in rivers is complex. A large sewage discharge to the Great Ouse near its source in Northamptonshire was found to have a negligible impact on the nitrate concentration but a significant impact on the phosphorus concentration, which varied seasonally (House and Denison, 1997). During the spring and summer there was a large uptake of P to the bed sediment and vegetation, which was associated with the calcite concentration of the sediment. Nitrate can be lost from rivers systems through denitrification, a process where nitrate is converted to nitrous oxide or nitrogen gas and can occur within periphyton dominated by *Cladophora* (Whitton and Kelly, 1998).

Other nutrients and vitamins

Apart from N and P algae have a requirement for other macro and micronutrients and vitamins (O'Kelley, 1974; Provasoli and Carlucci, 1974). Calcium, sulphur and iron concentrations are often measured by Water Management Bodies, but the status of other nutrients and vitamins is unknown as they are rarely monitored or even considered important.

Silica

Dissolved silica is utilised by diatoms to construct their frustules, however planktonic diatoms are unable to take advantage of pulses of silica and require a continuous supply to facilitate population increases (Sommer, 1988). Significant negative relationships can occur between phytoplankton abundance and silicate concentrations (Balbi, 2000) and silica limitation appears to limit the abundance of planktonic diatoms in the Rhine (Admiraal et al., 1993) and Trent (Skidmore et al., 1998). Silica limitation has been demonstrated in populations of the centric diatom *Stephanodiscus hantzschii*, both experimentally (Swale, 1963) and in the Severn (Swale, 1969). In both cases Swale identified the point where there was insufficient silica for the population to divide. Silica limitation, however, is not always easy to demonstrate, although a molecular Si:P ratio of less than 16:1 is thought to be diagnostic (Perkins and Underwood, 2000). Low silica concentrations can also limit planktonic diatom

abundance indirectly, through poorly developed frustules (Sommer, 1988) that render them more susceptible to fungal infection (Canter, 1979).

1.43 Biological interactions

Parasitism

Fungal and protozoan parasites can significantly impact on phytoplankton abundance. The majority of fungal parasites found in freshwaters belong to the Chytridiales or are simple biflagellate Phycomycetes. A flagellate stage in the chytrids and the flagellate habit of the phycomcetes allows widespread dispersal and infection. Once a host is located a chytrid attaches itself to the outside of the cell and digests its contents via a rhizoid, whereas a biflagellate invades the cell. Infection is followed either be the formation of a resting stage or a sporangia from which more zoospores are released (Canter, 1979). Fungal infection of phytoplankton can be severe and rapid. Chytrids can be abundant in the River Bure, Norfolk and Clarke (1989) identified a concurrent increase in the abundance of chytrids with a decline in the abundance of the centric diatom *Stephanodiscus hantzschii*, which occurred within a week. Holfeld (2000) found that fungal parasites could persist even when their host comprised of a small fraction of the total phytoplankton biovolume. Fungal infection is not restricted to diatoms and water born fungi can infect green algae, cryptomonads and dinoflagellates, also (Canter-Lund and Lund, 1996).

Protozoan parasites include the amoeba *Vampyrella* and the flagellate *Codosigma*, both of which attach themselves to the outside of algal cells (Finlay et al., 1988, Canter-Lund and Lund, 1996). Larger protozoa form a part of the zooplankton and can engulf some planktonic algae whole.

Zooplankton

The zooplankton of rivers often contains abundant rotifers (Gosselain et al., 1998) and protozoa (Foissner and Berger, 1996) and are less often dominated by cladocera and copepods (Sanderson, 1998), which can be abundant in standing waters (Fitter and Manuel, 1986). Many planktonic protozoa engulf their prey to form a food vacuole within their cytoplasm. Some species of protozoa are specialist feeders on diatoms or filamentous blue-green algae (Canter-Lund and Lund, 1996; Foissner and Berger, 1996). Rotifers either seize and engulf their food whole, or pierce and extract the cell contents (Pontin, 1978). Grazing by zooplankton has been cited as an important

controlling factor for phytoplankton abundance (Gosselain et al., 1994, 1998a, 1998b), although in other situations it appears that the abundance of rotifers is controlled by the availability of suitable food (Sanderson, 1998). Some larger phytoplankton are unsuitable prey for rotifers or ciliates and grazing pressure can select for colonial forms. In fact, the mere presence (without contact) of zooplankton can induce *Scenedesmus* to form larger coenobia more rapidly than when grazers are absent (Lürling and Van Donk, 1999). Gosselain et al. (1998a) noted an increase in 'inedible algae' (> 20μm GALD) in the Meuse during periods of grazing pressure from rotifers (up to 2500 ind. L^{-1}).

Bivalves and other invertebrates

Filtration by bivalve molluscs has long been thought a significant loss process to river phytoplankton (Swale, 1969). Bivalves feed mainly on suspended algae, detritus and organic matter, which are strained off by the gills where they become entangled in mucus and swept towards the mouth by cilia. Species of the genera *Anadonta* and *Unio* can filter up to 1.5 and 3.6 L h^{-1} respectively (Ellis, 1978). Over the past two centuries the zebra mussel (*Dreissena polymorpha*) has spread throughout much of northern and western Europe and is now locally common in many British rivers (Fitter and Manuel, 1986).

Low plankton abundance in the Rideau River, Ontario, was thought to result from filtration from *Dreissena polymorpha*, which were at a density greater than 1000 individuals m^2 (Basu and Pick, 1997). Experiments have shown that *D. polymorpha* can filter between 24 to 63 mL $mussel^{-1}$ h^{-1}. Clearance rate was found to be extremely variable, generally with unicellular taxa (e.g. unicellular *Microcystis aeruginosa*) being taken in preference to colonial and filamentous forms such as *Anabaena* sp. (Bastviken et al., 1998). From the above figures, it can be calculated that a thousand mussels could filter between 24 and 63 L h^{-1}, or a m^3 in under 16 h. It is therefore not surprising that when these organisms are numerous they can have a significant impact on phytoplankton abundance.

Numerous other invertebrate animals consume phytoplankton. Chironomid larvae can be very abundant in the sediments of lakes and rivers or attached to other substratum. *Chironomus plumosus* is thought to play a role in controlling phytoplankton abundance in shallow eutrophic lakes and enhance benthic metabolism and nutrient exchange following the sedimentation of phytoplankton (Hansen et al.,

1998). First instar larva of some species preferentially consume diatoms (Pinder, 1992), although selection is probably based on food size rather than diatoms *per se.* Pinder et al. (1992) found abundant chironomid larvae, cladocerans and copeopods attached to the leaves of *Nuphar lutea* in the Great Ouse, and this was the most numerous species of macrophyte in the middle and lower reaches of this river.

Brendelberge (1997) examined the feeding preferences of two gastropod species, *Radix peregra* and *Bithynia tentaculata. Radix* was found to browse generally whereas, *Bithynia* fed selectively on algae, with maximum assimilation efficiency for the green alga *Chlamydomonas.*

Macrophytes

The relationship between turbidity and aquatic macrophytes was reviewed by Scheffer (1999). Very turbid waters will limit the development of submerged macrophytes, but once vegetation is present water clarity improves and provides favourable light conditions for further development of aquatic vegetation (Yallop and O'Connell, 2000). Abundant submerged macrophytes can inhibit phytoplankton by interfering with the turbulent flow necessary for their maintenance in suspension. They can also provide a daytime refugium for zooplankton (Stephen et al., 1998) to avoid predation by fish and achieve population densities not otherwise attainable. Or, macrophytes can act as 'filters', as seen downstream of some lakes (Chandler, 1937). Submerged macrophytes can also reduce phytoplankton biomass by producing shade, reducing nutrient availability, through the production of allelopathic substances (Jasser, 1995) or the reduction of resuspension (Barko and James, 1998).

1.5 Abundance patterns of river phytoplankton

Introduction

River phytoplankton studies have covered a wide range of river types; from fast-flowing shallow systems (Holmes and Whitton, 1981; Jones and Barrington, 1985) to rivers which function as elongated shallow lakes of low retention (Welker and Walz, 1999). The key features taken from a range of these investigations are summarised in Table 1.1. Although the classifications used are a simplification of complex systems they permit a general appraisal of similarities in the abundance and periodicity of suspended algae.

Chlorophyll and/or cell counts are widely used in investigations, although some of

the studies did use other methods of biomass, such as cell volume (Reynolds and Glaister, 1992) and cell carbon content (Gosselain et al., 1994) .

Temporal and spatial trends

The shallow fast-flowing systems have low chlorophyll/cell counts and are dominated by 'benthic' forms compared to deeper slower rivers. Many of the deeper rivers are dominated by centric diatoms, which are particularly abundant during the spring. Many of the deeper systems also experience summer periods with low chlorophyll/cell counts compared to the major peaks and these periods are dominated by greens and sometimes blue-green algae. Few authors consider N or P to be limiting in lowland rivers, although silica depletion and filtration by zooplankton and mussels are often cited as restricting phytoplankton development. Not surprisingly, physical factors such as flow rate, temperature and light are universally considered as major impacts on phytoplankton.

Many systems exhibit a general downstream increase in algal biomass, although this trend does not necessarily occur throughout the year and in some instances increases to a point, thereafter tending to decline (Basu and Pick, 1997). Other systems have fluctuating stretches that correspond with river order, or downstream peaks that occur before upstream sites (Garnier, et al., 1995). Reynolds (1991) identified spatial differences in the algal biomass in the Severn, which were associated with a dead zone. Skidmore et al. (1998) examined the chlorophyll concentration at three points across the Trent. On two occasions, no difference was found between the samples, but on a third occasion the right-hand side of the river had a significantly lower concentration than the centre channel and left-hand side

Sources of river phytoplankton

Hynes (1970) proposed that river phytoplankton was a composite of several types: from bays and backwaters, the benthos and 'true' river phytoplankton, which grows and reproduces in flowing water. Reynolds and Descy (1996) reviewed the origin of phytoplankton in rivers, and defined potamoplankton as those species that are simultaneously able to grow and divide in the open-flow of river channels from an inoculum of cells that are somehow maintained or continuously renewed.

Table 1.1 Summary of key temporal and spatial features of 'river phytoplankton' selected from the literature. Classification is based on general impression and is not definitive. Y = yes, N = no, blank = not applicable or unknown.

Reference	River and/or location (country if not UK)	River/comment	Chlorophyll	Chlorophyll > 100 µg L^{-1}	Species composition	Total cell > 10000 mL^{-1}	Abundant centric diatoms	Abundant pennate	Dominant spring peak	Spring and Autumn peaks	Summer peak	Low summer phytoplankton	Greens Abundant in	Blue-greens abundant	N and P limiting	Si limiting	Physical impacts	Biological impacts	Downstream increase
Balbi, 2000	Nene		Y	Y					Y			Y			N	N	Y	N	Y
Basu & Pick, 1997	Rideau (Canada)		Y	N											N	N		Y	Y
Garnier et al., 1995	Seine (France)		Y	Y			Y					Y	Y		N	Y	Y	Y	
Gosselain et al., 1994	Moselle (France)		Y		Y		Y		Y			Y	Y						
Gosselain et al., 1998	Meuse and Moselle	Meuse	Y	Y	Y		Y			Y		Y	Y				Y	Y	
		Mosselle	Y	Y	Y		Y		Y			Y	Y				Y	N	N
Greenburge, 1964	Sacramento	See Hynes,			Y	Y	Y	Y	Y			Y							Y
Hindák & Makovinská,	Danube (Slovakia -		Y	Y	Y	Y	Y						Y	Y	Y		Y		Y
Holmes & Whitton, 1981	NE England	U/S Sites			Y	N		Y						N			Y		
		D/S sites			Y	Y								Y			Y		
Hutchings, 1996	Thames				Y	Y	Y		Y				Y	Y	N	N	Y		Y
Ibling et al., 1998	Rhine and Meuse	Meuse	Y	N	Y	Y	Y			Y		Y	Y	Y	N		Y	Y	Y
		Rhine	Y	Y	Y	Y	Y			Y		Y	Y	Y	N	Y	Y	Y	Y
Jones & Barrington, 1985	Derwent (Derbyshire)	U/S Sites	Y	N	Y	N		Y					Y		N	N	Y	N	
		D/S sites	Y	N	Y	N	Y				Y		Y		N	N	Y	N	
Kiss, 1996	Danube (Hungary)		Y	Y	Y	Y	Y		Y	Y	Y								
Köhler, 1993	Spree (Germany)		Y	Y			Y		Y					Y	N	N	Y		Y
Köhler, 1994	Spree (Germany)				Y					Y				Y	N	Y	Y	Y	Y
Kyong et al., 1998	Nakdong (St. Korea)		Y	Y	Y	Y	Y			Y			Y	Y	N	Y	Y		
Lack, 1971	Thames and Kennet	Kennet			Y	N	N	Y		N					N				
		Thames			Y	Y	Y			Y					N		Y		
Marker & Collett, 1991	Great Ouse		Y	Y	Y	Y	Y	N	Y			Y	Y	Y	N	Y			Y
Noppe & Prygiel, 1999	Artois-Picardie basin		Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y					
Pinder et al., 1992	Great Ouse		Y	Y	N							Y							
Pinder et al., 1997	LOIS rivers	Don	Y	Y							Y				N	N	Y		
		Ouse	Y	Y					Y						N	N	Y		
		Trent	Y	Y					Y	Y	Y				N	N	Y		
		Ure	Y	Y						Y					N	N	Y		
Reynolds & Glaister, 1992	Various larger UK				Y		Y		Y				Y				Y		Y
Rose & Balbi, 1997	Great Ouse		Y	Y	Y	Y	Y	N	Y			Y	Y	Y	N	N	Y	N	Y
de Ryter van Steveninck et	Rhine (Holland)		Y	Y			Y		Y						N	Y	Y	Y	Y
Skidmore, 1998	Trent		Y	Y	Y	Y	Y		Y			Y	Y	Y	N	Y	N	Y	Y
Swale, 1964	Lee				Y	Y	Y	Y		Y					N	N	Y		Y
Swale, 1969	Severn and Stour	Severn, U/S			Y	N	N	Y			Y		Y		N	N	Y	Y	Y
		Severn, D/S				Y	Y				Y		Y		N	Y	Y	Y	Y
		Stour, D/S			Y	Y	Y				Y		Y		N	N	Y		

'On-channel' lakes and backwaters within the river catchment can potentially influence the phytoplankton composition of the main river as suspended algae are continuously swept downstream or diffuse into the river. The greater retention time of lakes can often result in phytoplankton development when conditions in rivers are unsuitable. Marker and Collett (1991) observed the chlorophyll concentrations of three marinas connected to the Great Ouse and found they were relatively high compared to levels in the river.

The fate of phytoplankton entering rivers depends on the species and prevailing conditions. Holmes and Whitton (1981) found that suspended algae that originated from reservoirs did not persist in several fast-flowing rivers of north-east England. Chandler (1937) identified macrophytes as being responsible for 'filtering' lake phytoplankton within a river, while other studies have shown that lake phytoplankton can continue to grow when conditions in the lakes and river are similar, but decline when not (Köhler, 1994).

River size

Wehr and Descy (1998) reviewed the characteristics that might constitute a 'large river', and their relationship with river phytoplankton. Physical, ecological and cultural factors were considered and it was concluded that large rivers are of sixth order or greater.

1.6 Aims

The aim of this study was to identify the key factors controlling the abundance, species composition and periodicity of suspended algae in a small, lowland, nutrient-rich river and see how these compare to larger systems. It was also intended that the work would facilitate informed judgements about historic chlorophyll data and the evaluation of river phytoplankton as a monitoring tool.

The research will test the hypothesis that the key factors controlling phytoplankton populations in a small river are not dissimilar to those acting in larger systems.

The Nene was an ideal subject for this purpose because of its size, low stream order, considerable variation in flow and history of high chlorophyll concentrations at a downstream site.

2 GEOGRAPHICAL BACKGROUND

2.1 Introduction

The Nene rises (1° 13′ W, 52° 14′ N; 160 m.a.s.l.) a few kilometres south-west of the Northamptonshire town of Daventry and flows in a north-easterly direction for approximately 170 km before discharging into The Wash, near Sutton Bridge, Lincolnshire (0° 10′ E, 52° 45′ N). The river achieves third order at km 24.4 and remains so throughout its length (Smith and Lyle, 1979). Major tributaries, which join mainly from the north, include the Brampton Arm (also considered the Nene - Hart, 1971), River Ise and Willow Brook (Figure 2.1).

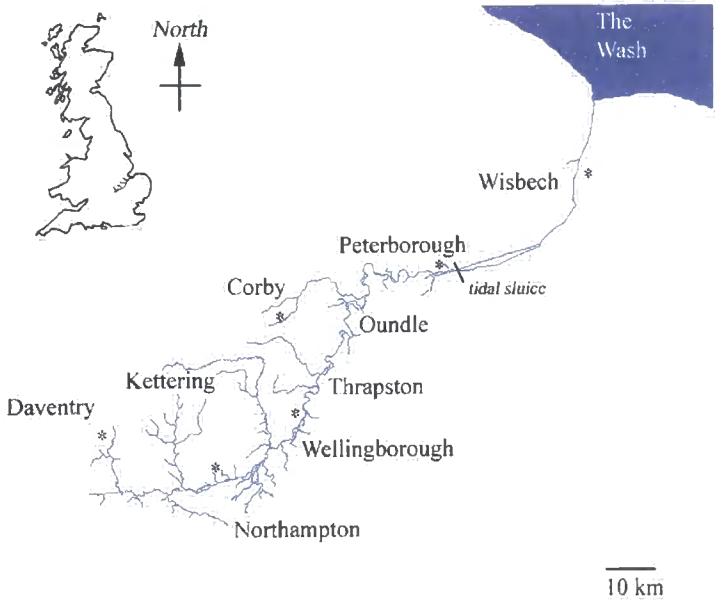


Figure 2.1 Map of the Nene. Showing, major towns and STW (*) and location of tidal sluice. Inset, UK location of Nene.

The Nene catchment is intensively agricultural and has a rapidly growing population, containing many large conurbations, and both of these factors impact on the river's water quality. The river has been utilised over many centuries for navigation, milling and water supply. The river is now the principal source for abstraction for Rutland Water, which is a major potable storage reservoir for the region. The Nene navigation, which extends downstream from Northampton, comprises 38 locks including a tidal sluice, which is situated several km downstream of Peterborough (National Rivers Authority, 1993). The changing nature of the river is summarised in

Figure 2.2.

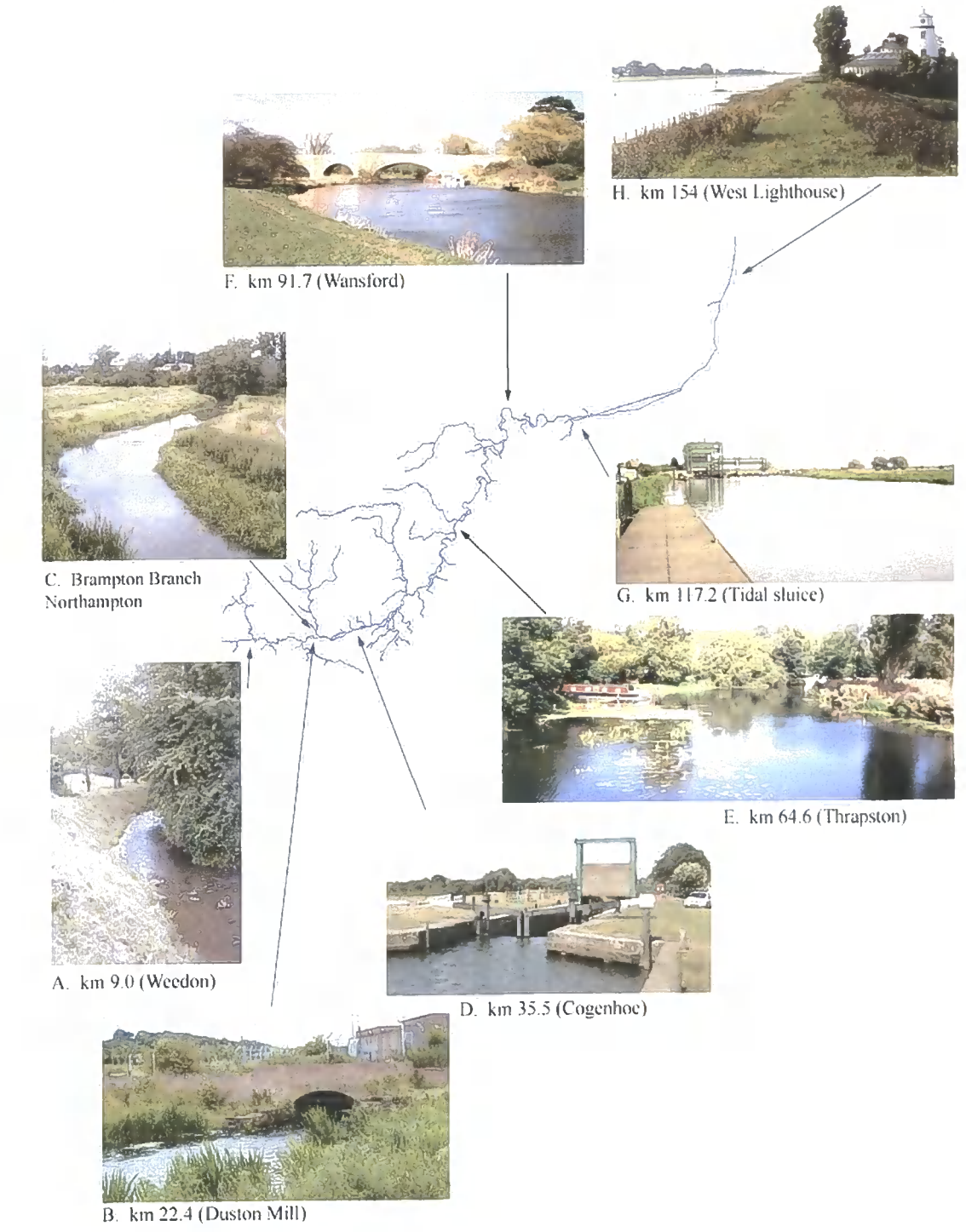


Figure 2.2 Photographic summary of the Nene from headwaters to estuary (labelled in a downstream direction).

2.2 Geology and hydrology

The geology of the Nene catchment has been described in detail (Ministry of Housing and Local Government, 1964; Hart, 1971) and the overlying features of the area are relatively recent compared to other parts of the UK. The main surface outcrops are all Jurassic in origin and were laid down below shallow seas or estuaries. The principal surface geology of the upper catchment consists of Lias clays with some Oolithic sand/ironstone and limestone. The middle reaches of the river flows mainly through Oolithic limestone, whereas downstream of Peterborough Alluvial fen and valley gravels become increasingly abundant.

The mixed geology of the Nene catchment results in a flow regime that is characterised by a mixture of surface and groundwater flow. This is reflected in a BFI (Shaw, 1998) of 0.52 at Peterborough (on a scale of 0 to 1), compared to 0.18 for the Wiske at Kirby Wiske (North Yorkshire) and 0.96 for the Itching at Highbridge (Hants) - which are examples of surface and base flow-dominated systems, respectively. These factors combined with relatively low rainfall (annual average = 635 mm) result in the Nene having a widely varied flow regime, with a seasonal contrast greater than many larger UK rivers (Lewin, 1981).

The Nene has seven principal gauging stations dating from the late 1930s and early 1940s, although most of them are situated in the upper catchment or on the tributaries (Figure 2.3). Recent additions such as Wootton Brook and others in the headwaters (not all shown in Figure 2.3) are of lesser interest here. Downstream discharge on the main Nene is calculated using a combination of gauged sites, Orton Staunch and Wansford, where the long-term (1975 - 1996) median discharge is 6 $m^3 s^{-1}$. The Orton station consists of a group of weirs, sluices and regulated bypass channels and records discharge up to about 38 $m^3 s^{-1}$. The complex nature of this station is necessary to allow boat passage and probably results in a less accurate flow record compared to a fixed weir system (D. Glenn, pers. comm.). Discharge of greater magnitude is recorded 12 km further upstream at Wansford gauging station (km 92.5), where discharge is based on level, which is calibrated using a fixed 'cable way' system (Shaw, 1998). Ultrasonic gauging equipment (Shaw, 1998) was installed at km 92.5 during the mid 1990s to gauge the main river and the Rutland Water abstraction, which is also located here. Data for this station are available from the beginning of 1997,

although the accuracy and reliability of the record is thought to be influenced by plant growth and siltation (D. Glenn, pers. comm.). River level loggers have also been installed recently at many of the locks (Figure 2.2 D. right hand foreground) and levels are recorded at 15 min intervals.

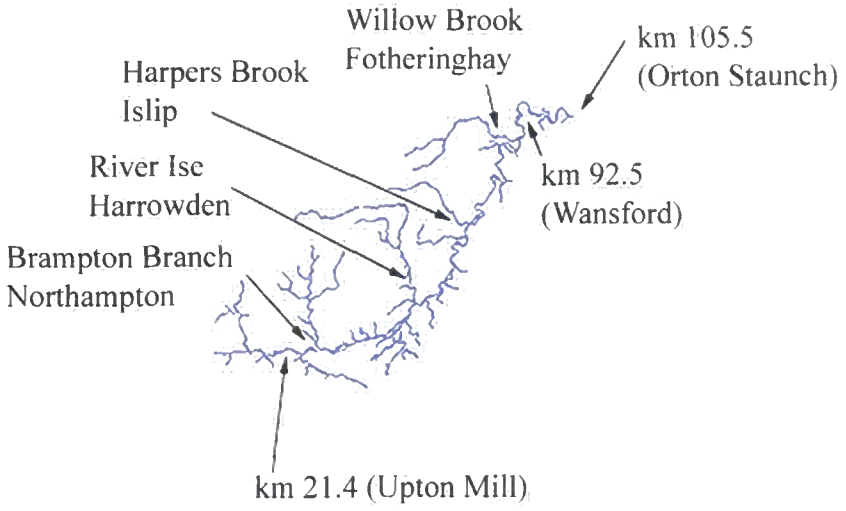


Figure 2.3 Location of gauging stations on the main Nene and tributaries.

2.3 Navigation and industry

Early accounts of navigation on the Nene include Vikings sailing their long-boats upstream to raid Northampton, the river having been made navigable to Peterborough by the Romans. Early navigation probably included the creation of temporary impoundments along the river, which were dismantled to allow boat passage. The first formal navigation was constructed in an upstream direction from Peterborough to Northampton, where it was officially opened in 1761 (Steane, 1974). The navigation consisted of thirty-four locks and twelve staunches. Unlike pen-locks which consist of a pair of gates, allowing manipulation of the water level over a small area, staunches have a single gate which, when closed, floods a large river section. The navigation was enhanced in 1815 when a branch of the Grand Union Canal (GUC) was extended to join the river at Northampton.

Modernisation of the navigation took place between 1936 and 1941, eliminating the necessity for nine of the staunches, by deepening the river, and conversion of the remaining staunches into pen-locks (Steane, 1974). The modernisation also included

the construction of a tidal sluice (Figure 2.2 G) a few kilometres downstream of Peterborough (Ministry of Housing and Local Government, 1964). Most of the present day locks consist of a pair of conventional pointing doors upstream with a vertical steel gate downstream (Figure 2.2 D). During periods of high flow the conventional doors can be opened and the vertical gate used as a regulatory sluice, thus rendering the navigation inoperative.

Today the freshwater navigation is used almost exclusively by pleasure craft. In 1962 the Dog-in-a-Doublet tidal sluice recorded the passage of 272 vessels, which included 80 stone barges, 55 corn barges and 130 pleasure craft. Although further corn barges were still entering the river at Northampton (from the GUC) and servicing the mill at Wellingborough.

There were once at least 43 water mills on the main river, extending from near the source at Newnham to Peterborough. This number was greatly reduced with the advent of turbine mills and later by electricity. Today, two commercial mills survive at Wellingborough and Bugbrooke but many of the old mill buildings and associated waterways remain.

The navigation section of the freshwater river has average channel dimensions of 27 m wide and 2 m deep and has little shading from trees, although overhanging vegetation is more abundant along the back and by-pass channels.

2.4 Meteorology

Long-term light data (1975-1997) were supplied by the Meteorological Office, Bracknell, Berkshire, UK. Light data (sunshine 'hours and tenths' from a Campbell-Stokes, universal sunshine recorder - Meteorological Office, 1982) were used from a combination of Wittering (TF 043 026), Monks Wood (TL 201 798) and Coningsby (TF 225 570). Light data were not available for the whole period (1975-1997) from a single station, so combinations of data were used, preferentially utilising Wittering (closest to km 91.7).

More recent meteorological data, including five-min solar radiation readings (kwatts m^2), were available from the Rutland Water Weather Station (SK 885 085) operated by the Environment Agency.

2.5 Standing waters

Standing waters within the catchment could potentially influence the chlorophyll concentration of tributaries and the main river. Numerous flooded gravel extractions occur along the Nene valley and several have through-flowing water that discharges to the main river. Some of these water bodies, such as Thrapston sailing lake, have been established for over thirty years (Ministry of Housing and Local Government, 1964), whereas others are of more recent origin.

Willow Brook has several lakes along its course the largest located near Blatherwycke (SP 975 965) is 25 ha and feeds directly into this tributary.

2.6 River maintenance

An ongoing maintenance programme is undertaken to reduce the probability of flooding and includes dredging and cutting of submerged macrophytes (National Rivers Authority, 1994). 'Weed' cutting is normally undertaken between July and September, working downstream from Northampton to the tidal sluice. The EA do not maintain records of the quantity of vegetation removed each year, but there is evidence of considerable inter-year variation (Brierley et al., 1989; Foster, 1990) and 1997 is thought to have had the greatest macrophyte growth for more than 20 years (T. Hill, pers. comm.).

2.7 Chemical monitoring and water quality

The Environment Agency and its predecessor organisations have monitored the Nene over many years as part of its water quality monitoring programme. Routine chemical determinands including temperature, N, P, silicate and chlorophyll, which have been recorded at Wansford (km 91.7) since early 1975, although the record is interrupted for chlorophyll and silicate. Data is also available for several other sites but these are of shorter duration and lower frequency than Wansford.

The water quality of the Nene is variable and several main river and tributary sites fail their 'River Ecosystem' or 'Coastal and Estuary Working Party' quality targets (Environment Agency, 1998). Significant quality failures are in the headwaters of Willow Brook and the tidal river and are attributed to eutrophication or drought. A headwater stream of Willow Brook also fails the EC Dangerous Substances Directive with respect to zinc, which originates from British Steels activities in Corby.

The Nene receives several inputs from large sewage treatment works (Figure 2.1 - serving populations > 100,000) and is considered to be eutrophic throughout its length (Rose and Balbi, 1997).

2.8 Biological monitoring and water quality

For many years the EA (and predecessors) have routinely monitored macroinvertebrate communities throughout the freshwater Nene and its tributaries. These surveys are undertaken as part of the statutory water quality monitoring programme and result in a biological assessment using the Lincoln Quality Index classification system (Extence and Ferguson, 1989), which is based on the work of Chester (1980). Biological water quality results often conflict with the results of the chemical surveys, with many river reaches achieving their targets biologically but failing chemically. This discrepancy probably results from the small number of determinands used to assess chemical quality (BOD, DO and ammonium concentrations), but where there are inconsistencies the results of chemical surveys often override the biological data. More recently, macroinvertebrates have been used to assess water quantity issues in the Nene catchment using a system devised by Extence at el. (1999).

Planktonic rotifers can be abundant in the Nene (Sanderson, 1998) and bivalve molluscs (e.g. *Anodonta cygnea*) are a common constituent of the benthos (EA, unpublished data). The Nene sustains a healthy coarse fishery, although there are concerns relating to the impact of poor water quality and restrictions to fish movements created by locks and weirs (Reeds et al., 1995).

Over recent years macrophyte and benthic diatom communities have been monitored in the Nene, and some of its tributaries, to assess nutrient status for the UWWT Directive submissions (Rose and Balbi, 1997).

2.9 Sample sites

A 69-km length of river was surveyed from km 22.4 (Duston Mill) to km 91.7 (Wansford) and consisted of eight main river and three tributary sites (Table 2.1 and Figure 2.4). Main river sites are identified by their distance from source (km) and name and tributaries are numbered (1-3) in a downstream direction (University of Durham tributary labelling system was not used). All sites were near bridges, locks or

weirs to facilitate ease of sampling from flowing water and to provide sufficient depth to measure light attenuation.

Table 2.1 Main river and tributary sample sites, showing downstream distance from source, tributary confluence distance from source of main river, watercourse, site name and NGR.

Site	River	Name	NGR
Main river			
km 22.4	Nene	Duston Mill	SP 729 596
km 34.0	Nene	Great Billing	SP 814 611
km 39.8	Nene	Hardwater Mill	SP 876 637
km 43.9	Nene	Wellingborough	SP 902 662
km 52.2	Nene	Irthlingborough	SP 957 706
km 64.6	Nene	Thrapston	SP 991 787
km 85.2	Nene	Elton	TL 830 939
km 91.7	Nene	Wansford	TL 075 991
Tributary			
1 (km 24.4)	Brampton Arm	Northampton	SP 749 615
2 (km 44.8)	Ise	Wellingborough	SP 907 674
3 (km 85.4)	Willow Brook	Fotheringhay	TL 063 935

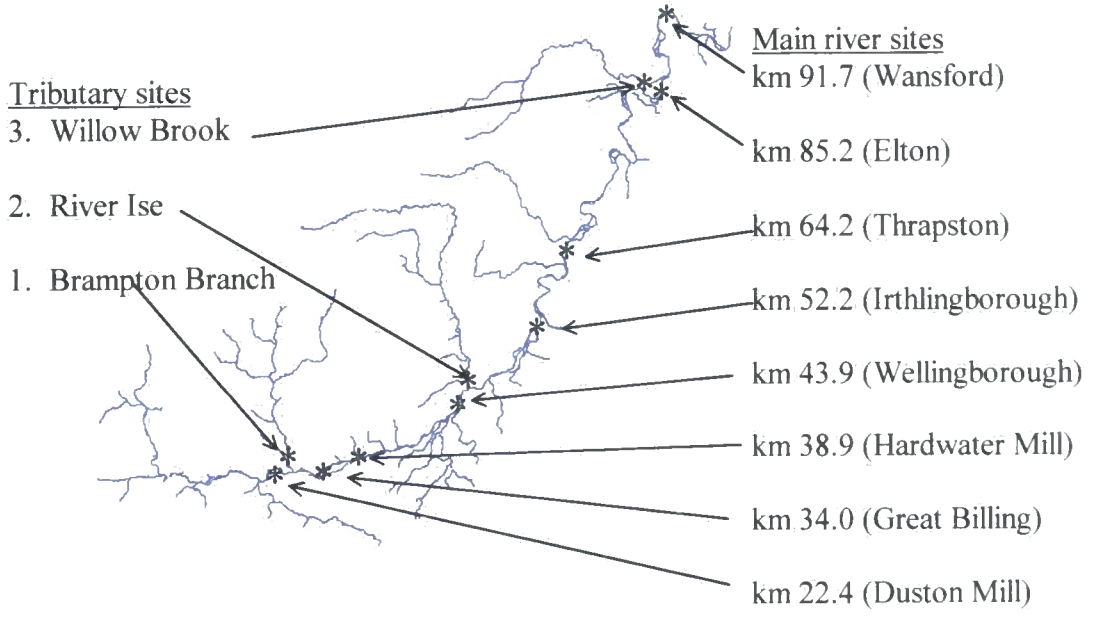


Figure 2.4 Location of routine sample sites (*). Tributary sites are numbered in a downstream direction and main river sites are labelled with distance from source and name.

3 Methods and materials

3.1 Field analysis

Surface water temperature and Secchi disc measurements (z_s - Standing Committee of Analysts, 1985) were recorded at all routine sites. On several occasions water column light attenuation was measured simultaneously with z_s, using a 'LI-COR' LI-250 light meter, to investigate their relationship (z_{eu} calculated as 1% surface light - Moss, 1988).

3.2 Physical data

3.21 Water temperature

The longitudinal temperature record for sites from km 34.0 (Great Billing) to km 85.2 (Elton) was augmented using estimated data, derived from linear regression models, based on km 91.7 (Wansford). Temperature data for km 91.7 were more numerous than other sites and these were used to extend the record elsewhere. Spot temperature records for each site were regressed on temperature data for km 91.7 and the resulting equations used to estimate longitudinal daily water temperatures (r^2 values ranged from 0.94 to 0.97; $p < 0.05$ for all).

3.22 Discharge

Most of the sample sites were not situated near to gauging stations (Figure 2.3 and Figure 2.4) or the discharge record was incomplete (Section 2.2). Discharge at km 91.7 could be derived using one of three options.

1. Scale downstream from gauged sites in the upper catchment, allowing for major discharges and abstractions (adapted from model produced by G. Watts, pers. comm.).
2. Use relationship between gauged sites in upper catchment and available ultrasonic data as a model (Upton, Harrowden and Fotheringhay produce best relationship; $r^2 = 0.91$, n = 578).
3. Scale upstream from Orton allowing for the Rutland Water abstraction.

Theoretically, option three should be the most accurate as Orton is closest to km 91.7, providing the available discharge and abstraction data are accurate. The options were evaluated by regressing daily discharge values calculated for 1997 from each method on daily values recorded using ultrasonic equipment at km 92.5 (Wansford gauging station), assuming that the latter record was most accurate of all. Methods one, two and three produced r^2 values of 0.81, 0.78 and 0.87 respectively (all: $p < 0.05$, n = 365). As upstream scaling produced the most significant relationship with the ultra-sonic record and it was decided to use these data throughout the research. However, the marginal difference between the methods possibly reflects the inaccuracy of the Orton Gauge and/or the Wansford abstraction data. Although consistency between the three methods evaluated and those recorded ultrasonically provides some confidence in downstream scaling and the accuracy of the recently installed ultrasonic equipment. With confidence established in both up and downstream scaling of flow it was decided to scale upstream from Orton to derive daily discharge for km 91.7 and km 85.2, and scale downstream from Northampton and gauged tributaries for all other sites. The use of km 85.2 as a transition point was investigated by regressing daily discharge values calculated in a downstream direction on those scaled upstream, during 1996. The two methods correlated significantly (r^2 0.79; n = 366) with a slope of 0.93, indicating that the upstream scaling was producing a slight underestimate compared to the downstream procedure.

3.23 Channel and catchment characteristics

Channel and catchment statistics are shown in Table 3.1. Slope was calculated as the difference in altitude (m.a.s.l.) between the upper and lower ends of the section, divided by the section length. Sinuosity was calculated as the section length divided by the shortest route along the section (Reynolds and Glaister, 1992). Approximate river channel dimensions were calculated from cross-sectional drawings held by the EA (navigation sections) or direct measurement over a range of flow conditions. Depth was determined as the average of several measurements across the whole river width and was based on median water levels for the navigation sections.

Table 3.1 Channel and catchment statistics of main river (above) and tributaries (below). Average channel dimensions and catchment area are common. Altitude, slope and sinuosity are shown for the Nene. Tributary length, lake area† and median velocity (1994 - 1996) are shown for tributaries. † Only lakes having a continuous discharge to tributary included; Slope = fall ÷ length; Sinuosity = reach length ÷ shortest distance between start and end of reach (Reynolds and Glaister, 1992).

Site	Depth (m)	Width (m)	Area (km^2)	Altitude (m)	Slope ($m\ m^{-1}$)	Sinuosity
km 22.4	0.2	13.0	299	61.0	---	----
km 34.0	1.7	18.8	604	49.8	0.0010	1.14
km 39.8	1.9	21.2	638	44.7	0.0009	1.24
km 43.9	2.0	29.9	729	39.0	0.0011	1.03
km 52.2	2.3	36.0	1107	35.3	0.0007	1.43
km 64.6	2.1	28.0	1128	28.4	0.0004	1.41
km 85.2	2.4	24.6	1393	15.8	0.0008	1.64
km 91.7	1.8	31.9	1529	8.1	0.0008	1.08
				Length (km)	Lake area (m^2)	Velocity ($m\ s^{-1}$)
Tributary 1	0.4	7.0	234	24	0.9	0.21
Tributary 2	0.4	17.0	226	46	8.8	0.20
Tributary 3	0.2	6.0	90	38	27.7	0.21

3.24 Velocity

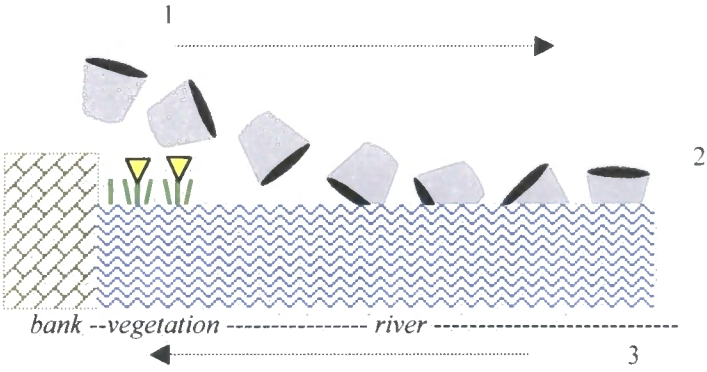
Average velocity at main river sites between km 34.0 and km 91.7 (inclusive) were calculated as the quotient of average daily discharge ($m^3\ s^{-1}$) and cross-sectional area (m^2, taken from drawings). The relationship between cross-sectional area and discharge, for each site, was established using daily average river levels (recorded every 15 min) and cross-sectional drawings. Independent current meter records were available for two sites (km 39.8 and km 91.7) and these were used to verify the velocity estimates here, with both comparisons producing highly significant results ($r^2 = 0.99$; $p < 0.05$; $n = 10$, for both). Estimated retention time at km 34.0, km 39.8 and km 43.9 were based on their distance from the commencement of navigation and estimated average daily velocity. River cross-sections and water level data were not available for all sample sites and in these cases the nearest location with suitable data was used. Median tributary velocity, for the period 1994 to 1997, were estimated using median and average discharge values, following a method described by Round et al., (1998).

3.3 Sample Programme

3.31 Routine collection, treatment and storage of water samples

Routine water samples were collected from the surface running water at a depth of about 10 cm, unless stated otherwise. Where necessary samples were taken from

bridges or using the technique described in Figure 3.1 to avoid contamination from bank-side vegetation or debris.



1. A clean empty bucket is thrown towards the desired sample point (retaining attached rope) in such a way that it rotates through the air and lands on the surface water face-down.

2. If carried out successfully, the bucket lands at the desired spot with its opening facing downwards and still rotating. The sample is collected and the bucket rights itself, with sample contained. The weight of the bucket and contained sample keep the bucket stable as it floats with its periphery above the surrounding water.

3. The bucket and contained sample are then carefully retrieved by gently withdrawing the rope.

Figure 3.1. Method of taking water sample from open/flowing water and avoiding contamination from bank-side vegetation and/or debris. Using a 15-L 'bottom-weighted' stainless steel bucket, with attached rope.

Water samples were subsampled for chlorophyll (1-L PET in light-tight container), phytoplankton counting (300-mL PET with Lugol's Iodine) and for live phytoplankton identification (300-mL PET). Additionally, chemical samples were collected to augment the routine EA chemical programme. These included, silicate (1-L PET), TP (300-mL PET) and FRP (60 mL site filtered through Whatman GF/C filter paper). Routine chemical samples were normally analysed within 24 h, although samples collected to evaluate sample heterogeneity and phaeopigment concentrations were analysed within 4 h.

Some chlorophyll samples were stored on frozen filter papers during periods of intensive sampling and analysed within 3 d of sampling. *In vivo* chlorophyll concentration was determined using a Turner 10-AU field fluorometer (Figure 3.5), following calibration against the routine chlorophyll method and adjustment for temperature. Live phytoplankton samples were examined on the day of collection and

fixed samples were processed as soon as possible. Diatom and rotifer samples (1-L PET with Lugol's Iodine) were also collected at km 91.7, mostly during 1995, 1996 and 1997.

The routine sampling programme was based on 2-weekly sampling at all main river and tributary sites between June 1993 and December 1997, although km 22.4 and tributary 1 (Brampton Branch) were introduced during 1994. The frequency of phytoplankton and chlorophyll sample collection was increased during the spring and summer (April to September) at km 91.7. Samples were normally taken weekly during the spring and summer of 1994, 1995 and 1997 and three times per week during 1996.

3.32 1994 flood event

Phytoplankton samples were collected from km 91.7 following two days of heavy rain that occurred on 14 and 15 September 1994, which followed three months of sustained low flow. Samples were collected on 15, 16, 17, 19, 20, 21 and 23 September. All taxa were identified and changes in abundance and diversity were used to evaluate the impact of increasing discharge.

3.33 Main river/bay comparison

A comparison was made between the phytoplankton occurring in a bay and the main channel at km 91.7 between 4 April and 28 September 1995 (Figure 3.2). Samples were collected at weekly intervals for phytoplankton counts and chlorophyll. Temperature and water transparency (Secchi disc) were also recorded.

3.34 Sediment trap

A sediment trap was used in the river at km 91.7 between 6 March and 11 October 1996 (location Figure 3.2) and was emptied every two weeks with the exception of 5 April, when it was retrieved after 24 h. The trap was constructed from a 0.5-L plastic measuring cylinder fitted to a heavy steel base-plate and connected to a chain for lowering and retrieving from the river bed (Figure 3.3). The trap had a total height of approximately 40 cm and was positioned at a depth of 2.8 m, on the far side of the river from the routine sample point.

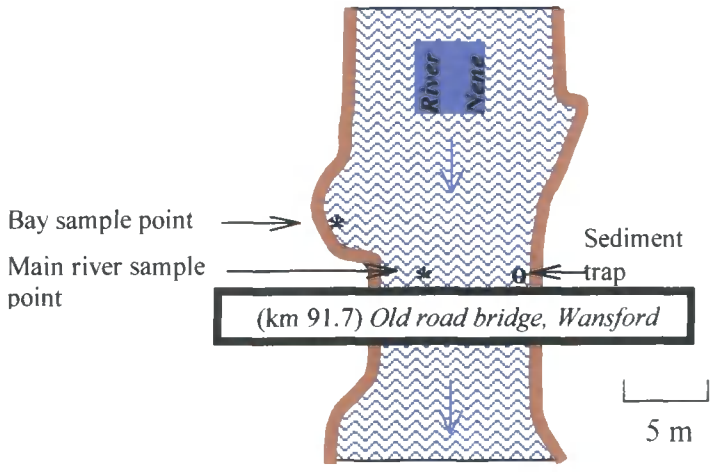


Figure 3.2 Schematic representation of km 91.7 sample site, showing location of main river and bay sample points (*) and sediment trap (o). Solid arrows indicate direction of flow.

Figure 3.3 Sediment trap. Also showing cylinder cap, retaining chain and other materials used during collection of samples.

The sediment trap was implemented and emptied using the following steps.

Implementing sediment trap

1. Add 10 mL Lugol's Iodine to clean cylinder
2. Fill cylinder with deionised water
3. Fit cylinder with lead cap
4. Lower sediment trap carefully to the river bed (ensuring chain lies to one side)
5. Allow to stand for at least 5 min
6. Carefully remove cap

Emptying sediment trap

1. Carefully retrieve sediment trap from river bed
2. Empty contents into clean bucket and rinse with deionised water
3. Transfer contents of bucket to a labelled 1-L PET bottle and re-fix
4. Clean cylinder and base removing any invertebrates or debris

3.35 High frequency sampling

Samples were taken at high frequency (1 h to 4 h) to assess short-term trends in chlorophyll and phytoplankton abundance.

Short-term temporal change

Samples were collected for phytoplankton and chlorophyll analysis at two depths (10 cm and 1 m) from km 91.7 at 4-hourly intervals for 24 h, on the 4 and 5 April 1994. Water temperature and Secchi depth were also recorded with each sample (where possible).

Four-hourly samples were also collected using a Hobo automatic sampler (Figure 3.4) positioned within the Wansford gauging station (km 92.5 - TL 081 996), which is located 800 m downstream from the routine site, km 91.7. The sample tube was suspended from a buoy (Figure 3.5) at a depth of 0.5 m in a total water depth of approximately 2 m and was routed underground to the sampler.

Figure 3.4 Hobo automatic water sampler (open) and Grant YSI water quality monitor. Both located inside Wansford gauging station at km 92.5.

Figure 3.5 Nene at km 92.5, adjacent to Wansford gauging station. Showing supporting buoy for sample tube and Grant YSI water quality probe and field fluorometer, battery and tubing.

The Hobo sampler purged and flushed the sample tube prior to each sample collection, which took several minutes to complete. The sampler was used on three occasions during the spring and summer of 1997, as follows.

1. Samples every four h between 08:00 31 March and 08:00 4 April.
2. Samples every h between 08:00 14 April and 13:00 18 April.
3. Samples every two h between 00:00 6 May and 06:00 9 May.

Samples were collected for phytoplankton and chlorophyll and the latter was either determined photometrically or using a fluorometer. Samples stood for a maximum of 14 h after being either filtered and frozen on site or determined fluorometrically. Water temperature was recorded using a Grant YSI water quality logger at 15-min intervals over a 12-month period. The temperature probe was positioned at a depth of 0.8 m and located on the same buoy used for the automatic sampler. During the second survey (14 to 18 April 1997) silicate, ammonium, TON and TRP samples were also collected every four h for 48 h.

3.36 Cross sectional profiles and spot sampling

River profiles were investigated for chlorophyll, temperature and velocity at km 91.7 and km 92.5 on the 17 April 1997. Additionally, the heterogeneity of the surface chlorophyll concentration of the main river and bays was explored around the upstream site.

A series of vertical profiles were undertaken across the river at 2- m intervals at km 92.5 (16 profiles) and 1-m intervals at km 91.7 (9 profiles). Each profile consisted of four individual sample points, 0.2, 0.4, 0.6 and 0.8 of the total river depth. At each point the chlorophyll concentration and temperature were determined using a fluorometer and velocity was measured using an Ott RC2 electro-magnetic current meter.

3.37 Picoplankton

Samples were collected for picoplankton analysis from km 91.7 at 2-weekly intervals between 10 July 1997 and 28 August 1998. Samples were stored in a 300 mL PET bottle and preserved in 2% (final concentration) buffered formalin (Hawley and Whitton, 1991) and stored in the dark below 5°C.

3.38 Rotifer

Samples were collected for rotifer identification and enumeration at km 91.7 three times a week between April and September 1996. Samples were stored in a 1-L PET bottle and preserved in Lugol's Iodine.

3.39 Planktonic diatoms

Several samples were collected at km 91.7 for the identification of diatoms, following acid digestion. These samples were stored in a 1-L PET bottle and preserved with Lugol's Iodine.

3.310 Influence of Billing marina and Thrapston sailing lake

The influence of Billing marina (20 m upstream of km 34.0) and Thrapston sailing lake (500 m downstream of km 64.6) on the phytoplankton of the main river was investigated between 10 April and 14 August 1996, following pilot studies during 1994/5. Billing marina and Thrapston sailing lake are both flooded gravel workings, but they differ in that Billing has no flow to the main river and Thrapston usually has a continuous through-flow. Both sites were sampled on a 2-weekly basis for phytoplankton and chlorophyll.

Billing

In addition to the routine samples at km 34.0 (which is situated approximately 20 m downstream of the marina) one sample was taken at the mouth of the marina and another 500 m further upstream. The upstream sample point was located upstream of Billing lock to eliminate any possibility of back-flow from the marina.

Thrapston

The outflow water of Thrapston sailing lake was sampled, although flow was restricted for maintenance during some of the period.

3.311 Spot surveys

In addition to the surveys covered above several spot investigations were undertaken to supply important background or supporting information for the wider study (Table 3.2).

Table 3.2 Spot samples and surveys undertaken.

Kinewell lake outfall (SP 975 753)
Summer Leys outfall (SP 889 642)
Spatial survey of tributary 3 (Willow Brook) 7 sites

3.312 Phaeopigment surveys

Chlorophyll was not routinely corrected for phaeopigments, although a small study was carried out to assess the relative contribution of degradation products, which were determined using acidification and neutralisation (Marker, 1992). Samples were collected at km 91.7 on 10 April 1997, 5 August 1997 and 8 January 1998, n = 5 for each.

3.313 Sample and subsample error

Numerous phytoplankton and chlorophyll samples were taken at km 91.7 to assess sample and subsample error, sample treatment and the accuracy of analysis carried out by the EA NLS.

Sample error

Samples were collect at 2-min intervals to assess sample error for phytoplankton and chlorophyll. Samples were taken from the main river at km 91.7 (18/8/94, 21/4/95, 7/8/95, 13/5/96, 11/7/95, and 15/5/96) and one from the bay (21/4/95). n = 5 for all tests.

Subsample error

Primary-subsample error was assessed by taking five subsamples for phytoplankton and chlorophyll from a single sample (17/8/94,18/8/94, 20/4/95, 13/5/96, 15/5/96 and 5/8/97).

Analysis of five subsamples from a single primary-subsample assessed secondary-subsample error. Primary and secondary-subsample errors were also assessed on the 14/5/98 using in-vivo fluorescence. On this occasion two 1-L primary-subsamples were taken from a single 10-L sample and the *in-vivo* chlorophyll was measured in 10 secondary-subsamples from each 1-L bottle.

Chlorophyll sample treatment

The influence of different storage durations and treatments were evaluated along with parallel analyses carried out by the NLS. Samples were stored for varying periods prior to analysis, either as whole water samples or as filtered samples on frozen filter paper (Table 3.3).

Table 3.3 Chlorophyll sample storage treatments and parallel runs with NLS. Light-tight bottles were used for storage of whole water samples. All samples were taken at km 91.7. Water samples were stored < 5°C , except 10/6/97 which was stored at ambient temperature.

Date	Treatment
17/8/94	10 1-L samples collected. Odd numbered samples were site filtered and frozen. Even numbered samples were kept in bottles. All samples analysed after 27 h.
18/8/94	10 1-L samples collected. Odd numbered samples sent for analysis at NLS. Even numbered samples analysed within four h.
20/4/95	10 1-L samples collected. Five samples site filtered and frozen. Five samples stored in bottles.
7/11/95	25 1-L samples collected. 500-mL site filtered and frozen from each of the 25 1-L bottles and remaining 500-mL was left in the bottle. Five each of the site filtered and whole water samples were selected at random and analysed at 4 h, 24 h, 48 h, 72 h and 144 h.
13/5/96	25 1-L samples collected. Five samples sent for analysis by NLS. Five samples analysed after four h. 10 1-L were site filtered and frozen and remaining 10 1-L stored in bottles. Five samples from each treatment were selected at random and analysed after 16 d and 31 d.
10/4/97	10 1-L samples collected. Odd numbered samples sent for analysis at NLS. Even numbered samples analysed within 4 h.
10/6/97	10 1-L samples collected. Five samples site filtered, frozen and analysed same day. Five samples stored overnight in Wansford gauging station at ambient temperature and analysed following day. Both sets of samples were analysed for phaeopigment concentrations.

3.314 Additional data

Chemical

A range of chemical determinands was available for Nene sites, collected as part of the EA routine monitoring programme. These data were readily available from 1991 onwards from a database at Peterborough. Additional data for km 91.7 were obtained from two other sources, archive tape held by the EA at Peterborough (1981 to 1990) and microfiche held by the EA at Lincoln (1975 to 1980). The km 91.7 data were compiled to form what will be referred to as the long-term data set. Unfortunately, the long-term data were incomplete for two important determinands for this research (silicate and chlorophyll).

Rotifers

Rotifer abundance data were obtained for the Nene at Wansford Railway Station (TL 093 979), which was situated 2.6 km downstream from km 91.7. 10-L samples

were taken from the open water, filtered through 53-μm mesh and preserved in 4% formaldehyde (R. J. Sanderson, pers. comm.).

Macrophyte data

Macrophytes (including filamentous algae) were recorded and percentage cover of each taxon estimated from 100-m stretches at several Nene sites between 1993 and 1997, as part of the UWWT Directive eutrophication assessment. Surveys were carried out using the EA standard methodology (Environment Agency, 1999), which uses the nine-point scale for estimating abundance of macrophytes (based on, Standing Committee of Analysts, 1987).

Summary of sampling programme

Key elements of the sample programme described above are shown in Table 3.4.

Table 3.4 Summary of sample programme. Showing determinand, median sample frequency (Freq.) and comments (occ. = occasionally).

Determinand	Freq.	Comment
Long-term study: km 91.7 1975 to 1998		
Temperature	6 d	
NH_4-N	7 d	
NO_3-N	7 d	
NO_2-N	7 d	
TRP	7 d	
Chlorophyll	7 d	Chlorophyll data augmented by current study
Current study: eight main river and three tributary sites 1993 to 1998 (Figure 2.4)		
Secchi disc depth	14 d	
Temperature	14 d	
FRP	14 d	Higher frequency at km 91.7 for chlorophyll
TP	14 d	and phytoplankton: 7 d 1994 and 1995 (Apr
Chlorophyll and phytoplankton	14 d	to Sep), 3 d 1996 (Mar to Oct)
Additional studies: chlorophyll and phytoplankton		
Spate flow	1 d	September 1994 at km 91.7
Bay/main river comparison	7 d	April to September 1995 at km 91.7
Sedimentation	14 d	March to October 1996 at km 91.7
Light attenuation	occ.	km 91.7
Rotifers 1996	3 d	March to October at km 91.7
High frequency sampling	4 h	Four occasions at km 91.7 and km 92.5
Picoplankton	14 d	1997 to 1998 at km 91.7
Influence of Billing marina	14 d	Mar to Sep 1996
Influence of Thrapston lake	14 d	Mar to Sep 1996
Willow Brook	-----	1996
Additional data from EA and Meteorological Office		

3.4 Laboratory analysis

3.41 Chemical

Nutrient and silicate concentrations were determined photometrically following standard methodologies. TRP, FRP and TP were analysed using the phosphomolybdenum blue method (Standing Committee of Analysts, 1992: based on Murphy and Riley, 1962). Silicate was determined as molybdate reactive silicon following reduction by means of 1-amino-2-naphthol-4-sulphonic acid (Standing Committee of Analysts, 1992: based on Webber and Wilson, 1964). NH_4-N was determined as indophenol blue after reacting with hypochlorite and salicylate ions in solution in the presence of sodium nitroprusside (Standing Committee of Analysts, 1981a: based on Chaney and Marchbach, 1962). TON, NO_3-N and NO_2-N were measured using continuous flow analysis (Standing Committee of Analysts, 1981b: based on Bendschneider and Robinson, 1952). TON was measured as NO_2-N, following the reduction of NO_3-N to NO_2-N by hydrazine. NO_3-N was determined as TON minus NO_2-N. TN was calculated as the product of NH_4-N, NO_3-N and NO_2-N and N:P ratio as the quotient of TN and TRP. Likewise, the P:silicate ratio was calculated as the quotient of TRP and SiO_2-Si.

All chlorophyll samples were analysed using hot methanol extraction and photometric determination at wavelengths 665 nm and 750 nm, the latter to compensate for 'background turbidity' (Standing Committee of Analysts, 1980) Phaeopigments were determined using acidification and neutralisation (Marker, 1992).

Dissolved inorganic carbon (DIC) was estimated from total alkalinity using a method described by (Wetzel and Likens, 1991), using contemporaneous temperature and pH values.

3.42 Biological

Planktonic diatom treatment and enumeration

Diatom samples were allowed to sediment in clean 1-L glass cylinders for sufficient time for the smallest specimens to settle completely (Furet and Benson-Evans, 1882). Following settlement the supernatant was carefully siphoned off and the remaining sediment transferred to a 50-mL centrifuge tube for the digestion of organic matter, following the method described by Kelly (1996). This method was too disruptive for some plankton samples, which contained pennate diatoms with poorly

developed frustules. These diatom samples were digested by ignition, where a concentrated droplet of sample was placed on a clean microscope slide, which was placed on a hot-plate for several hours. All samples were mounted in Naphrax prior to examination under oil immersion at × 1000 magnification.

Slides were enumerated by transects (avoiding the edges of the sample) counting at least 300 specimens.

Picoplankton enumeration

Picoplankton enumeration was based on the methods described by Hawley and Whitton (1991). Fully mixed samples were pre-filtered through a 3-μm Whatman cellulose nitrate filter papers, before being filtered on 0.2-μm Whatman polycarbonate (hydrophilic) filter paper, which had been pre-stained with irgalan black (Ceiba-Geigy, 2 g L^{-1} in 2% acetic acid). Filter papers were placed on a clean microscope slide, held in position with a little emersion oil, and fitted with a cover slip.

Enumeration was carried out in a darkened room using oil emersion at ×1000 magnification and illuminated using epifluorescence light with blue and green filtration. Cells were counted in random fields of view and classified according to their size and colour of filter under which they fluoresced most brightly.

Phytoplankton enumeration

The basic counting method employed during this work, which was based on the techniques described by Utermöhl (1958) is described and evaluated in Chapter 5. The standard method was carried out on routine samples collected from km 91.7, and adaptations of this method and other procedures were used elsewhere, depending on the desired information and accuracy required.

In addition to km 91.7, three main river sites (km 34.0, km 43.9, km 64.6) were selected for evaluation of phytoplankton abundance and species composition. At these sites 'key taxa' (those which were most dominant or of greatest interest) were counted during periods when the chlorophyll concentration > 10 μg L^{-1}. High frequency, tributary and special survey samples were also treated in this way and/or using a new approach (spaced fields) which provides greater accuracy with narrower confidence intervals (Chapter 5).

Live samples were concentrated by centrifugation (where necessary) and examined at × 400 magnification. The species were recorded and their abundance classified according to a semi-quantitative scale (Table 3.5).

Table 3.5 Semi-quantitative abundance categories.

Abundance category	Description
present (P)	1 specimen in sample
scarce (S)	2 to 10 specimens in sample
common (C)	11 to 100 specimens in sample
abundant (A)	101 to 1000 specimens in sample
very abundant (V)	more than 1000 specimens in sample

Zooplankton enumeration

The 1-L zooplankton samples were concentrated in clean glass cylinders and allowed 48 h to settle. The supernatant was carefully siphoned off and the sediment was transferred to a clean measuring cylinder and made up to a known volume with deionised water. The concentrated sample was fully mixed and a subsample transferred to a clean 10-mL sedimentation chamber. Sediment chambers were allowed 20 min to settle, which was found by experimentation to be the necessary time for all the rotifers to settle. This technique reduced the quantity of smaller sediment, which was still in suspension, thus improving the accuracy of the count.

The whole chamber was then scanned in a series of horizontal adjacent transects (Hasle, 1981) at × 100 magnification using a cross-hair graticule (Chapter 5), on an inverted microscope. Zooplankton and rotifer eggs encountered during the scans were counted as they passed the vertical line of the cross-hair. Organisms falling on the upper horizontal line of the cross-hair were counted and those on the lower line ignored, being counted in the next transect. At least 300 individuals were counted per sample (where possible).

Sediment treatment and enumeration

Sediment samples (with the exception of the 5/4/96) were thoroughly mixed and divided into two equal quantities, one for evaluation of dry weight and LOI and the other for identification and counting. Dry weight was achieved by placing the sample in

a Perspex beaker in an oven at 110°C until weight became stable. LOI was calculated as the dry weight minus the weight following combustion in a muffle furnace for 24 h.

The remaining aliquots and the sample collected on the 5 April 1995 were made up to a known volume, using deionised water. These aliquots were then subsampled into clean 10-mL sedimentation chambers and allowed to settle for at least 24 h before counting. The samples were counted at × 1000 magnification under oil immersion in between 50 and 100 random fields, attempting to count at least 300 individuals, although the quantity of inorganic sediment in some samples made this impossible.

All results were converted to a percentage and expressed as units day^{-1} by dividing by the number of days the sediment trap was deployed.

3. 5 Statistical methods and treatment of data

3.51 General procedures and transformations

General statistical analysis was carried out using MINITAB software (Ryan et al., 1985). Statistical distributions were examined prior to analysis and where necessary data were either transformed (Table 3.6) to approximate normality (Fry, 1996) or non-parametric techniques were used. Normally distributed or successfully transformed data facilitates the use of parametric techniques, such as product moment correlation and regression analysis (Elliott, 1983). Chlorophyll values < 1µg L^{-1} were excluded from the subsequent analysis, $\log_{10}$ transformation resulting in negative values.

Table 3.6 Data transformations.

Variable (x)	Transformation	Variable (x)	Transformation
7 d CuSum Sun-light	None	Phytoplankton	$\log_{10}(x + 1)$
75 SF counts	None/ $\log_{10}(x)$	Rotifers	$\log_{10}(x)$
Chlorophyll	$\log_{10}(x)$	SiO_2-Si	None
Discharge	$\log_{10}(x)$	TRP	$\sqrt{(x)}$
FRP	$\sqrt{(x)}$	Temperature	None
Macrophytes	$\log_{10}(x)$	TN	None
N:P ratio	$\log_{10}(x)$	TON	None
NH_4-N	$\log_{10}(x)$	Velocity	1/(x)
NO_3-N	None	z_{eu}	$\log_{10}(x)$
NO_2-N	$\sqrt{(x)}$	z_s	$\sqrt{(x)}$

Confidence intervals for small samples (n < 30; e.g. sample error evaluation and phytoplankton counts in diameter transects) were calculated using $\log_{10}$ transformed data and the arithmetic mean (Elliott, 1983). Confidence intervals for larger samples

($n \geq 30$) were calculated using the Student's t statistic or from predetermined relationships (Chapter 5).

3.52 Physical and chemical

Euphotic depth

The relationships between chlorophyll concentration and z_s and z_s and z_{eu} were explored using regression analysis. The data were restricted to the periods April to September (1994 – 1996) when the influence of inorganic suspended solids on light attenuation was thought to be least.

Long-term trends

Long-term trends in discharge, water temperature, light, NH_4-N, NO_3-N, NO_2-N, TN, N:P and TRP (1975 -1996) at km 91.7 were investigated by regressing annual medians on t and by comparison of the medians from the periods 1975 to 1986 and 1987 to 1998. This was undertaken using Mood's median test, which was also used to investigate inter-year variation.

3.53 Chlorophyll

Temporal trends

The relationships between discharge and water chemistry and physical and chemical variables and chlorophyll concentration were investigated using regression analysis. Additionally to evaluating relationships using the full chlorophyll data set, subsets were created to ease elucidation of the factors influencing temporal chlorophyll concentration.

Spatial trends

Downstream trends in chlorophyll concentration were investigated by plotting velocity, temperature and chlorophyll concentration (1994 - 1996) for main river sites (km 22.4 to km 91.7) against downstream distances from source. This was completed for chlorophyll concentrations expressed as 3-monthly averages, annual maxima at downstream sites (km 85.2 and km 91.7) and annual maxima for the study section. Downstream chlorophyll trends were further investigated using multiple-regression

analysis, using velocity, temperature and downstream distance from source as predictors.

Differences in tributary chlorophyll concentration were examined using Mann-Whitney U tests and results were evaluated with respect to relevant physical variables. The influence of tributary 3 on the main river was investigated by examination of contemporaneous chlorophyll concentrations at up and downstream sites (km 85.2 and km 91.7). Comparison was made using the whole data set and a restricted set, consisting of tributary chlorophyll concentrations that exceeded those of the main river.

Derivation of chlorophyll data subsets

The exploration of chlorophyll data subsets was undertaken using a specially written computer program (PEEP), which aided rapid manipulation and comparison of large data sets. The PEEP software enabled rapid production of data subsets and facilitated the examination of relationships between variables, either as selected time periods (e.g. all between April to June) or ranges (e.g. all between a temperature range of 5 to 20°C). Furthermore, where daily data were available (e.g. discharge and sunlight) chlorophyll could be correlated against variables expressed in a variety of different ways. For example, average, cumulative-sum, minimum, etc could be calculated over varying numbers of days (2 - 15) proceeding and including the sample date. The principal objective of this process was not to produce the most significant relationships between chlorophyll concentration and other variables but to extract the most useful information. Following preliminary exploration, five chlorophyll sub-sets were created (Table 3,7).

Table 3.7 describes low chlorophyll summer periods, which were further investigated by examination of the annual physical variables for the period July to September. Statistical comparison was made between data from years with 'low chlorophyll summer periods' and other years.

3.54 Phytoplankton

Temporal trends at km 91.7

Temporal trends in phytoplankton abundance were explored by examination of time series, through correlation with chemical and physical variables and using multivariate statistics. Initially all phytoplankton taxa from the routine sampling

programme were collated and each taxon expressed as a percentage occurrence. Taxa groups were then selected based on abundance/biovolume, periodicity and ecology. Group selection was partly a subjective process based on experience gained during the phytoplankton counts and personal observation, but their validity was assessed during the analysis and reclassifications made as necessary.

Table 3.7 Chlorophyll data sub-sets created to investigate the relationship between chlorophyll concentration and physical and chemical variables.

Sub-set name	Definition
January to spring chlorophyll maximum	All data from 1 January to spring chlorophyll maximum (maximum occurring between January and June)
Low chlorophyll summer period	Period (in excess of 30 d) following the spring chlorophyll peak that starts when the chlorophyll concentration falls below 10 $\mu g\ L^{-1}$ (between June and August) and ends when it increases above 20 $\mu g\ L^{-1}$ or 31 December, whichever is sooner
All minus low chlorophyll summer period	All chlorophyll data minus the 'low chlorophyll summer period'
January to June	All data from 1 January to 30 June
July to December	All data from 1 July to 31 December

Major groups were plotted as time series to aid qualitative assessment. The PEEP software (described above) was also used to explore the relationships between important taxa groups and physical and chemical variables. During this analysis phytoplankton data were transformed and any resultant negative values excluded.

All major groups were also analysed using the CANOCO software (ter Braak and Smilauer, 1998) where the ordination of the taxa can be assessed against environmental variables, using the CCA routine (ter Braak and Verdonschot, 1995). The CCA analysis was undertaken using symmetrical biplot scaling with log transformed taxa data and down-weighing of the influence of rare taxa. The initial analysis was carried out using manual forward selection of environmental variables with Monte Carlo permutation tests for significance. Those environmental variables that had a non-significant influence during the initial analysis were removed from the model and the final analysis was run with tests for axes significance. The resulting ordinations were printed as separate biplots of samples and environmental variables and taxa and environmental

variables (ter Braak and Verdonschot, 1995). The taxa data was analysed as a complete set and separated into two subsets, 1 January to 30 June and 1 July to 31 December.

Short-term trends at km 91.7

A pilot survey was undertaken during 1996 and three more extensive surveys during 1997. Throughout the 1997 surveys discharge, light and temperature were recorded at 30 min, 10 min and 15 min intervals respectively and chlorophyll and phytoplankton at varying intervals between 1 h and 4 h, using the auto-sampler.

Temporal change in chlorophyll and phytoplankton were investigated using regression analysis, with discharge, temperature and light as predictors. Temperature and discharge were averaged over varying periods preceding the sample and light was integrated in the same way.

Spatial trends

Direct comparison of time series of spatial chlorophyll was complicated by seasonality, and this problem was addressed by decomposition of the series using time-series analyses (Minitab, 1996). Seasonality of data was removed and liner trends used as underlying values for site comparisons using Mann-Whitney U tests.

3.55 Zooplankton

The influence of physical and biological variables on rotifer and ciliate abundance was investigated using regression analysis.

3.56 Sediment

The relationship between the abundance of taxa in the sediment traps and the phytoplankton was investigated using regression analysis.

3.57 Macrophyte

The percentage cover values of submerged macrophytes were summated for each site and evaluated against discharge, temperature and chlorophyll. Variables were expressed as the annual averages up to the data of, or the nearest sample preceding the macrophyte survey and transformed as necessary. Data sets were pooled following standardisation, which was achieved by dividing each value by the site average and multiplying by 100.

4 TAXONOMY

4.1 Introduction

This chapter contains details of suspended algae, zooplankton and submerged macrophytes. Details of diatom and picoplankton size classes used, centric diatoms in digested samples and the morphological impacts of silicate deficiency are also included.

4.2 Suspended algae at km 91.7

Samples were treated in one of four ways; examined live, fixed in Lugol's Iodine, fixed in buffered formalin or digested/combusted to allow the frustules of diatoms to be examined. The latter being examined using a light microscope and SEM. A single method of treatment was unsuitable for the identification of all taxa. For example, if movement is diagnostic then the examination of live material is necessary, whereas diatom taxonomy is usually based on the morphology of the frustule, which cannot be resolved without the removal of organic material. The taxa list for km 91.7 (Wansford), that follows, was compiled using a combination of techniques and contains representative taxa from all sites found throughout the study (Table 4.1).

Table 4.1 List of suspended algae at km 91.7. Codes, nomenclature and authorities were taken from Whitton et al. (1998). Abbreviations and other conventions follow those given by Brummitt and Powell (1992).

Code	Genus	Species	Authority
01020000	ANABAENA		Bory
01020090	*Anabaena*	*flos-aquae*	(Lyngb.) Bréb.
01040020	*Aphanizomenon*	*flos-aquae*	(L.) Ralfs
01130000	CHROOCOCCUS		Nägeli
01320000	GOMPHOSPHAERIA		Kütz.
01320010	*Gomphosphaeria*	*aponina*	Kütz.
01320020	*Gomphosphaeria*	*lacustris*	Chodat
01430000	LYNGBYA		C.Agardh
01460000	MERISMOPEDIA		Meyen
01490000	MICROCYSTIS		Kütz.
01430000	OSCILLATORIA		Vaucher
01430010	*Oscillatoria*	*agardhii*	Gomont
01430160	*Oscillatoria*	*limnetica*	Lemmerm.
01430230	*Oscillatoria*	*redekei*	Goor
04010000	COLACIUM		Ehrenb. 1838
04020000	EUGLENA		Ehrenb. 1830
04020310	*Euglena*	*tripteris*	(Dujard.) G.A.Klebs

04070000	PHACUS		Dujard. 1841
04100000	TRACHELOMONAS		Ehrenb. emend Deflandre 1926
04100140	*Trachelomonas*	*hispida*	(Perty) Stein
04100340	*Trachelomonas*	*volvocina*	Ehrenb.
04100360	*Trachelomonas*	*volvocinopsis*	Swirenko
04020000	CHROOMONAS		Hansg. 1884
04040000	CRYPTOMONAS		Ehrenb. 1838
04040040	*Cryptomonas*	*marsonii*	Skuja
04040040	*Cryptomonas*	*ovata*	Ehrenb.
04100000	RHODOMONAS		Karsten 1898
05100012	*Rhodomonas*	*lacustris* var. *nannoplanktica*	Pascher et Ruttner. (Skuja) Jav.
06020000	CERATIUM		Schrank 1793
06020040	*Ceratium*	*hirundinella*	(O.F.Müll.) Dujard.
06040000	GLENODINIUM		(Ehrenb.) F.Stein
06070000	GYMNODINIUM		F.Stein 1878 em. Kofoid et Swezy
06110000	PERIDINIUM		Ehrenb. 1830
09130042	*Chrysococcus*	*rufescens*	G.A.Klebs
09230000	DINOBRYON		Ehrenb. 1834
09230070	*Dinobryon*	*sertularia*	Ehrenb.
09310000	MALLOMONAS		Perty 1841
09310030	*Mallomonas*	*akrokomos*	Ruttner in Pascher
09430000	SYNURA		Ehrenb. 1834
10040010	*Centritractus*	*belonophorus*	Lemmerm.
10090020	*Goniochloris*	*mutica*	(A.Braun) Fott
12020010	*Actinocyclus*	*normanii*	(Greg. ex Grev.) Hust. 1947
12030000	AULACOSEIRA		G.H.K.Thwaites 1848
12030060	*Aulacoseira*	*granulata*	(Ehrenb.) Simonsen 1979
12060040	*Cyclostephanos*	*dubius*	(Fricke) Round 1982
12060040	*Cyclostephanos*	*invisitatus*	(Hohn et Hellermann) Theriot
12070000	CYCLOTELLA		Kütz. ex. Bréb. 1838
12070040	*Cyclotella*	*atomus*	Hust. 1937
12070280	*Cyclotella*	*meneghiniana*	Kütz. 1844
12070340	*Cyclotella*	*pseudostelligera*	Hust. 1939
12070370	*Cyclotella*	*radiosa*	(Grunow) Lemmerm. 1900
12110000	MELOSIRA		C.Agardh 1824
12110080	*Melosira*	*varians*	Agardh 1827
12160000	SKELETONEMA		R.K.Grev. 1864
12160020	*Skeletonema*	*potamos*	(Weber) Hasle 1976
12160030	*Skeletonema*	*subsalsum*	(A.Cleve) Bethge 1928
12180000	STEPHANODISCUS		Ehrenb. 1844
12180090	*Stephanodiscus*	*hantzschii*	Grunow in Cleve et Grunow 1880
12180092	*Stephanodiscus*	*hantzschii* fo.	Grunow in Cleve et Grunow

		tenuis	1880
12180160	*Stephanodiscus*	*neoastraea*	Håk. et Hickel 1986
12180190	*Stephanodiscus*	*parvus*	Stoermer et Håk. 1984
12190140	*Thalassiosira*	*pseudonana*	Hasle et Heimdal 1970
12190200	*Thalassiosira*	*weissfloggii*	(Grunow) Fryxell et Hasle 1977
13010000	ACHNANTHES		Bory 1822
13010270	*Achnanthes*	*haynaldii*	Schaarschm. 1881
13040000	AMPHORA		Ehrenb. ex Kütz. 1840
13040180	*Amphora*	*libyca*	Ehrenb.
13040280	*Amphora*	*ovalis*	Kütz. 1844
13040290	*Amphora*	*pediculus*	(Kütz.) Grunow in Schmid et al. 1874
13080010	*Asterionella*	*formosa*	Hassall 1844
13160000	COCCONEIS		Ehrenb. 1837
13160080	*Cocconeis*	*pediculus*	Ehrenb. 1838
13160100	*Cocconeis*	*placentula*	Ehrenb.1838
13160104	*Cocconeis*	*placentula*	Ehrenb. 1838
13190012	*Ctenophora*	*pulchella*	(Ralfs ex. Kütz.) Kütz. 1844
13210000	CYMATOPLEURA		W.Sm. 1841
13220000	CYMBELLA		C.Agardh 1830
13220122	*Cymbella*	*cistula*	(Ehrenb.) Kirchner 1878
13220330	*Cymbella*	*lanceolata*	(Ehrenb.) Kirchner 1878
13220600	*Cymbella*	*tumida*	(Bréb.) Grunow in Van Heurck 1880
13260000	DIATOMA		Bory 1824
13260042	*Diatoma*	*tenue*	Agardh 1812
13260040	*Diatoma*	*vulgare*	Bory 1824
13310170	*Encyonema*	*minutum*	(Hilse in Rabenh.) D.G.Mann in Round et al. 1990
13370000	FRAGILARIA		H.C.Lyngb. 1819
13370034	*Fragilaria*	*capucina*	Desm. 1824
13370040	*Fragilaria*	*crotonensis*	Kitton 1869
13390040	*Frustulia*	*vulgaris*	(Thwaites) De Toni 1891
13410000	GOMPHONEMA		Ehrenb. 1832
13410080	*Gomphonema*	*augur*	Ehrenb. 1840
13410310	*Gomphonema*	*parvulum*	(Kütz.) Kütz. 1849
13410390	*Gomphonema*	*truncatum*	Ehrenb. 1832
13420000	GYROSIGMA		Hassall 1844
13420010	*Gyrosigma*	*acuminatum*	(Kütz.) Rabenh. 1843
13420040	*Gyrosigma*	*attenuatum*	(Kütz.) Cleve 1894
13410000	MERIDION		C.Agardh 1824
13420000	NAVICULA		Bory 1822
13420340	*Navicula*	*capitatoradiata*	Germain 1981
13420410	*Navicula*	*cincta*	(Ehrenb.) Ralfs in A.Pritch. 1861

13420440	*Navicula*	*cryptocephala*	Kütz. 1844
13420480	*Navicula*	*cryptotenella*	Lange-Bert. 1984
13421070	*Navicula*	*gregaria*	Donkin 1861
13421240	*Navicula*	*incertata*	Lange-Bert. 1984
13421390	*Navicula*	*lanceolata*	(Agardh) Ehrenb. 1838
13421600	*Navicula*	*menisculus*	Schumann 1866
13421860	*Navicula*	*phyllepta*	Kütz. 1844
13422010	*Navicula*	*radiosa*	Kütz. 1844
13422100	*Navicula*	*rhynchocephala*	Kütz. 1844
13422730	*Navicula*	*tripunctata*	(O.F.Müll.) Bory 1822
13422740	*Navicula*	*trivialis*	Lange-Bert. 1980
13422840	*Navicula*	*veneta*	Kütz. 1844
13422880	*Navicula*	*viridula*	(Kütz.) Ehrenb. 1836
13440000	NITZSCHIA		Hassall 1844
13440020	*Nitzschia*	*acicularis*	(Kütz.) W.Sm. 1843
13440090	*Nitzschia*	*amphibia*	Grunow 1862
13440140	*Nitzschia*	*archibaldii*	Lange-Bert. 1980
13440180	*Nitzschia*	*bacillum*	Hust. 1922
13440260	*Nitzschia*	*capitellata*	Hust. in A.Schmidt et al. 1922
13440370	*Nitzschia*	*dissipata*	(Kütz.) Grunow 1862
13440480	*Nitzschia*	*filiformis*	(W.Sm.) Van Heurck 1896
13440490	*Nitzschia*	*flexa*	Schumann 1862
13440442	*Nitzschia*	*frustulum*	(Kütz.) Grunow in Cleve et Grunow 1880
13440440	*Nitzschia*	*fruticosa*	Hust. 1947
13440610	*Nitzschia*	*gracilis*	Hantzsch 1860
13440640	*Nitzschia*	*heufleriana*	Grunow 1862
13440730	*Nitzschia*	*intermedia*	Hantzsch ex Cleve et Grunow 1880
13440842	*Nitzschia*	*linearis*	(Agardh) W.Sm. 1843
13440900	*Nitzschia*	*microcephala*	Grunow in Cleve et Möller 1878
13440980	*Nitzschia*	*palea*	(Kütz.) W.Sm. 1846
13440990	*Nitzschia*	*paleacea*	Grunow in Van Heurck 1881
13441040	*Nitzschia*	*perminuta*	(Grunow) M.Perag. 1903
13441110	*Nitzschia*	*pumila*	Hust. 1944
13441140	*Nitzschia*	*recta*	Hantzsch ex. Rabenh. 1861
13441210	*Nitzschia*	*sigmoidea*	(Nitzsch.) W.Sm. 1843
13490000	PINNULARIA	PINNULARIA	Ehrenb. 1843
13491240	*Pinnularia*	*viridis*	(Nitzsch) Ehrenb. 1843
13670010	*Reimeria*	*sinuata*	(Greg.) Kociolek et Stoermer 1987
13680000	RHOICOSPHENIA		Grunow 1860
13680010	*Rhoicosphenia*	*abbreviata*	(C.Agardh) Lange-Bert. 1980
13770020	*Staurosira*	*elliptica*	(Schumann) D.M.Williams et

			Round 1987
13800000	SURIRELLA		Turpin 1828
13800100	*Surirella*	*brebissonii*	Krammer et Lange-Bert. 1987
13800230	*Surirella*	*helvetica*	Brun 1880
13800320	*Surirella*	*minuta*	Bréb. 1838
13810000	SYNEDRA		Ehrenb. 1830
13810010	*Synedra*	*acus*	Kütz. 1844
13810180	*Synedra*	*ulna*	(Nitzsch) Ehrenb. 1836
13820000	TABELLARIA		Ehrenb. 1840
13820010	*Tabellaria*	*fenestrata*	(Lyngb.) Kütz. 1844
13840070	*Tryblionella*	*debilis*	Arnott in O'Meara 1873
14040010	*Nephroselmis*	*olivacea*	Stein
16030010	*Aulacomonas*	*submarina*	Skuja
16060000	CARTERIA		Diesing
16180000	CHLAMYDOMONAS		Ehrenb.
16180030	*Chlamydomonas*	*cingulata*	Pascher
16180032	*Chlamydomonas*	*cingulata*	Pascher
16190000	CHLOROGONIUM		Ehrenb.
16260000	EUDORINA		Ehrenb.
16260010	*Eudorina*	*elegans*	Ehrenb.
16330000	GONIUM		O.F.Müll.
16330020	*Gonium*	*sociale*	(Dujard.) Warming
16470000	PANDORINA		Bory
16470010	*Pandorina*	*morum*	(O.F.Müll.) Bory
16600000	PTEROMONAS		Seligo
16600032	*Pteromonas*	*angulosa*	(N.Carter) Lemmerm.
16680010	*Spermatozopsis*	*exsultans*	Korshikov
17020000	ACTINASTRUM		Lagerh.
17020010	*Actinastrum*	*hantzschii*	Lagerh.
17040000	ANKISTRODESMUS		Corda
17040030	*Ankistrodesmus*	*falcatus*	(Corda) Ralfs
17040040	*Ankistrodesmus*	*fusiformis*	Corda
17130000	CHLORELLA		Beij.
17130060	*Chlorella*	*vulgaris*	Beij.
17170000	CLOSTERIOPSIS		Lemmerm.
17170020	*Closteriopsis*	*longissima*	(Lemmerm.) Lemmerm.
17200000	COELASTRUM		Nägeli
17200020	*Coelastrum*	*microporum*	Nägeli
17200040	*Coelastrum*	*pseudomicroporum*	Korshikov
17240000	CRUCIGENIA		Morren sensu Komárek
17240030	*Crucigenia*	*tetrapedia*	(Kirchner) W. et G.S.West
17260010	*Crucigeniella*	*apiculata*	(Lemmerm.) Komárek
17330000	DICTYOSPHAERIUM		Nägeli
17330010	*Dictyosphaerium*	*chlorelloides*	(Nauman) Komárek et Perman

17330020	*Dictyosphaerium*	*ehrenbergianum*	Nägeli
17330040	*Dictyosphaerium*	*pulchellum*	H.C.Wood
17430020	*Golenkinia*	*radiata*	(Chodat) Wille
17410000	KIRCHNERIELLA		Schmidle
17410040	*Kirchneriella*	*obesa*	(W.West) Schmidle
17440000	LAGERHEIMIA		(De Toni) Chodat
17440020	*Lagerheimia*	*ciliata*	(Lagerh.) Chodat
17440040	*Lagerheimia*	*genevensis*	(Chodat) Chodat
17440070	*Lagerheimia*	*wratislaviensis*	Schröder
17470000	MICRACTINIUM		Fresenius
17470010	*Micractinium*	*pusillum*	Fresenius
17480000	MONORAPHIDIUM		Komárková-Legnerová
17480010	*Monoraphidium*	*arcuatum*	(Korshikov) Hindák
17480020	*Monoraphidium*	*contortum*	(Thur.) Komárková-Legnerová
17480070	*Monoraphidium*	*komarkovae*	Nygaard
17620000	NEPHROCHLAMYS		Korshikov
17640000	OOCYSTIS		Nägeli ex A.Braun
17640060	*Ooocystis*	*marssonii*	Lemmerm.
17640120	*Oocystis*	*parva*	W. et G.S.West
17680000	PEDIASTRUM		Meyen
17680020	*Pediastrum*	*biradiatum*	Meyen
17680040	*Pediastrum*	*duplex*	Meyen
17680090	*Pediastrum*	*tetras*	(Ehrenb.) Ralfs
17800000	RAPHIDOCELIS		Hindák em. Marvan et al.
17810000	SCENEDESMUS		Meyen
17810020	*Scenedesmus*	*aculeotatus*	Reinsch
17810030	*Scenedesmus*	*acuminatus*	(Lagerh.) Chodat
17810040	*Scenedesmus*	*acutus*	Meyen
17810060	*Scenedesmus*	*apiculatus*	(W. et G.S.West) Corda
17810080	*Scenedesmus*	*armatus*	(Chodat) Chodat
17810090	*Scenedesmus*	*arthrodesmiformis*	Schröder
17810120	*Scenedesmus*	*bicaudatus*	Dedusenko
17810160	*Scenedesmus*	*communis*	Hegewald
17810192	*Scenedesmus*	*denticulatus*	Lagerh.
17810240	*Scenedesmus*	*grahneisii*	(Heynig) Fott
17810282	*Scenedesmus*	*intermedius*	Chodat
17810320	*Scenedesmus*	*obliquus*	(Turpin) Kütz.
17810340	*Scenedesmus*	*opoliensis*	P.Richter
17830000	SCHROEDERIA		Lemmerm. em. Korshikov
17860000	SELENASTRUM		Reinsch
17870020	*Siderocelis*	*ornata*	(Fott) Fott
17910000	SPHAEROCYSTIS		Chodat
17910010	*Sphaerocystis*	*planctonica*	(Korshikov) Bourr.
17960000	TETRAEDRON		Kütz.

17960010	*Tetraedron*	*caudatum*	(Corda) Hansg.
17960020	*Tetraedron*	*incus*	(Teiling) G.M.Sm.
17960030	*Tetraedron*	*minimum*	(A.Braun) Hansg.
17970000	TETRASTRUM		Chodat
17970020	*Tetrastrum*	*glabrum*	(Roll) Ahlstrom et Tiffany
17970040	*Tetrastrum*	*staurogeniaeforme*	(Schröder) Lemmerm.
18010020	*Treubaria*	*triappendiculata*	Bernard
24170000	GLOEOTILA		Kütz.
24340000	STICHOCOCCUS		Nägeli
24380000	ULOTHRIX		Kütz.
24010000	ELAKATOTHRIX		Wille
24030000	KOLIELLA		Hindák
24030010	*Koliella*	*longiseta*	(Vischer) Hindák
27040000	CLOSTERIUM		Nitzsch 1817 ex Ralfs 1848
27040040	*Closterium*	*acutum*	(Lyngb.) Bréb. in Ralfs 1848
27040440	*Closterium*	*moniliferum*	(Bory) Ehrenb. ex Ralfs 1848
27040400	*Closterium*	*parvulum*	Nägeli 1849
27040000	COSMARIUM		Corda ex Ralfs 1848
27040160	*Cosmarium*	*bioculatum*	Bréb. in Ralfs 1848
27370000	STAURASTRUM		Meyen 1829 em. Ralfs 1848

4.3 Diatom identification

Identification of diatoms was not normally possible during counting (with some exceptions, such as filamentous centric forms like *Melosira varians* and colonial pennates like *Nitzschia fruticosa*). Diatoms were therefore normally classified according to size. Centric diatoms were grouped according to the diameter of their valves and pennate diatoms according to their length (Table 4.2). On occasions *Stephanodiscus hantzschii, Stephanodiscus hantzschii* fo. *tenuis* and *Cyclotella meneghiniana* were readily identifiable and recorded during the counts.

Table 4.2 Size classifications used for unidentifiable diatoms. Sizes are in µm and classifications used are identified 'X'.

	≤ 5	> 5 to ≤ 10	> 10 to ≤ 20	> 20 to ≤ 30	> 30 to ≤ 40	> 40
Centric ∅	X	X	X	X		
Pennate GALD		X	X	X	X	X

During 1995 diatoms were identified following digestion and the proportions of species found then compared with the counts of size classes. The four most abundant centric diatoms were *Stephanodiscus hantzschii, Stephanodiscus hantzschii* fo. *tenuis, Cyclotella meneghiniana* (two size classes) and *Cyclotella radiosa. Stephanodiscus*

hantzschii fo. *tenuis* has recently been identified as a poorly silicified form of *Stephanodiscus hantzschi* (E.Y. Haworth, pers. comm.). Morphological changes relating to silicate deficiency have been recorded from other centric diatoms (Belcher et al., 1966), although taxonomists often group 'morphotypes' together (Krammer and Lange-Bertalot, 1991).

The four most abundant centric diatom species constituted the majority of centric diatoms found throughout the survey (Table 4.3). *Stephanodiscus hantzchii* was generally smaller than *S. hantzschii* fo. *tenuis* and the two taxa have an inverse abundance relationship, with *S. hantzschii* fo. *tenuis* becoming most abundant during times of probable silicate limitation.

Table 4.3 Percentage abundance of centric diatoms found in digested samples during 1995 and their average valve diameter (µm).

	Stepanodiscus hanztschii	*Stepanodiscus* fo. *tenuis*	*Cyclotella meneghiniana*	*Cyclotella radiosa*	Others
21 March	66	22	11	0	1
4 April	70	20	10	0	0
21 April	87	9	0	4	0
2 May	53	31	1	11	4
31 May	4	93	0	1	2
7 June	5	93	0	2	0
28 June	5	77	0	15	3
11 July	39	44	0	17	0
6 September	71	2	27	0	0
∅ (µm)	8.80	11.06	10.90	12.0	
SD	1.07	1.16	1.84	1.36	
n	30	30	10	10	

The abundance of centric diatoms ≤ 5 µm ∅ varied in the counts from 3.1% to 87% but were scarce in the digested samples (Table 4.3). Some centric diatoms ≤ 5 µm were found in the digested samples (e.g. *Cyclotella stelligera* and *C. pseudostelligera*) but were not abundant.

4.4 Morphological impact of low silicate concentration

Some species of pennate diatom, particularly *Nitzschia acicularis*, exhibited twisting and splitting of the frustule. This phenomenon occurred mostly during the spring when centric diatoms were abundant and was attributed to poorly developed frustules, resulting from rapid growth in a silicate depleted environment. Twisting or

splitting was, however, rarely seen in live samples and this condition could have been exacerbated by fixation. Acidified Lugol's solution is thought to be responsible for dissolving diatoms during long-term storage (S. Juggins, pers. comm.). However, it is unlikely that the acid contained in Lugol's solution was wholly responsible for the deterioration of frustules as other diatoms exhibited well formed frustules using the same fixative at other times.

4.5 Picoplankton identification

Picoplankton (≤ 3 μm GALD) were counted using phase-contrast microscopy during routine analysis and under fluorescence illumination during a special investigation, the former approach allowing estimation of colonial forms (Stockner et al., 2000). Under the light microscope cells were classified by size, shape and number of cells per colony (Figure 4.1). Examination of picoplankton under fluorescence illumination was carried out using green (ca. 500-550 nm) and blue (ca. 420-490 nm) filtration. Those cells that fluoresced most persistently under green filtration were classified as blue-green algae and those fluorescing using blue filtration as green algae (Hawley and Whitton, 1991). This analysis was based respectively on the photosynthetic pigments characteristics of phycoerythrin and chlorophyll *a* (Hall and Rao, 1972). Within these broad distinctions picoplankton cells were classified according to size (Table 4.4).

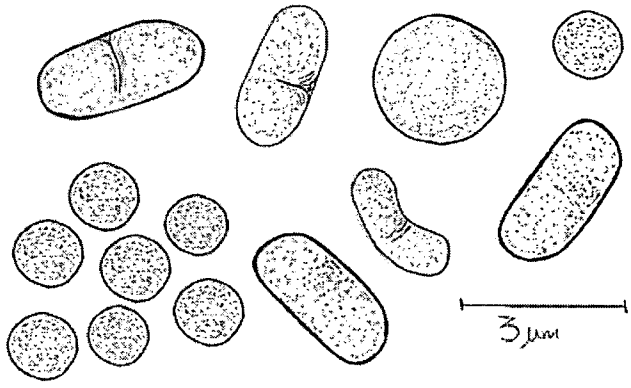


Figure 4.1. Drawings of picoplankton observed under light microscopy at km 91.7 on 28/6/94, although these are typical of several occasions. Showing spherical and rod shaped cells ≤ 3μm diameter/GALD. Some rod cells appear to be dividing.

Table 4.4 Size and classification of picoplankton under the fluorescent microscope. Classifications used are identified 'X'.

	> 1 µm ∅	1 µm ∅	2 µm ∅	3 µm GALD
Blue filtration	X	X	X	
Green filtration	X	X		X

4.6 Zooplankton

Zooplankton are listed in Table 4.5, taxa codes, nomenclature and authorities were taken from Maitland (1977).

Table 4.5 Zooplankton recorded at km 91.7 and from R. J. Sanderson (pers. comm.).

Code	Taxa/Genus	Species	Authority
None	CILIATES		
08000000	ROTIFERA		
08040900	*Brachionus* sp.		
08040901	*Brachionus*	*angularis*	Gosse
08040902	*Brachionus*	*calyciflorus*	Pallas
08040903	*Brachionus*	*leydigii*	Cohn
08040904	*Brachionus*	*quadrientatus*	Hermann
08040907	*Brachionus*	*urceolaris*	Müller
08081010	*Cephalodella*	*gibba*	Ehrenberg
08042409	*Colourella*	*uncinata*	Müller
08040403	*Epiphanes*	*senta*	Müller
08041608	*Euchlanis*	*pyriformis*	Gosse
08041602	*Euchlanis*	*dilatata*	Ehrenberg
08140404	*Filinia*	*terminalis*	Plate
08042101	*Kelicottia*	*longispina*	Kellicott
08041901	*Keratella*	*cochlearis*	Gosse
08041904	*Keratella*	*quadrata*	Müller
08060100	*Lecane sp.*		
08042408	*Lepadella*	*patella*	Müller
08042400	*Notholca* sp.		
08042204	*Notholca*	*squamula*	Müller
08042203	*Notholca*	*labis*	Gosse
08042201	*Notholca*	*accuminata*	Ehrenberg
08042201	*Notholca* (Argonotholca)	*foliacea*	Ehrenberg
08120100	*Polyartha* sp.		
08120106	*Polarthra*	*remata*	Skorikov
08120101	*Polyarthra*	*delichoptera*	Idelson
08030309	*Rotaria*	*neptuna*	Ehrenberg
08120200	*Synchaeta* sp.		
08120210	*Synchaeta*	*oblonga group*	Ehrenberg
08120211	*Synchaeta*	*pectinata group*	Ehrenberg
08140108	*Testudinella*	*patina*	Herm
08090101	*Trichocerca* sp.		

08040701	*Trichotria*	*pocillum*	Müller
None	Loose eggs		
24000000	CLADOCERA		
24030601	*Bosmina*	*longirostris*	Müller
24030000	*Daphnia* sp.		
26000000	COPEPODA		
None	Adult		
None	Nauplii		

4.7 Submerged macrophytes

Those taxa classified as submerged macrophytes are listed in Table 4.6, taxa codes and nomenclature of angiosperms were taken from Holmes et al. (1978), authorities from Chapman et al. (1987) and code, name and authority of *Cladophora* from Whitton et al. (1998).

Table 4.6 List of 'submerged' macrophytes recorded at Nene sites during the study (EA unpublished data).

Code	Taxa/Genus	Species	Authority
	ALGAE		
20030030	*Cladophora*	*glomerata*	(L.) Kütz
	MONOCOTYLEDONS		
382103	*Elodea*	*nuttallii*	Planchon
384004	*Potamogeton*	*compressus*	L.
384011	*Potamogeton*	*lucens*	L.
384014	*Potamogeton*	*pectinatus*	L.
384016	*Potamogeton*	*perfoliatus*	L.
384018	*Potamogeton*	*praelongus*	Wulfen
	DICOTYLEDONS		
361100	*Callitriche* spp.		L.
361401	*Ceratophyllum*	*demersum*	L.
364403	*Myriophyllum*	*spicatum*	L.

4.8 Taxonomic literature

Taxonomic literature used for the identification of algae, zooplankton and macrophytes are listed in Table 4.7.

Table 4.7 Taxonomic literature used for the identification of algae, zooplankton and macrophytes.

Barber and Haworth, 1981
Belcher and Swale, 1978
Belcher and Swale, 1979
Bourrelly, 1966
Bourrelly, 1968

Bourrelly, 1970
Chapman et al., 1987
Cox, 1996
Desikachary, 1959
Geitler, 1932
Komárek and Anagnostidis, 1999
Krammer and Lange-Bertalot, 1988
Krammer and Lange-Bertalot, 1991
Pentecost, 1984
Pontin, 1978
Prescott, 1962
West et al., 1927
Whitton et al., 2000

4.9 Discussion

The numerous algae recorded for km 91.7 were the result of four different identification methods, including live examination and acid digestion of diatoms. Unfortunately, this level of taxonomy could not be carried out on routine counts, where algae were treated with preservative. Examination of live algae and the pilot study of diatoms provides some confidence regarding species composition, but complete quantitative assessments at species level was not always possible. The classification of centric diatoms by diameter measurements was not altogether satisfactory. However, the results indicate a concurrent species and size class shift, from *Stephanodiscus hantzshii* to *S. hantzshii* fo. *tenuis* and from centric diatoms less than to greater than 10 µm ∅. There is also some evidence that this apparent shift may be occurring at times with a reduced silicate concentrations. Additionally, the size class and species shift is supported by the literature. Swale (1964), found that *S. hantzschii* to have an average valve diameter of 9.9 µm in two UK rivers, whereas Krammer and Lange-Bertalot (1991) identify *S. hantzshii* fo. *tenuis* as having a diameter larger than 10 µm.

Discrepancies in the quantity of centric diatoms ≤ 5 ∅ found in the digested and Lugol's-preserved samples does give cause for concern and are either the result of loss during concentration or mechanical damage during treatment. The complete sedimentation of very small centric diatoms can be problematical (Furet and Benson-Evans, 1882) and they could easily become re-suspended during siphoning, or may not sediment at all due to circulatory currents in tall cylinders. Other methods should be explored, such as concentration through centrifugal force or siphoning of the settled algae rather than the supernatant.

Reduced silicate concentration do appear to impact on the frustule development of *Nitzschia acicularis*. A poorly developed frustule can result from rapid division or growth in a low ambient silicate concentration (E.Y. Haworth, pers. comm.). *Nitzschia acicularis* had an average length of 76 µm (n = 30) during the spring diatom peak of 1997. This is relatively small for this species, the length of which ranges from 30 µm to 150 µm (Krammer and Lange-Bertalot, 1988), and this could indicate rapid growth or adaptation for life in the planktonic.

Picoplankton taxonomy is specialised and as a group have received increasing attention over recent years, particularly single celled taxa (Stockner et al., 2000). The two approaches tried during this study are complimentary. Colonial forms would be overlooked in the fluorescent technique, some being removed during pre-filtration, although single cells would be mostly missed under the light microscope at × 400 magnification. The Fluorescent microscopy allowed singled celled picoplankton to be counted, classified and a tentative distinction made between those taxa belonging to the classes Chlorophyceae and Cyanobacteria.

4.10 Summary

1 Phytoplankton taxa were examined in four ways, including acid digestion and live examination, and taxa found at km 91.7 represented all locations.

2 Diatoms were classified by size during routine counts, centric forms by diameter and pennate forms by length. The determination of centric forms was approximated to species identified in acid digested samples which were examined under a light microscope and SEM.

3 Reduced silicate appeared to impacted on the development of centric diatoms and the pennate diatom *Nitzschia acicularis.*

4 Picoplankton were identified during routine counts and using fluorescence microscopy, the latter technique permitting the classification of blue-green and green algae.

5 Suspended algae, zooplankton and submerged macrophytes are listed.

5 COUNTING: DESCRIPTION, EVALUATION AND DEVELOPMENT

5.1 Introduction

It was considered necessary to undertake a thorough appraisal of the accuracy and reproducibility of phytoplankton counts in sedimentation chambers, as preliminary evaluations had produced unreliable results (National Rivers Authority, 1993).

Routine phytoplankton enumeration undertaken during this research was based on the sedimentation procedures described by Utermöhl (1958). The Utermöhl, or sedimentation technique, uses glass bottomed counting chambers with an approximate diameter of 25 mm, where the sample is concentrated, through sedimentation, and examined from below using an inverted microscope. The majority of phytoplankton counts were undertaken in accordance with the methods used by the Anglian Region of the EA (Environment Agency). These methods were implemented during 1993 following a project undertaken to establish standardised phytoplankton methods (National Rivers Authority, 1993). Although the standard or 'basic' methods were thought sufficient for routine analysis, I endeavoured to develop a new approach that would produce more accurate results and permit the identification of small changes that would otherwise be undetectable.

This chapter describes the basic counting method, evaluates its accuracy and precision empirically and by computer simulations. The description, development and evaluation of a new counting technique, 'spaced fields', is also presented.

5.2 Description of basic counting method

Water samples, fixed with Lugol's Iodine and normally contained in 300-ml PET bottles, were thoroughly mixed and an aliquot transferred to a clean 5 or 10-mL sedimentation chamber and allowed to settle for a minimum of 24 h. Following sedimentation, the chamber was carefully transferred to an inverted microscope and counted using the three tiered approach described in Table 5.1.

The counting area of sedimentation chambers and eyepiece graticules were measured prior to use. The counting area of individually identifiable 5 and 10-ml sedimentation chambers were calculated by measuring the diameters of each chamber using the vernier scales on the microscope stage (n = 5). The diameter and area (πr^2) of

each chamber was recorded for use during count calculations. Eyepiece graticules were calibrated using a stage micrometer. The Whipple graticule perimeter was measured at × 200 and × 400 magnification and the vertical length of the cross-hair graticule at × 200 magnification. The counting area of a transect being calculated as the width of the cross-hair graticule multiplied by the diameter of the chamber.

Table 5.1 Counting strategy of 'basic' method.

Method	Description
Full chamber count	Full chamber count in strips (Hasle, 1981) at × 80 magnification counting the 'larger' taxa (e.g. *Closterium* sp.) *
Diameter transects	5 to 9 evenly spaced diameter transects (Hasle, 1981) at × 200 magnification counting 'intermediate' sized taxa (e.g. *Scenedesmus communis*)*
Fields	50 to 100 'randomly' placed fields of view at × 400 magnification, counting the 'smaller' taxa (e.g. *Stephanodiscus hanzschii*) †

* The upper and lower limits of the counting area were defined using a cross-hair graticule placed in one of the microscopes eyepieces (Lund et al., 1958)
† The limits of the counting area were defined as the perimeter of a Whipple graticule (Guillard, 1981) placed in one of the microscope's eyepieces. Field placement was in an ad hoc fashion and not necessarily placed randomly. Counts at × 200 an × 400 were undertaken using phase-contrast microscopy

The volume used in each count was varied according to the abundance of algae and usually ranged from 0.25 to 10-mL and normally provided sufficient numbers of the most abundant taxa to produce a count exceeding 100 units (cells, filaments or colonies). The main exception to this was when there was an abundance of non-algal material present in the samples (e.g. inorganic sediment).

Fields were placed by 'blind' movements of the microscope stage (not truly random). All algal cells falling within the Whipple field, or on two of the predetermined edges, were counted and all other algae were excluded. Spaced diameter transect counts were positioned at the left-hand edge of the chamber, at its widest point, and then the chamber was slowly moved so that the graticule transversed the chamber. Algae falling within the graticule, or on one of the predetermined lines, were counted as they passed the vertical line of the cross-hair. All other algae were excluded. Transects were evenly spaced although the position was approximate, plus or minus a few degrees in either direction.

The abundance of each taxa recorded was converted into 'algal units' mL^{-1}, as follows.

$$\text{Units } mL^{-1} = \left(\frac{\text{No. of algal units counted}}{\text{No. of fields/transects counted}}\right) \times \left(\left[\frac{\text{area of chamber}}{\text{area of field/transect}}\right] \div \text{sample volume}\right)$$

Where units are cells, filaments or colonies.

When algal units were filaments or colonies these were converted to cells mL^{-1} by multiplying the number of units counted by the average numbers of cells in 30 units (where possible). Conversion of counts to cells/units mL^{-1}, data storage and manipulation was facilitated using a specially written computer program and database (LIMNDAT).

5.3 Evaluation of basic method and development of a new technique

During this evaluation counting error was evaluated in two ways. Firstly by trial counts using fixed cultures, and secondly using a specially written computer program that simulated counting (SIMCOUNT), the latter of which provided a mechanism for evaluating alternative techniques.

5.31 Counting Trials

Introduction

When the whole chamber is enumerated the counting accuracy that results will depend on personal counting error. If the subsample counted can be shown to come from a Poisson distribution then the accuracy of the subsample is proportional to the number of units counted (Lund et al., 1958). However, others have shown that Poisson based confidence intervals may underestimate the true error by between 5% and 15% (Edgar, 1993).

When the counting chamber is subsampled, using either transects or fields, the accuracy of the result will depend on the distribution of the algae within the chamber. Some plankton workers calculate subsamling errors based on the assumption that cells are randomly distributed within the counting chamber (Venrick, 1981). Others suggest that a homogenous distribution of plankton can never be guaranteed (Nauwerk, 1963).

Method

Two cultures, *Scenedesmus communis* and *Chlamydomonas augustea*, were obtained from CCAP. These cultures were diluted with distilled water and fixed in Lugol's Iodine. Estimates of cell concentrations were made using a Lund Chamber (Lund, 1959), and final concentrations were made, using distilled water, to achieve approximately 1500 and 1200 cells mL^{-1} respectively for the *Chlamydomonas* and *Scenedesmus* cultures. The quantity of algae in these cultures was investigated further by full chamber counts (n = 3). The fixed cells of *Chlamydomonas* and *Scenedesmus* had approximate dimensions of 9×7 μm and 42×25μm, respectively (including spines for *Scenedesmus*, n = 30).

Chlamydomonas was enumerated in trials of 50, 75 and 100 Whipple graticule fields at a magnification of × 400. *Scenedesmus* was counted in 5, 7 and 9 evenly spaced diameter transects at a magnification of × 200. Counts were made over a range of volumes, and cell counts were recorded as individual fields or transects and expressed as cells mL^{-1}. Individual transects and fields, including the full chambers transects, were replicated and where the error exceeded 5% the results were rejected. Below this value differences were averaged. Complete counts were also replicated within chambers (on the same distribution of algae). This procedure was intended to identify the difference between errors created by the counting method and those caused by the distribution of cells.

Estimates were evaluated according to their distance from the population mean, their success of accurately predicting the population and their distribution around the population. The average distance of estimates from the population produced by the different counting methods was used as a measure of accuracy. Confidence intervals (95% level) were assigned to field counts (n > 30), as follows.

$$95\% \text{ confidence interval} = \text{average cells per fields} \pm 1.96 \sqrt{\frac{S^2}{n}}$$

Confidence intervals for transect counts (n < 30) were calculated by applying $\log_e$ transformed data to the arithmetic mean (Elliott, 1983).

$$95\% \text{ confidence interval} = \text{average cells per transect} \times \div t \sqrt{\frac{\text{variance of transformed counts}}{n}}$$

The distribution of estimates about the population was evaluated using the χ^2 statistic, assuming (H_o) that an unbiased method would produce estimates randomly distributed around the population. χ^2 was calculated as follows:

$$\chi^2 = \Sigma \frac{(f_o - f_e)^2}{f_e}$$

The significance of the resultant χ^2 values were assessed against statistical tables (Gravetter and Wallnau, 1985) at the 95% level with one degree of freedom.

Results

Full chamber counts produced reasonably consistent results (Table 5.2). However, the distributions that resulted from the counts were variable (Figure 5.1). If the cells within these chambers were distributed at random then the bar charts in Figure 5.1 would exhibit a semicircular appearance, reflecting the shape of the chamber. The skewed appearance of most graphs, particularly *Chlamydomonas* plot 3, suggests that the distributions are non-random.

The results of transect and field counts are shown in Figure 5.2 and Figure 5.3. The results of the counts indicate a very wide range of estimates with the number failing to predict the population exceeding that expected by the 95% confidence intervals (1 in 20). As expected, an increased n produces a reduction in error.

Table 5.2 Full chamber counts (standardised to 1 mL).

	Replicates			
Stock	1	2	3	Mean
Chlamydomonas	1621	1641	1533	1598
Scenedesmus	1269	1341	1258	1289

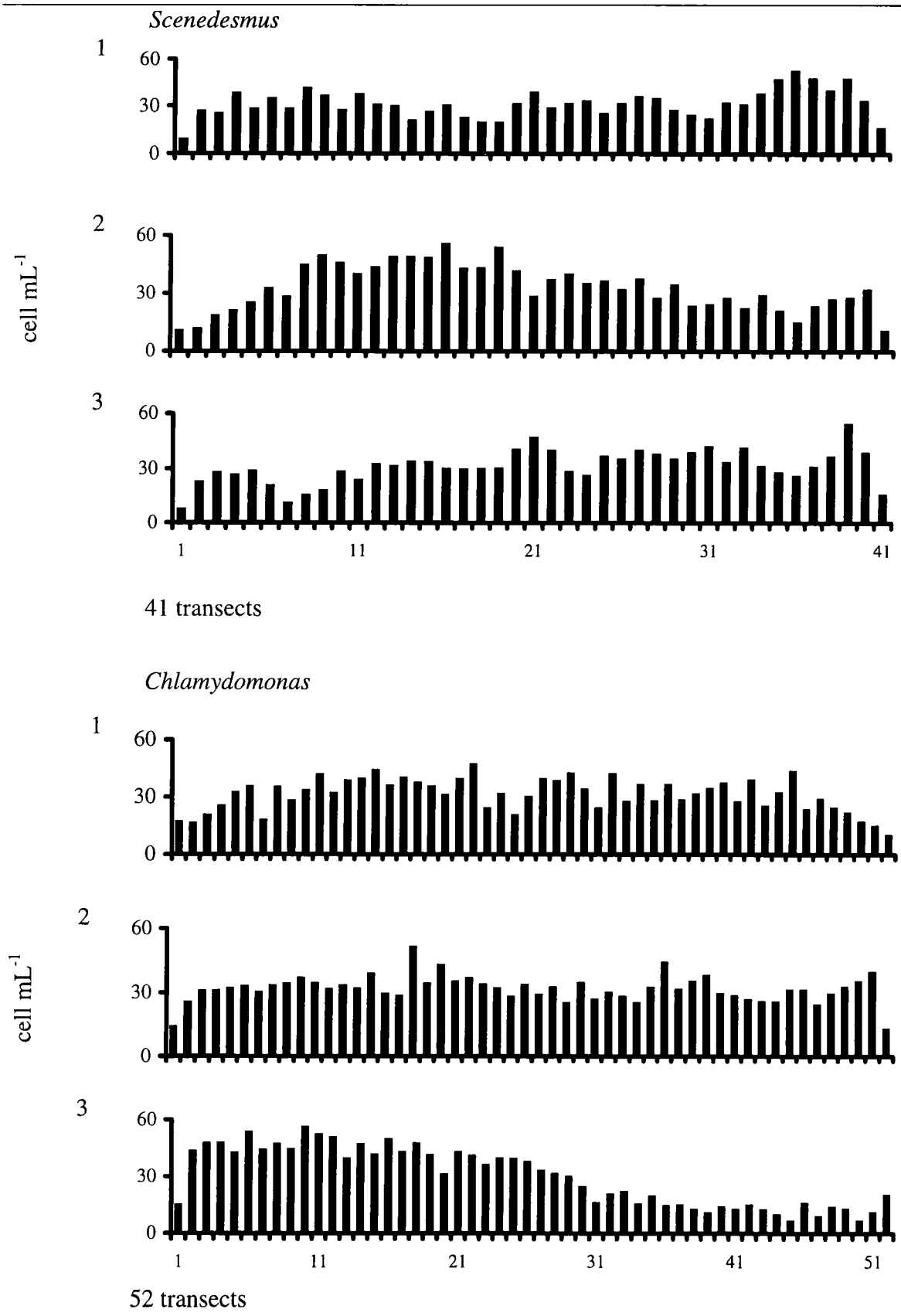
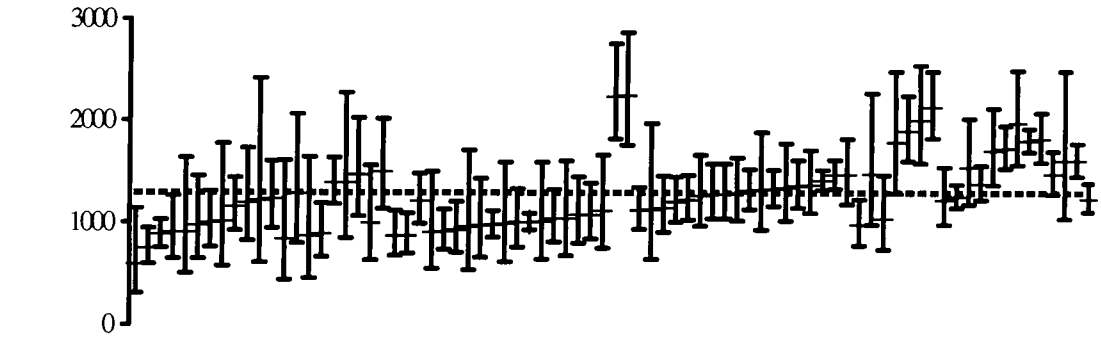
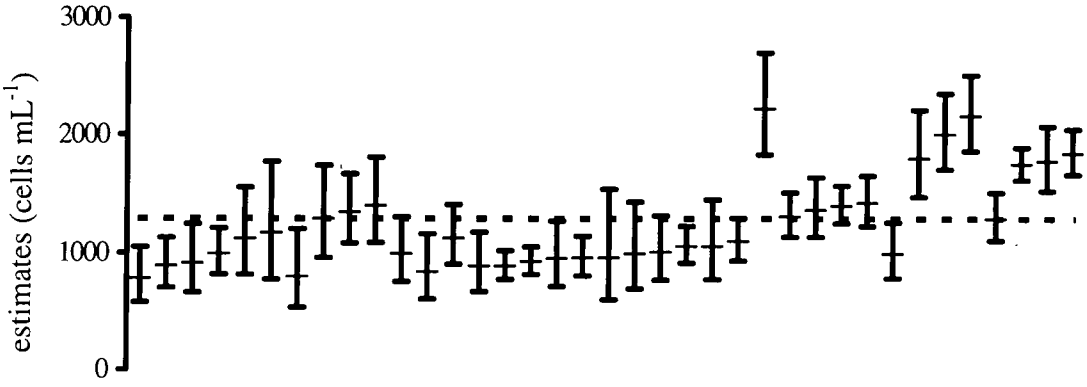


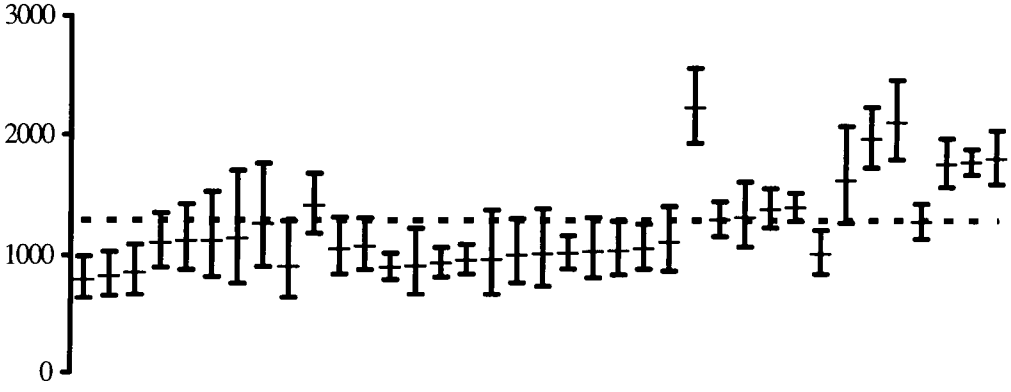
Figure 5.1 Full chamber counts.



5 transects: 65 counts ranging from 23 to 1526 (mean = 241). Mean error = 30%. 32% failure

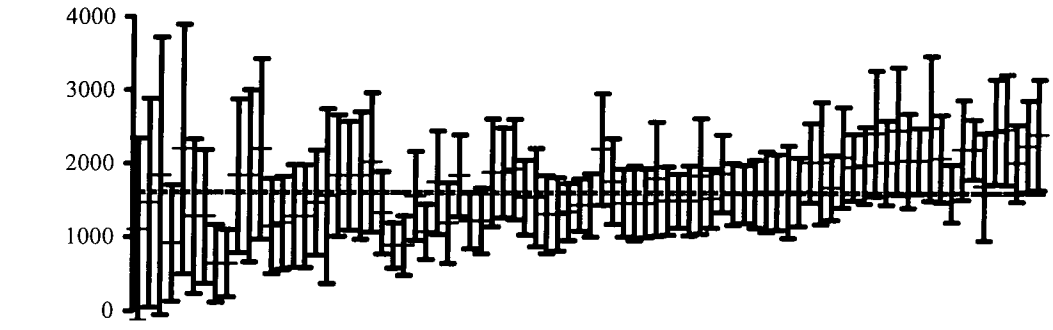


7 transects: 37counts ranging from 42 to 797 (mean = 265). Mean error = 24%. 54% failure

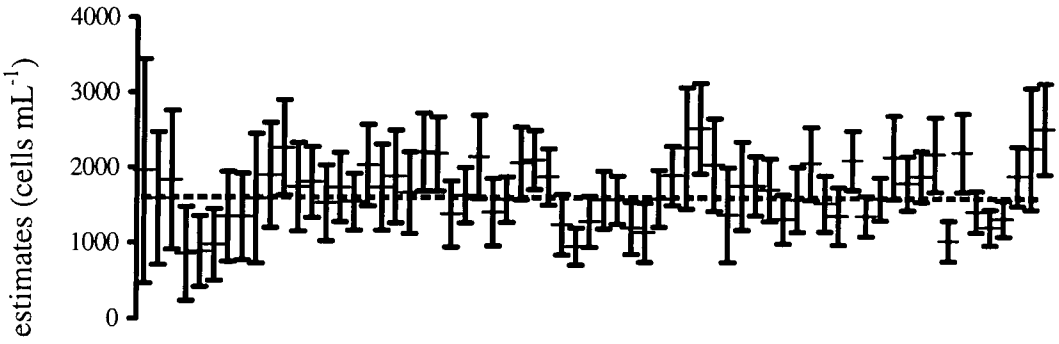


9 transects: 37 counts ranging from 55 to 1000 (mean = 339). Mean error = 20%. 51% failure

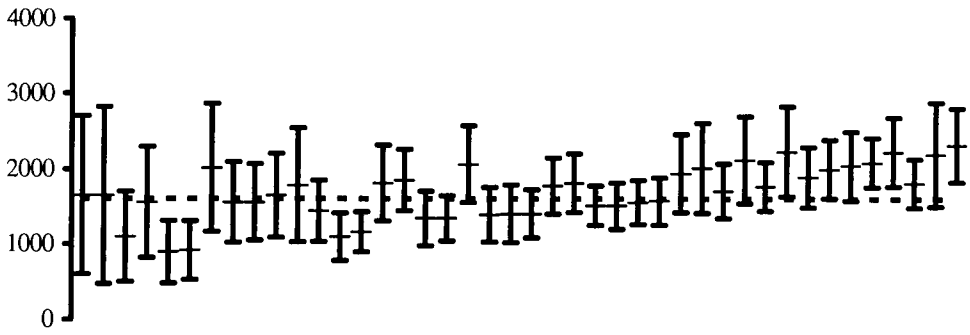
Figure 5.2 Estimates and 95% confidence intervals for 5, 7 and 9 diameter transects at × 200 magnification. Estimates arranged from smallest to greatest count, left to right. Also showing, count range, mean error and percentage of counts failing to accurately estimate the population.



50 fields: 84 counts ranging from 3 to 118 (mean = 44). Mean error = 42%. 11% failure



75 fields: 66 counts ranging from 7 to 165 (mean = 77). Mean error = 31%. 14% failure



100 fields: 42 counts ranging from 9 to 203 (mean = 87). Mean error = 29%. 21% failure

Figure 5.3 Estimates and 95% confidence intervals for 50, 75 and 100 fields at × 400 magnification. Estimates arranged from smallest to greatest count, left to right. Also showing, count range, mean error and percentage of counts failing to accurately estimate the population.

The results of the distribution of estimates around the population are shown in Table 5.3. None of the tests were significant but transect counts all exhibited an underestimation.

Table 5.3 Results of the distribution of estimates around the population. Showing counting method, percentage of estimates above population, χ^2 and probability. df = 1 for all.

Method	% above	χ^2	p
5 transects	39	3.66	NS
7 transects	38	2.19	NS
9 transects	38	2.19	NS
50 fields	51	0.76	NS
75 fields	50	0.00	NS
100 fields	50	0.00	NS

Replicate counts within an undisturbed chamber exhibit a greater level of consistency than replicates between newly settled chambers (Figure 5.4) and it is likely that this phenomenon relates to differences in subsample volume and/or subsample distribution. Significant differences were not evident between any of the replicates, using either method, but significant differences do exist between chambers.

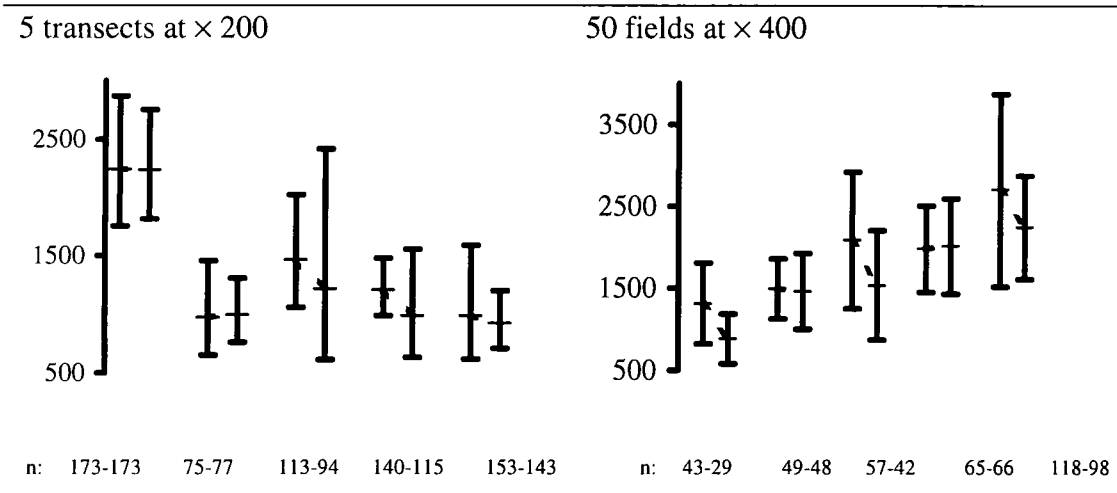


Figure 5.4 Estimates (cells mL^{-1}) and 95% confidence intervals for five replicate, transect and field counts (within the same chamber on the same distribution). Also showing the count (n) for each estimate. Note: Sample volume varies between and within methods.

When the confidence of the estimates is increased to ±50% then the majority of trials accurately predict the population (Figure 5.5). Counts greater than 100 and 30 for 5 transects and 50 field respectively produced estimates that accurately predicted the population.

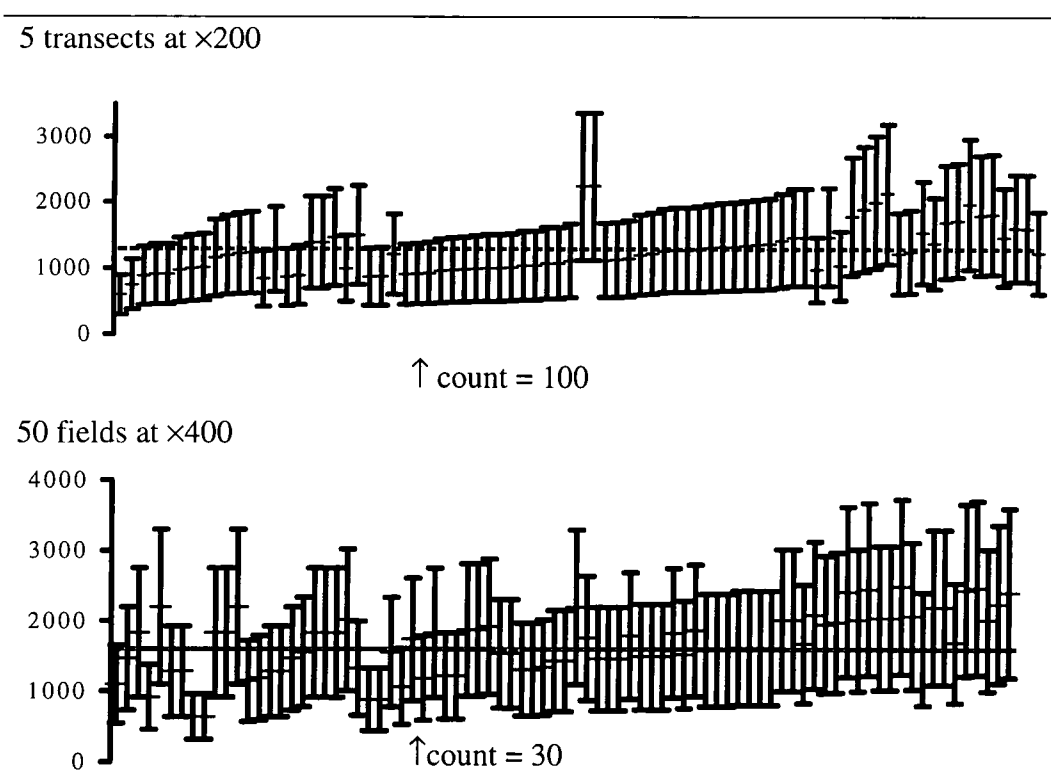


Figure 5.5 Population estimates (cells mL^{-1}) ± 50% for 5 transects and 50 fields.

Because of the uncertainty regarding the influences of cells distribution and subsample error, it was decided to investigate counting error further using computer simulation. This approach would also allow the evaluation of novel counting techniques.

5.32 Simulation

Introduction

The aim of the simulation was to accurately reproduce the process of counting algae in sedimentation chambers while maintaining control over the number of cells and their distribution within each chamber. This approach would provide a mechanism of evaluating the significance of cell numbers and distributions and facilitate the exploration of alternative counting techniques.

Method

Chamber and graticule dimensions were reproduced accurately within the simulation (Table 5.4). The simulation operated at a resolution of 1 μm, which was the size of the simulated taxa used (each cell occupying an area of 1 μm^2).

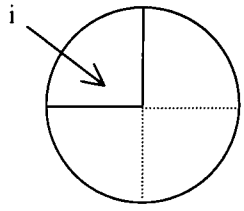
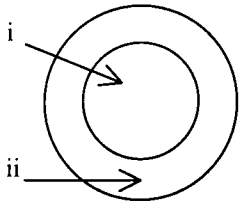
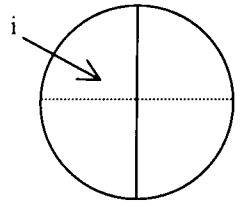
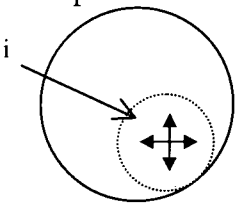
Table 5.4 Simulated chamber and graticule dimensions.

Dimension	Length/area
Chamber diameter	25.00 mm
Chamber area	490.87 mm^2
Whipple area at × 400 magnification	2.96 mm^2
Whipple area at × 200 magnification	11.83 mm^2
Vertical length of 'cross hair' graticule	0.64 mm
Transect area at × 200 magnification	15.90 mm^2

Five simulated distributions were used (Table 5.5) and these were based on observation, experience and the literature. Observations and the results of full chamber counts (above) indicate that algae can accumulate in sections of the chamber and this was the basis for producing the sector and semicircle distributions. Sandgren and Robinson (1984) observed that algae tend to accumulate around the periphery of a chamber, this was supported by personal observation and resulted in the inclusion of a 'two-strata' distribution. Observations of clumped distributions of algae occurring anywhere within chambers was allowed for by the 'spot' distribution, the random placement of these spots being more likely to occur towards the edges of chambers. Chambers with a random distribution were also included.

The four non-random distributions each had four levels of increasing contagion (A to D - Table 5.5). For example, with single sector distribution at level 'B' one of the four chamber sectors was selected at random (i) and 35% of cells were placed randomly within that sector. The remaining 65% cells were then distributed at random throughout the whole chamber. Likewise, with a 'spot distribution at level 'D' 45% of the cells were placed randomly within the a 626 μm radius of the centre of the randomly selected 'spot' and the remaining 55% of cells were distributed at random throughout the whole chamber.

Table 5.5 Contagious distributions used during simulations. Details of the four contagious distributions (1-4) and the four levels of contagion within each distribution (A-D).

Distribution	Description
1. Single sector 	Randomly selected sector i = 25% of chamber area Distributions A. 30% of cells in 'i' - 70% at random throughout chamber B. 35% of cells in 'i' - 65% at random throughout chamber C. 40% of cells in 'i' - 60% at random throughout chamber D. 45% of cells in 'i' - 55% at random throughout chamber
2. Two strata 	Two fixed strata i = 25% of chamber area - ii = 75% of chamber area Distributions A. 20% of cells in 'i'- 80% of cells in 'ii' B. 15% of cells in 'i'- 85% of cells in 'ii' C. 10% of cells in 'i'- 90% of cells in 'ii' D. 5% of cells in 'i' - 95% of cells in 'ii'
3. Semicircle 	Randomly selected semicircle i = 50% of chamber area Distributions A. 60% of cells in 'i' - 40% at random throughout chamber B. 70% of cells in 'i' - 30% at random throughout chamber C. 80% of cells in 'i' - 20% at random throughout chamber D. 90% of cells in 'i' - 10% at random throughout chamber
4. Spot 	Randomly selected spot (centre anywhere within chamber) i = 25% of chamber area Distributions A. 30% of cells in 'i' - 70% at random throughout chamber B. 35% of cells in 'i' - 65% at random throughout chamber C. 40% of cells in 'i' - 60% at random throughout chamber D. 45% of cells in 'i' - 55% at random throughout chamber

Four counting methods were evaluated during the simulations. These included random fields, pseudo-random fields, spaced transects and an experimental approach called spaced fields (Table 5.6). Random fields and spaced fields were evaluated at simulated magnifications of × 200 and × 400, pseudo-random fields at × 400 and spaced transects at × 200. Pseudo-random fields were a simulation of ad hoc field placement used in the count trials as a substitute for actual random fields.

Table 5.6 Counting methods evaluated during the simulations.

Method	Description of simulation
Spaced transects	Evenly spaced diameter transects. Commencing from a randomly chosen point on the chamber's circumference. Transects were thereafter spaced evenly within a few degrees.
Random fields	Fields placed at random. Each field had an equal chance of being placed anywhere in the chamber.
Pseudo-random fields	Commencing from a randomly chosen position, subsequent fields were placed in any direction between 0.4 and 4.0 mm of the previous field. These field placements were interspersed with placements of between 4 and 12 mm of the previous field, and occurred at a frequency of between every 5 to 15 fields
75 Spaced fields	Starting from a randomly chosen position on the chambers circumference 10 evenly spaced diameter transects were placed, each transect had either 6 or 9 fields placed along its length (Table 5.7). Transect placements were within a few degrees
All cells falling within or on the edges of the fields or transects were counted	

A fuller explanation of spaced fields method is shown in Table 5.7. The spaced fields method was developed following some experimentation and its purpose is to produce a consistent coverage of the chamber and to eliminate the possibility of over or under-sampling contagion within the chamber. Spaced fields do not cover the chamber evenly, as they under-sample the chambers periphery compared to the centre, but the distribution of fields is the result of a compromise between even coverage of the chamber and practicalities of field placements.

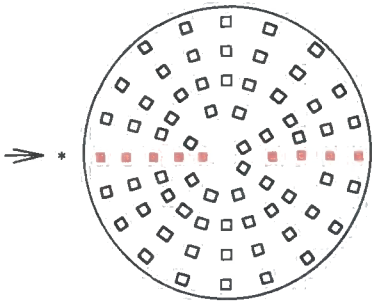
The four contagious distributions were used in equal proportions at contagion level 'B' combined with random distributions at a ratio of 4:1, contagious to random. This combination was chosen by comparing the slope and intercept of fitted lines produced by plotting the variance against the count for actual and simulated data (Table 5.8). The contagion level 'B' combined with random distributions at 2:1 fitted the actual slope most accurately but underestimated the variance up to a count of 275. Therefore, the 'B' distribution combined with random distributions at 4:1 was chosen, this combination producing a variance equal to the actual at a count of 125, thereafter overestimating the variance. This combination was used for simulations (n = 1000) where counting methods were compared over a range of cell quantities. The methods

were also evaluated over a range of contagion (n = 400), from a random distribution to contagion levels A to D.

Table 5.7 Placement of 75 spaced fields. a: Schematic of field placement. Arrow indicates transect 1 (red), other transects clockwise from here. b: Details of field numbers in each transect. For example, transect 1 (red) uses nine fields, of the ten evenly spaced positions, number 6 being omitted. Omitted fields indicated 'X'.

Arrangement of transects and fields for 75 spaced fields

a



b Fields

transect	a	b	c	d	e	f	g	h	i	j
1*	■	■	■	■	■	X	■	■	■	■
2				X	X	X	X			
3					X					
4				X	X	X	X			
5						X				
6				X	X	X	X			
7					X					
8				X	X	X	X			
9						X				
10				X	X	X	X			

The latter approach was also tested using Kruskal-Wallis (a non-parametric alternative to a one-way analysis of variance - Ryan et al., 1985), with the null hypothesis that the counting method does not produce difference in the population estimates.

Simulations were evaluated in terms of their accuracy, precision and distribution of estimates around the population. Where necessary, confidence intervals were adjusted to conform to the requirements of the 95% level and the relationship between error and count calculated.

Table 5.8 Fitted lines for variance regressed onto count, for actual field counts and simulated pseudo-random fields at × 400 magnification.

Distribution	n	slope$^{\Psi}$	intercept$^{\Psi}$	r$^{\Phi}$	p
Actual	42	0.019	-0.057	0.801	0.000
B 2:1*	600	0.020	-0.330	0.541	0.000
B 4:1*	500	0.022	-0.427	0.598	0.000

*Combination of four distributions shown in Table 5.5 with random distributions, at the ratio shown. Ψ Lines fitted using resilient line method (Ryan et al., 1985).

Φ Spearman rank correlation

Results

The simulation of pseudo-random fields and spaced diameter transects, at × 200 and × 400 magnification respectively, performed poorly compared to random fields and spaced fields, the latter producing greatest accuracy and precision. Figure 5.6 shows the percentage of counts that did not accurately predict the true population. Confidence intervals were calculated at the 95% level, therefore a failure level of 5% is acceptable.

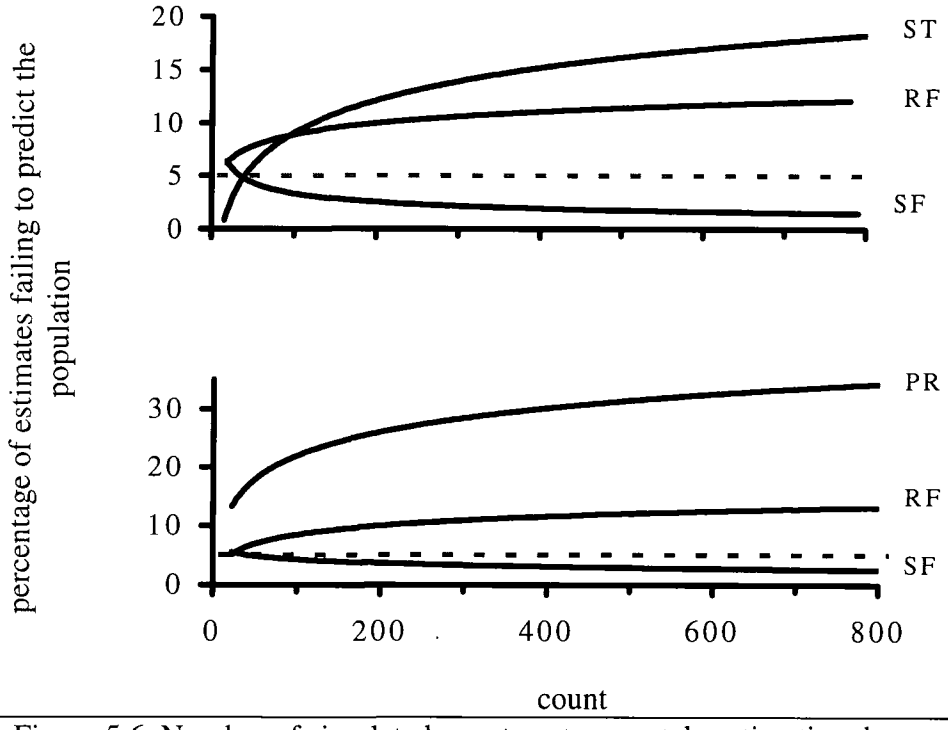


Figure 5.6 Number of simulated counts not accurately estimating the population plotted against increasing count. Counts were at × 200 magnification (above) and × 400 (below), using the methods: spaced diameter transects (ST), random fields (RF), pseudo-random fields (PR) and spaced fields (SF). Each line is fitted to eight points and each point consists of the four contagious (at level 'B') and a random distribution, combined at a ratio of 4:1 (n = 1000 for each point). All lines are significant at the 95% level and were fitted using natural logarithms of the count. Broken line = 5% level.

The results indicate that the spaced fields were the only method evaluated that conformed to the requirements of the 95% level, with less that 5% of the estimates failing to predict the actual population. Pseudo-random fields had the poorest precision with between 13% and 32% of the counts not accurately predicting the true population. The average error produced by the spaced fields method was also the least of all the methods evaluated. Figure 5.7 shows the percentage error (half intervals) produced following adjustment to satisfy the requirements of the 95% level.

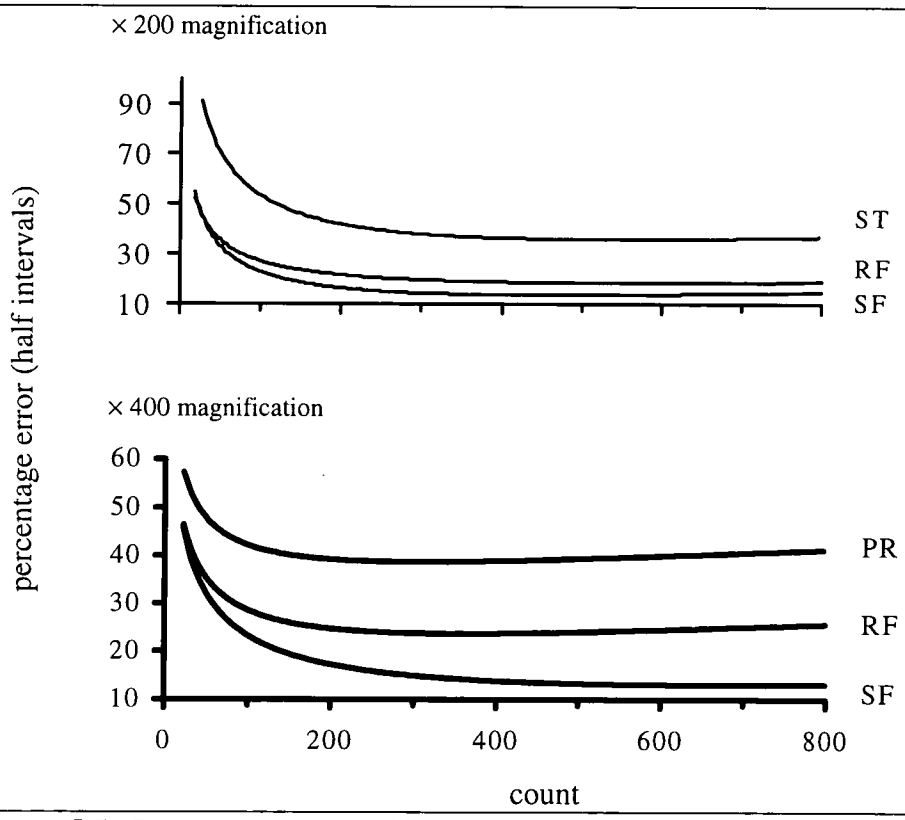


Figure 5.7 Percentage error (half intervals) plotted against increasing counts. Counts were at × 200 magnification (above) and × 400 (below), using the methods spaced diameter transects (ST), random fields (RF), pseudo-random fields (PR) and spaced fields (SF), using the same data described in Figure 5.6. Lines were fitted using a 2nd order logarithmic function, which fits the data more closely than a standard logarithmic function, especially over the count range of 50 to 200. All lines are significant at the 95% level (Table 5.10).

The results indicate there is a large difference in the level of error produced by the different methods. At × 200 magnification a count of 125 produces errors of ± 49%, 23% and 18% for spaced transects, random fields and spaced fields, respectively. Likewise, at × 400 magnification a count of 125 produces errors of ± 41%, 27% and 21% for pseudo-random fields, random fields and spaced fields, respectively. All methods produced error half intervals within ± 50% of estimate and spaced fields performed the most favourably.

The equations for the fitted lines in Figure 5.7 are shown in Table 5.9 and these could be used to estimate error. A second order logarithmic function was used to fit the lines in Figure 5.7 because it fitted the data more closely through the critical count range of 50 to 200. This results in overestimates in the error at high counts (>300), particularly for pseudo-random and random fields at ×400.

Table 5.9 Parameters used for the fitted lines in Figure 5.7 and r^2 values. The parameters shown can be used in the following equation to calculate error (y) for a given count (x), within the range 50 to 800. $y = y_o + a\ln x + b(\ln x)^2$.

Method	y_o	a	b	r^2
×200 magnification				
Spaced transects	275.1	-75.98	6.03	0.98
Random fields	133.9	-36.48	2.88	0.99
Spaced fields	161.5	-48.31	3.94	0.98
×400 magnification				
Pseudo-random fields	128.4	-31.32	2.73	0.91
Random fields	127.2	-35.20	2.99	0.93
Spaced fields	132.4	-36.65	2.82	0.99

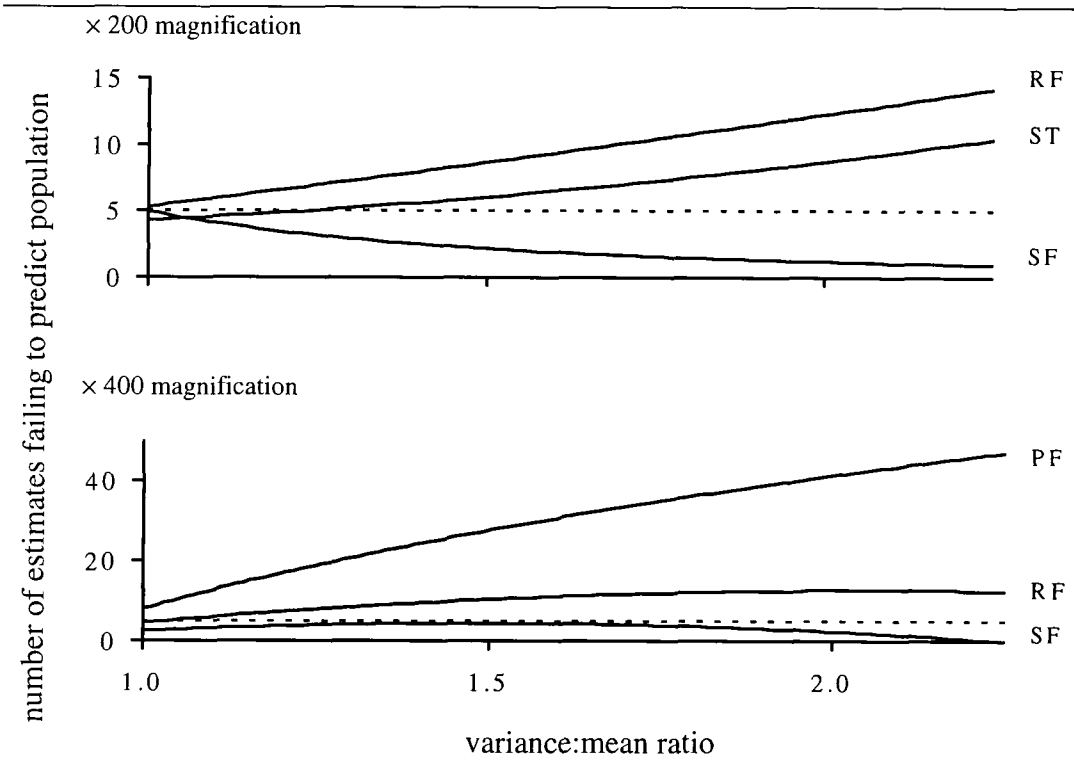


Figure 5.8 Number of simulated counts not accurately estimating the population plotted against variance:mean ratio. Counts were at × 200 magnification (above) and × 400 (below), using the methods: spaced diameter transects (ST), random fields (RF), pseudo-random fields (PR) and spaced fields (SF). Each count was approximately 150 and 140 at × 200 and × 400 magnification, respectively. Each line is fitted to five points, which consist of a random distribution and the four contagious distributions in equal proportions at levels 'A', 'B', 'C' and 'D' (n = 400 for each point).

The performance of the counting methods was assessed across a range of distributions, from random to contagious, using the four contagious distributions in

equal proportions at level 'A', 'B', 'C' and 'D' (Figure 5.8). All methods, except spaced fields, exhibited an increased number of failures with increasing contagion, although random fields appear to stabilise at 14 failures. Spaced fields conform to the 95% confidence limits throughout.

The results of the Kruskal-Wallis tests support those presented above and provide further insight into the behaviour of the simulated counts (Table 5.10).

Table 5.10 Probability values (adjusted for ties) for Kruskal-Wallis tests for 5 spaced transects (ST), 75 random fields (RF), 75 pseudo-random fields (PR) and 75 spaced fields (SF). Tests carried out on simulated populations of 1000 for ST, 8000 for RF and SF at × 200 magnification and 30000 at × 400 magnification. Significant results are boxed, n = 100 for each test.

Distribution and level	×200 magnification			×400 magnification		
	ST	RF	SF	PF	RF	SF
Random	0.982	0.735	0.991	0.711	0.431	0.907
1A	1.000	0.289	0.956	**0.000**	0.766	0.914
2A	1.000	**0.001**	0.999	0.338	**0.002**	0.204
3A	0.398	**0.007**	1.000	**0.000**	**0.001**	1.000
4A	0.644	**0.000**	0.831	**0.000**	**0.000**	0.974
1B	0.610	**0.001**	0.999	**0.000**	**0.020**	0.994
2B	0.398	0.088	0.700	0.252	0.617	0.987
3B	0.398	**0.007**	1.000	0.543	**0.000**	1.000
4B	**0.020**	0.058	0.946	**0.000**	**0.001**	0.961
1C	1.000	**0.000**	0.999	**0.000**	**0.002**	0.934
2C	0.492	**0.000**	1.000	**0.005**	0.313	0.851
3C	1.000	**0.000**	1.000	**0.000**	**0.000**	1.000
4C	**0.001**	**0.001**	0.803	**0.000**	**0.080**	0.180
1D	1.000	**0.000**	0.990	**0.000**	**0.000**	1.000
2D	0.442	0.764	1.000	**0.000**	0.536	1.000
3D	0.364	**0.000**	1.000	**0.000**	**0.000**	1.000
4D	**0.002**	**0.000**	0.999	**0.000**	**0.000**	0.672

The spaced fields method was non-significant throughout, as were all methods using random distributions. The spaced transects exhibited a better overall performance than random fields but produced significant results on three distribution levels ('B', 'C' and 'D'). Spaced transects are likely to produce variable results with the 'spot' distribution when it occurs towards the centre of the chamber. The bias produced by spaced transects is also evident when the distribution of counts around the population is examined (Table 5.11). All methods are non-significant with the random

distribution with the exception of spaced transects, which produced a significant underestimation. The 'A' level of contagion was non-significant with spaced transects, but levels 'B', 'C' and 'D' produced increasingly greater underestimates. Spaced transect produced the most significant deviation from the mid-point (50%). Other methods did exhibit significant differences, but no pattern was evident and these deviations were distributed around the mid-point.

Table 5.11 Distribution of estimates around the population. Showing percentage of counts above the population and significance of χ^2 test. Contagion was produced using a combination of distributions 1 to 4, in equal proportions, at the level of contagion shown. Counting methods were spaced transects (ST), random fields (RF) and spaced fields (ST). df = 1, n = 400 for each test.

		Contagion level				
		Random	A	B	C	D
× 200 magnification						
ST	% above	39	51	44	43	39
	p	<0.005	NS	<0.025	<0.005	<0.005
RF	% above	49	45	56	53	50
	p	NS	NS	<0.025	NS	NS
SF	% above	51	56	54	58	56
	p	NS	<0.025	NS	<0.005	<0.025
× 400 magnification						
PR	% above	48	49	45	43	43
	p	NS	NS	NS	<0.005	<0.005
RF	% above	53	52	55	55	57
	p	NS	NS	NS	NS	<0.01
SF	% above	49	48	43	51	47
	p	NS	NS	<0.025	NS	NS

The average distances of estimates from the population are shown in Table 5.12. All methods exhibited increased average distances of estimates from the population with increased contagion. Spaced transects and pseudo-random fields have the greatest distances and spaced fields the least. Direct comparison cannot be made between spaced transects and the other methods at × 200, or between the results at × 200 and × 400. Nevertheless, the comparisons indicate that the spaced fields method produces results that are consistently closer to the population and are less influenced by increasing contagion.

Table 5.12 Average distances and standard deviations of simulated estimates. Contagion was produced using a combination of distributions 1 to 4, in equal proportions. Counting methods were 5 spaced transects (ST), 75 random fields (RF), 75 pseudo-random fields (PF) and 75 spaced fields (SF). n = 400 for each value.

× 200 magnification			× 400 magnification		
Method & distribution	Average distance	SD	Method & distribution	Average distance	SD
ST			PF		
Random	63	50	Random	1937	1506
A	73	58	A	3938	3147
B	108	86	B	4995	3951
C	135	108	C	5244	4480
D	147	128	D	5957	4828
RF			RF		
Random	751	375	Random	2060	1538
A	772	605	A	2894	2042
B	751	577	B	3180	2470
C	915	749	C	3371	2620
D	920	713	D	3916	3030
SF			SF		
Random	528	402	Random	1867	1346
A	545	399	A	2091	1631
B	536	411	B	2210	1751
C	526	417	C	2339	1739
D	570	415	D	2084	1632

Further count trials

Following the evaluation of errors and performances of the different counting methods resulting from the simulations it was decided to conduct further counting trials comparing the spaced fields technique with those used in the 'basic method'. Counts at × 400 magnification were carried out using the *Chlamydomonas* culture described above. However, the remaining quantity of *Scenedesmus* culture was inadequate for further work and counts at × 200 magnification were carried out on a natural sample collected on the 4 November 1997 from West Lake, Whisby Nature Park, Lincolnshire (NGR, SK 905 665) that contained abundant *Cryptomonas* sp. The number of *Cryptomonas* cells per mL were estimated by counting three complete chambers, as explained above (Figure 5.9).

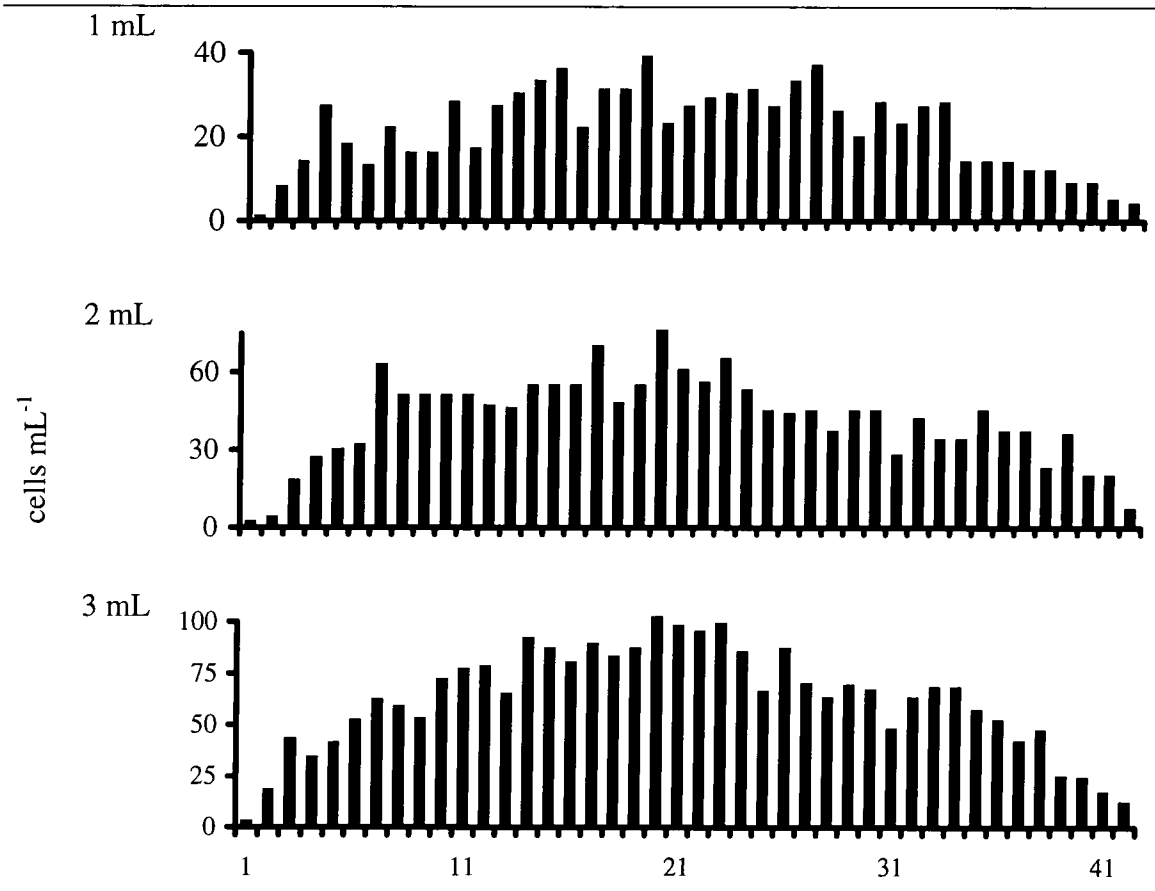


Figure 5.9 Full chamber counts for *Cryptomonas* in 41 transects. Counts are 910, 1744 and 2598 for volumes 1, 2 and 3 mL samples, respectively. Average = 883 cells mL^{-1}.

The test species were used to evaluate the spaced fields method against spaced transects at × 200 magnification and pseudo-random fields at × 400 magnification. Each counting method was repeated twenty-four times, consisting of twelve counts at one of two volumes (Figure 5.10). The twelve counts were derived from four replicates from three chambers. Two of the four replicates were carried out on the same distribution and the second two on the same subsample following re-suspension and complete settlement of the original sample. This was done in an attempt to identify the differing influences of distribution and subsampling on the different counting methods. Estimates and confidence intervals were calculated as described above. Kruskal-Wallis tests were also carried out on each method, comparing the medians at each volume between counts (n = 2), between chambers (n = 3) and between distributions within the same chamber (n = 6).

None of the spaced field counts failed to estimate the population whereas both the spaced transects and the pseudo-random fields failed to predict more frequently than would be expected by the 95% confidence intervals. The influence of subsampling error (difference between replicates from the same chamber before and after re-suspension) is not evident in any of the spaced field counts although there is a suggestion of pairing in the spaced transects and pseudo-random fields, although none of these are significant.

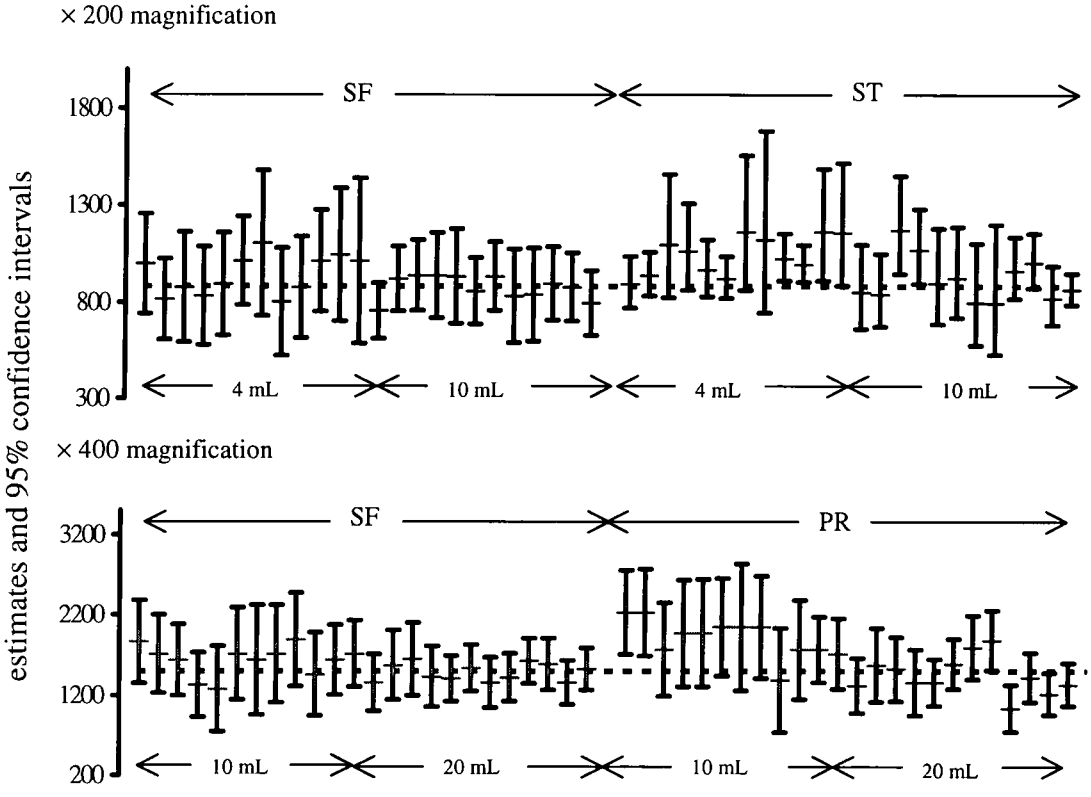


Figure 5.10 Count trials at × 200 and × 400 magnification for 75 spaced fields (SF), 5 spaced transects (ST) and 75 pseudo-random fields (PR). Each counting method consist of 24 trials, 12 from one of two volumes. Average counts for 4 and 10 ml samples at × 200 magnification are 62 and 139 (SF) and 643 and 1359 (ST) respectively. Average counts for 10 and 20 ml samples at × 400 magnification are 64 and 116 (SF) and 75 and 113 (PR) respectively.

The Kruskal-Wallis tests identified spaced fields as being non-significant at all levels and spaced transects and pseudo-random fields as significant at the distribution and/or chamber level (Table 5.13). Thus indicating that distribution is a major influence on the accuracy of counts rather than absolute number of cells within the chamber.

Table 5.13 Kruskal-Wallis probability values for count trials comparing 75 spaced fields (SF) with 5 spaced transects (ST) and 75 pseudo-random fields (PF) at two magnifications and volumes. Tests were between counts (n = 2), between chambers (n = 3) and between re-distributed samples within the same chamber (n = 6). Significant results are boxed.

	× 200 magnification				× 400 magnification			
	4 mL		10 mL		10 mL		20 mL	
	SF	ST	SF	ST	SF	PF	SF	PF
All	0.818	0.348	0.626	0.089	0.292	0.135	0.581	[0.026]
Chambers	0.921	0.294	0.547	0.186	0.065	0.215	0.051	[0.002]
Distributions	0.967	0.052	0.818	[0.004]	0.149	[0.021]	0.189	[0.011]

The distance of estimates from the population was calculated separately for each volume and had a similar trend to that seen in the simulations, with spaced fields producing consistently closer estimates to the population than other methods (Table 5.10). The influence of increasing count is also evident in all counting methods.

Table 5.14 Average distances and standard deviations of estimates at two volumes, as Figure 5.10. n = 12 for each value.

	×200 magnification				×400 magnification			
	Spaced fields		Spaced transects		Spaced fields		Pseudo-random fields	
	4 mL	10 mL	4 mL	10 mL	10 mL	20 mL	10 mL	20 mL
Average	102	52	159	89	206	97	432	193
SD	65	37	99	79	98	44	202	135

5.4 Discussion

The simulations identified cell distribution as a key factor influencing the accuracy of estimates, in the absence of any variation in cell number that might result from subsampling. However, it appears that the simulated distributions produced a greater degree of contagion than exists naturally. This is not altogether surprising, as the arbitrary choice of four contagious distributions combined in equal proportions and mixed with random distributions is unlikely to simulate reality, where distributions would probably vary more gradually. This discrepancy is not detrimental to the exercise with the greater contagion created by the simulations producing a cautious approach to the methods evaluated.

The counting trials identified the inherent dangers of assuming randomness in the distribution of settled algae and the use of parametric statistics to calculate confidence

intervals. As some workers suggest (Nauwerk, 1963), a random distribution can never be relied upon. Utermöhl (1958) suggested many ways of subsampling chambers and also identified the dangers of a contagious distribution within sedimentation chambers. He did not, however, identify the influence that different counting methods could have on the final result. Sandgren and Robinson (1998) identified a bias from using diameter transects, caused by an 'edge effect' with more algae settling towards the edge of chambers, and proposed that fields should be placed disproportionately at the edge of the chamber. This work confirms that diameter transects introduce bias to the results not only with an edge effect, but even with a random distribution. Indeed a slight edge effect appears to improve the accuracy of diameter transects, presumably by compensating for the under-sampling of the chambers periphery, that is inherent in the method.

The key components of a successful counting method must include accuracy (how close the estimate is to the population), acceptable precision (confidence intervals that estimate the population within known limits) and a minimum amount of error (percentage difference between the estimate and confidence intervals). Spaced transects produce large confidence limits, compared to other methods, because of their small n. Therefore spaced transects have fewer estimates failing to accurately predict the population compared to pseudo-random fields, because the latter produce narrower confidence intervals.

The results generally support those previously stated for the EA 'basic method' (National Rivers Authority, 1993). With a count of 100 algal units in 5 spaced diameter transects (at × 200 magnification) producing an error of $< \pm 50\%$ and a count of 50 algal units in 50 'ad hoc' field placements (at × 400 magnification) producing an error of $< 40\%$. Errors of $< 50\%$ are acceptable when gross changes in populations are under investigation (Lund et al., 1958). However, this makes the 'basic method' unsuitable for work where the identification of smaller differences in populations need to be identified, such as small spatial differences or short-term temporal trends. The spaced fields method appears to provide a mechanism capable of accurately identifying small changes in populations, normally within $\pm 20\%$.

The spaced fields method, however, presents a number of problems. Systematic sampling does not conform to normal statistical practice. The main purpose of random sampling is to make available an estimate of error, using probability theory (Cassie,

1962). Parametric confidence intervals should not be assigned to any of the counting methods evaluated here except random fields, where they do not appear to work satisfactorily. A well-planned systematic sampling procedure in which samples are placed in a regular pattern would almost invariably give a much more precise estimate, but the degree of precision would by unknown (Cassie, 1962). Improved accuracy of estimates does occur with the systematic placement of spaced fields and the results of the simulations provide a method of assigning confidence intervals, which appear to work in the count trials. Alternatively a non-parametric method of assigning confidence could be used (Ryan et al., 1985).

Cell distribution appears to be the greatest influence on the accuracy of results and differences in subsample quantity of lesser significance. Errors can occur at all stages, from taking the sample, subsamples and counting (Venrick, 1981). Some studies have identified sampling error as being the principal constituent of overall error (Irish and Clarke, 1984). It appears that counting and secondary subsample error can be adequately accounted for using the methods described here and any differences identified outside assigned confidence limits will originate from the sample or primary subsample. This subject will be discussed further in the next chapter.

5.5 Summary

1. A basic counting method is described and evaluated using counting trials and computer simulation, the latter approach was also used to evaluate a new technique called spaced fields.

2. The basic method exhibited a similar performance during the simulation as the count trials whilst spaced fields had the best overall performance in the simulations and secondary count trials.

3. Variations in distribution were more significant than differences in cell quantity between samples.

4. A count of 100 algal units in 5 spaced diameter transects (at ×200 magnification) produces an error of $< \pm 50\%$ and a count of 75 algal units in 50 'ad hoc' field placements (at ×400 magnification) an error of $< \pm 40\%$.

5. A count of 100 algal units in 75 spaced fields at either ×200 or ×400 magnification produces an error of $< \pm 25\%$.

6 SAMPLE ERROR AND TREATMENT

6.1 Introduction

This section explores sources of error additional to those described in the previous chapter. Stages from sample collection to analysis (Figure 6.1) can all introduce error and all these stages will also contain a degree of random or experimental error. Sample and subsample errors are explored using chlorophyll, fluorescence and counting, although counts used for this purpose will also contain secondary-subsample and counting error. All the subsequent analyses were undertaken during periods of relatively stable discharge and not within 7 d of spate flows.

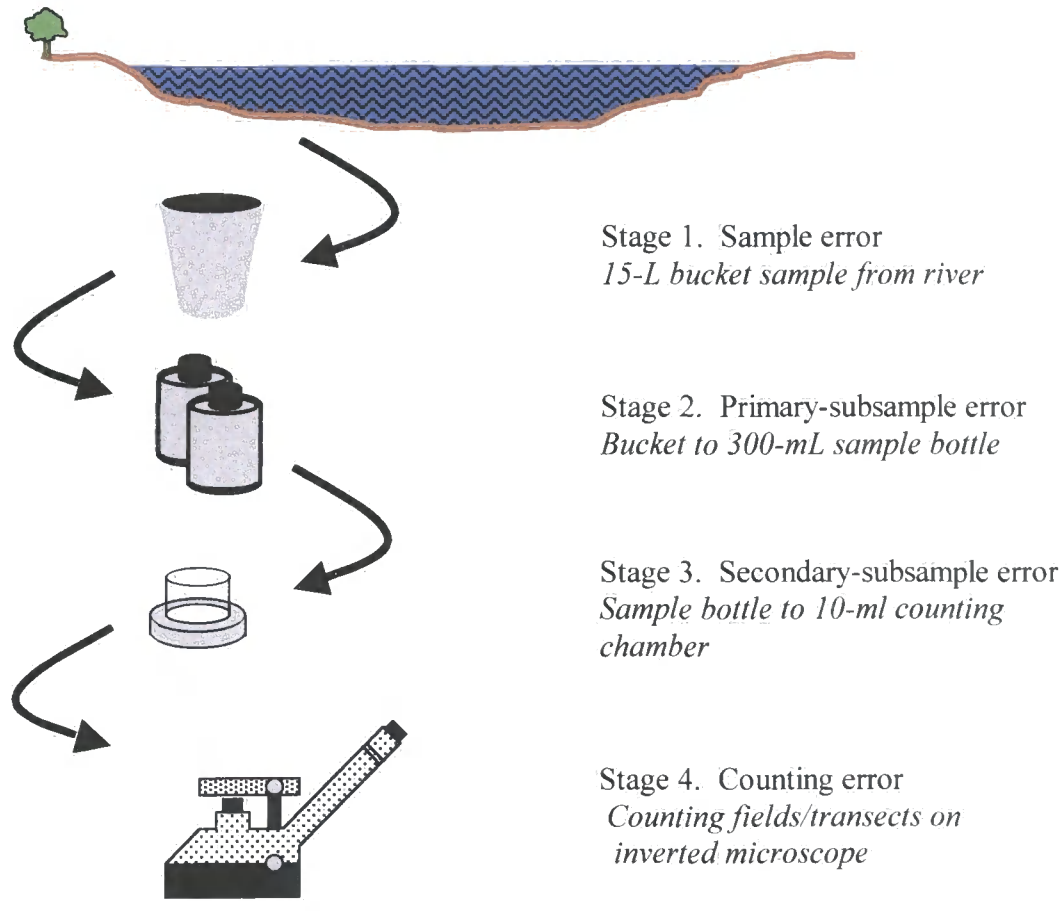


Figure 6.1 Schematic representation of the stages in phytoplankton sampling and sub-sampling. Showing principal sources of errors.

6.2 Sample error

Sample error, chlorophyll

Five samples were collected at 2-min intervals over a range of chlorophyll concentrations, from an average of below 10 μg L^{-1} to more than 200 μg L^{-1} and error ranged from 0.8% to 14.7% (Table 6.1). Percentage error does not appear to be connected to magnitude, with high chlorophyll concentrations producing a range of error values. The results indicate an acceptable level of consistency in sampling from the main river and bay at km 91.7 (Wansford). It is assumed that these error levels are transferable to other locations within the system.

Table 6.1 Chlorophyll sample error. All samples were collected from the main river at km 91.7 except (b) which were collected from a bay. Showing mean, upper and lower 95% confidence limits, standard deviation, n and percentage error. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983).

	18/8/94	21/4/94	21/1/94[b]	11/7/95	13/6/96	15/5/96
mean	6.4	130.6	143.5	13.1	207.6	174.5
UCL	6.4	149.9	151.6	13.8	240.4	182.2
LCL	6.3	113.7	135.8	12.4	179.3	167.1
SD	0.1	14.1	6.3	0.6	18.3	6.0
n	5	5	5	5	4	5
% error	0.8	13.9	5.5	5.3	14.7	4.3

Sample error , phytoplankton

Counts of *Nitzschia acicularis* from five 2-min samples were highly consistent indicating minimal variation in samples (Figure 6.2).

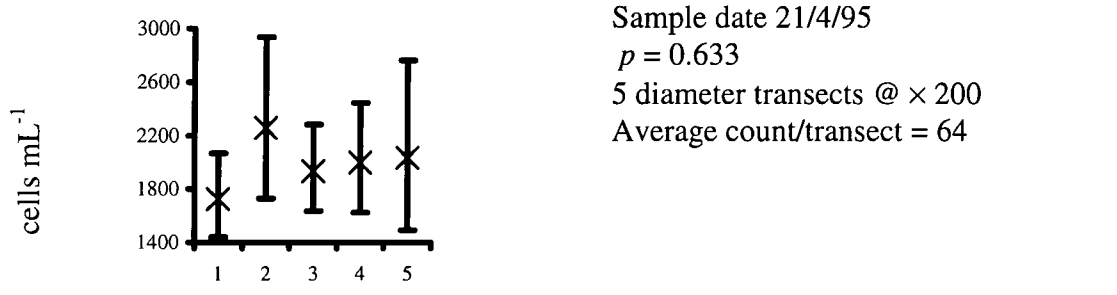


Figure 6.2 Phytoplankton counts and confidence limits for samples taken every 2-min at km 91.7. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983). *p* was calculated using Mood Median Test.

Primary-subsample error, chlorophyll

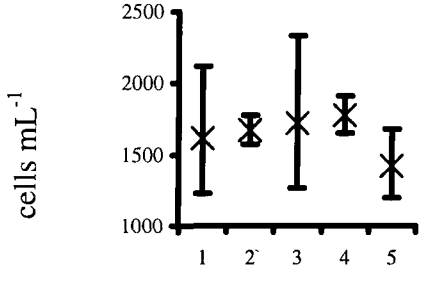
Five subsample taken from a single sample were used to assess primary-subsample error, which was generally similar to sample error ranging from 1.5% to 10.6% (Table 6.2). This suggests that much of the variation results from analytical or random error rather than chlorophyll variability *per se*.

Table 6.2 Chlorophyll primary-subsample error. All samples were collected from the main river. Showing mean, upper and lower 95% confidence limits, standard deviation and percentage error. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983). The samples taken on 14/5/98 were primary-subsamples from a single sample and were analysed using in-vivo fluorescence.

	1994		1996		1994		1998		
	17/8	20/4	13/6	15/5	10/4	10/6	8/1	14/5	14/5
mean	6.4	125.8	213.0	171.8	126.2	44.5	8.4	85.1	83.6
UCL	6.8	139.8	221.5	186.2	129.3	47.9	9	86.8	84.9
LCL	6.0	113.1	204.9	158.5	123.1	41.3	7.8	83.3	82.3
SD	0.3	10.5	6.7	10.9	2.5	2.7	0.3	2.4	1.8
n	5	5	5	5	5	5	5	10	10
% error	6.3	10.6	3.9	8.1	2.5	7.4	7.1	2.0	1.5

Primary-subsample error, phytoplankton

Phytoplankton primary-subsample counts for *Nitzschia acicularis* were non-significant. These data support the finding of the chlorophyll analysis, indicating a minimum of sample error (Figure 6.3).



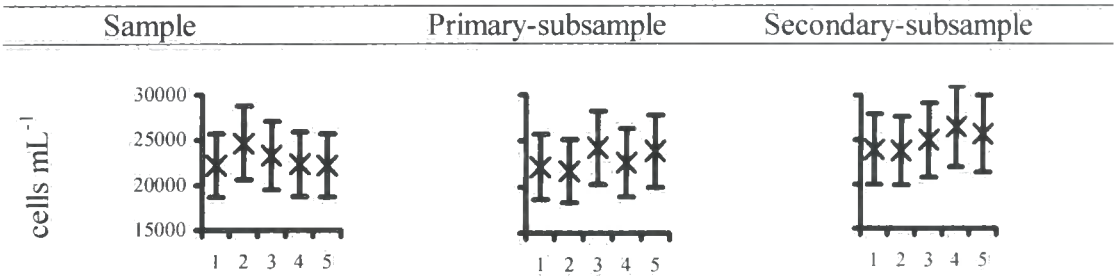
Sample date 20/4/95
p = 0.067
n = 5
5 diameter transects
Average count/transect = 54

Figure 6.3 Phytoplankton counts and confidence limits for primary-subsamples taken from a single sample from km 91.7. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983). p was calculated using Mood Median Test.

Sources of error

The contribution of variance from sample, primary-subsample and secondary-subsample were evaluated by repeating 75 spaced field counts throughout the levels, with five counts at each level (Figure 6.4). No significant difference was found between

the levels and the contribution of variation from the sample was similar to that of the primary and secondary-subsamples.



Level	SS	df	MS	*p*
Samples (1)	808.4	14	57.7	0.634
Primary-subsample (2)	575.0	9	63.9	0.559
Secondary-subsample (3)	151.7	4	37.9	0.752

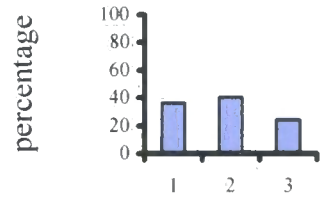


Figure 6.4 Sources of variance from sample to counting. Comprising five samples, five primary-subsamples from a single sample and five secondary-subsamples from a single primary-subsample (above, left to right). Table shows the distribution of variance between levels, with sum of squares (SS), degrees of freedom (df), mean square (MS) and *p* value from ANOVA. Proportion of variance components shown as a percentage of total variance (bottom right). Counts were 75 spaced fields for centric diatoms > 5 ≤ 20 µm ∅ collected from km 91.7 on 15/5/96. Average count per field were 14.2, 14.2 and 15.4 for sample, primary and secondary-subsamples, respectively.

6.3 Sample storage

Chlorophyll

In the absence of definitive guidance on the storage of chlorophyll samples it was decided to conduct a range of experiments to evaluate deterioration during storage of whole water samples and samples stored on frozen filter papers (Figure 6.5). These evaluations would provide guidance for routine sample treatment and indicate how best to handle samples during high-frequency sampling. The results indicate gradual deterioration of chlorophyll with time which appears to be delayed by filtering samples and storing on frozen filter papers. Samples stored for 24 h in Wansford gauging station (km 92.5) at ambient were non-significant, although the increase of degradation product was almost significant.

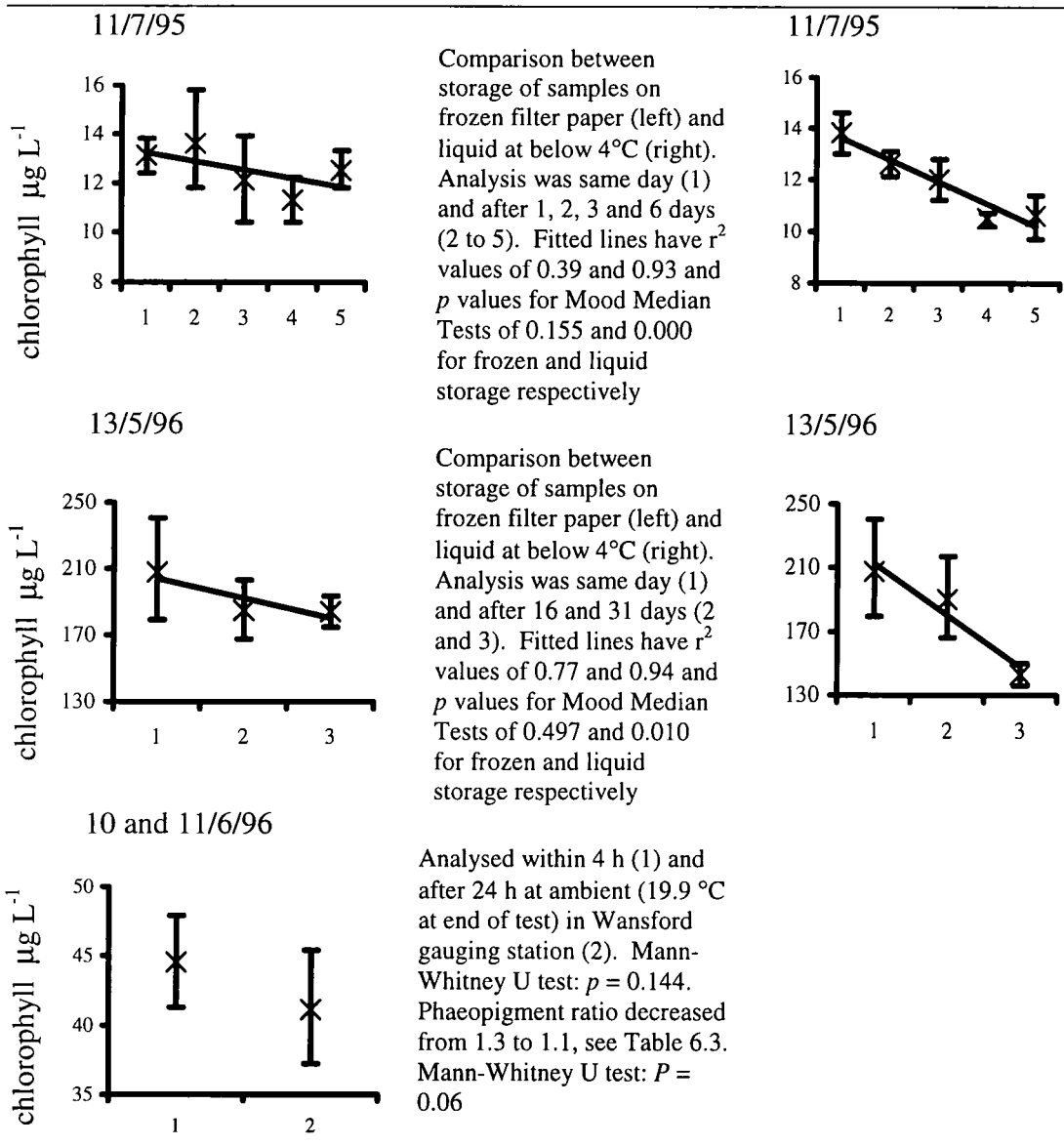


Figure 6.5 Comparisons and evaluations of chlorophyll storage techniques. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983) and all samples were n = 5. Lines were fitted using least-squares method.

Phytoplankton

Phytoplankton preservation using Lugol's iodine was assessed by counting preserved samples prior to and following storage. Figure 6.6 shows two replicate counts for samples that had been in storage for over 16 months. No significant difference were found between the treatments for either species.

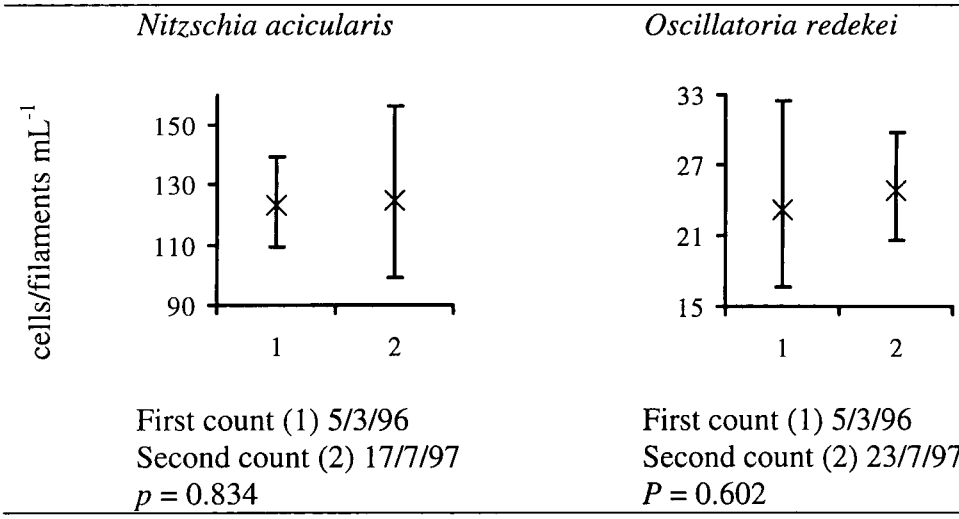


Figure 6.6 Preservation efficiency for two species fixed with Lugol's iodine. Comparisons are for 5 transect counts at × 200 magnification for original (1) and replicate (2) counts. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983) and all samples were n = 5. *p* values are from Mann Whitney U Tests.

6.4 Inter-laboratory calibrations

Chlorophyll

As the majority of chlorophyll samples were analysed by the EA National Laboratory Service (NLS - and its predecessors) it was thought necessary to evaluate the results of self and NLS analyses (Figure 6.7). One of the three comparisons made is significant but the trend is inconsistent, with the remaining evaluations being non-significant. Without further information about the treatment of individual samples if is difficult to draw further conclusions.

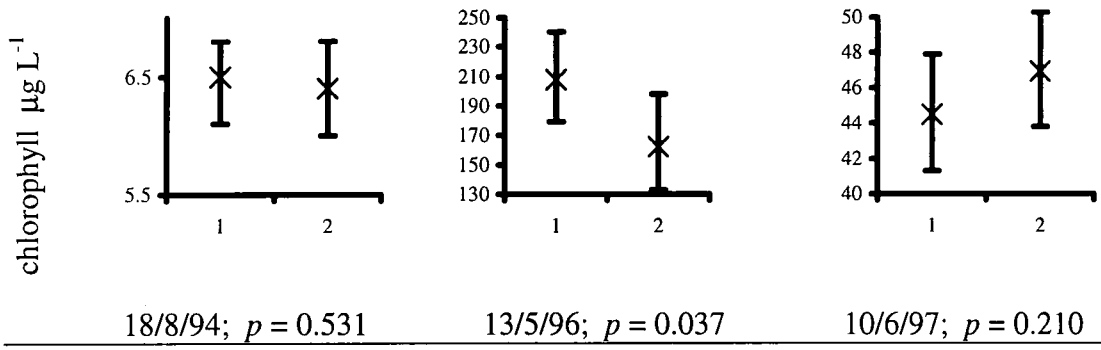


Figure 6.7 Comparison between self (1) and NLS (2) chlorophyll analysis. Confidence limits were calculated using $\log_{10}$ transformed data and arithmetic mean (Elliott, 1983) and all samples were n = 5. Sample date and *p* values from Mann-Whitney U tests are shown below each plot.

6.5 Phaeopigment

Phaeopigment estimates (Table 6.3) indicate that the proportion of degradation product increases through the year, being minimal during the spring peak and becoming most abundant during the winter.

Table 6.3 Total chlorophyll and phaeopigment concentrations on three occasions at km 91.7. Degradation absent A_n/A_m = approximately 1.6, degradation complete A_n/A_m = 1.0 (Marker, 1992).

	Chlorophyll ($\mu g\ L^{-1}$)			
	Total (A_n)	Degraded (A_m)	A_n/A_m	n
10/4/97	127.2	82.1	1.54	5
05/8/97	8.7	7.6	1.13	5
08/1/98	11.1	10.8	1.07	5

6.6 Discussion

In standing waters sample variance is often cited as the principal source of error, with subsample and counting errors being small by comparison (Irish and Clarke, 1984). In the Nene, it appears that phytoplankton can be considerably homogeneous. A phenomenon that probably results from the turbulent and unidirectional nature of rivers, especially during periods of relative stability. It would be expected that sample variation would be far more heterogeneous during spate flows or following other physical disturbance, such as the passage of boat traffic or 'weed' cutting. Although small localised influxes of algae would soon become 'diluted' by turbulent flow.

The results here indicate a very different situation to standing waters with similar variation in sample, primary and secondary-subsample (Figure 6.4). This is further supported by the sample and primary-subsample counts, which were all non-significant (Figure 6.2 and Figure 6.3). Such counting consistency indicates homogeneity at all levels, which is confirmed by the analysis of variance. However, smaller counts, particularly in transect or pseudo-random fields would probably result in significant differences, relating to the counting method (Chapter 5). The influence of sample frequency and sample location are explored in Chapters 8 and 9, respectively.

The results from the chlorophyll storage evaluations vindicate the methods employed during this research. Samples stored below 4°C will usually have insignificant deterioration, providing they are analysed within a few days. Samples

stored on frozen filter papers exhibited reduced deterioration compared to whole-water samples, and this method was therefore more suitable for long-term storage (longer than 48 h). Storing chlorophyll samples on frozen filters would have reduced bacterial activity, grazing or parasitism that could continue in water samples stored below 4°C, albeit more slowly than normal. Filter papers can be frozen 'on site' by placing them (in sealed and labelled bags) between ice-blocks in a cool-box. However, storing samples on frozen filters has the disadvantages of prolonging sampling time and the requirement for additional field equipment. Also, extra care is needed at the analysis stage as filter papers can become brittle and must be completely defrosted before analysis. The comparison of a freshly analysed sample and one stored at ambient in the gauging station (at km 92.5) for 24 h provides validity for using the auto-samples, where samples could stand for up to 14 h before treatment.

The inter-laboratory calibrations of chlorophyll samples indicate a reasonable level of consistency, although some samples do give cause for concern. Intra-laboratory consistency suggests that differences were due to treatment rather than differences in chlorophyll *per se*. For example, some samples sent to the NLS may have been stored for longer than usual (e.g. 13/5/96), but without details of each sample analysed it is not possible to speculate further.

As chlorophyll samples were not routinely corrected for phaeopigment. The pilot survey provides an important contribution to the understanding of chlorophyll dynamics in the Nene, which follow a similar pattern to those seen in the Kennet and Thames (Kowalczewski and Lack, 1971). During the spring chlorophyll peaks degradation product appears to be minimal but becomes increasingly important as the year progresses. The high proportion of phaeopigment in the winter provides justification for removing samples with chlorophyll concentrations ≤ 1 µg L^{-1} for statistical analysis of trends, with normalisation (log_{10}) resulting in negative values.

6.7 Summary

1. Sample error was consistent throughout the levels of treatment, being similar to the primary and secondary-subsamples.

2. Sample storage evaluations provide justification for treatments used throughout this research. Although prompt analysis of samples is preferable, the analysis of chlorophyll samples within 24 h of collection and preserved phytoplankton

3. Samples within a few months of collection should not result in significant deterioration. Chlorophyll samples stored for longer than 48 h exhibit reduced deterioration if stored on frozen filter paper.

4. Inter-laboratory chlorophyll analysis tests were within acceptable limits although some comparisons had a significant difference.

5. Phaeopigment exhibit seasonal variation and the pilot study provides valuable information for the interpretation of chlorophyll data.

7 ENVIRONMENTAL BACKGROUND AND LONG-TERM TRENDS

7.1 Introduction

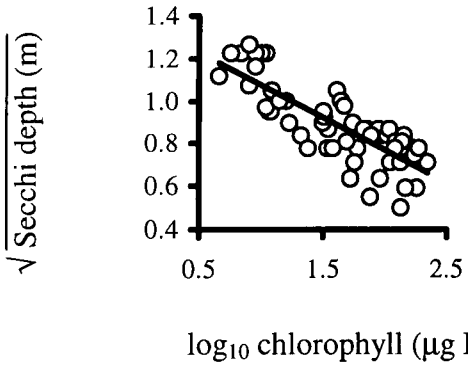
This Chapter examines environmental background information and long-term trends at km 91.7 (Wansford).

7.2 Light attenuation

Light attenuation measurements were not available for the whole period therefore chlorophyll concentration was utilised as an estimate for z_{eu} (using z_s as an intermediate). Figure 7.1 shows the relationships between chlorophyll concentration and Secchi disc depth and Secchi disc depth and euphotic depth. Chlorophyll concentrations of 10, 30 and 50 μg L^{-1} result in estimated z_{eu} of 2.3, 1.8 and 1.6 m, respectively. These results indicate that benthic photosynthesis would be inhibited throughout much of the navigation section of the Nene at chlorophyll concentrations greater than 30 μg L^{-1}, as the average depth is greater than 1.8 m (Table 3.1).

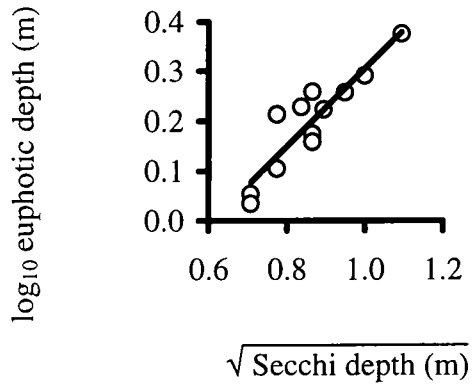
7.3 Environmental trends and background

All physical, chemical and biological variables have significant inter-year variation, which is most pronounced in discharge (Table 7.1, Figure 7.2 and 7.3). Drought periods are evident particularly during 1976 and in the early 1990s and during 1996. Temperature and light varied throughout but both have higher values in the latter twelve year period. Ammonium exhibits the most consistent change through time, the 1976 peak was possibly exacerbated by the drought but this was followed by a consistent downward trend. This pattern is also evident to a lesser extent in NO_2-N, TON, NO_3-N, TN levels. Silicate concentrations were consistent throughout, with a slight decrease during the latter periods possibly reflecting lower discharge. TRP concentrations also reflect varying discharge, although increasing concentrations up to 1991 could reflect an increasing population in the area and lower concentrations thereafter could partly reflect P removal at major STW. Chlorophyll concentrations were significantly higher in the earlier period, even when the influence of severer drought years (1975 and 1976) is removed.



Secchi disc depth (m) on chlorophyll concentration (µg L^{-1})

$z_s = (-0.31 \times \log_{10}[chl] + 1.39)^2$
$r^2 = 0.66$
$p = < 0.05$
n = 65



Euphotic depth (m) on Secchi depth (m)

$z_{eu} = 10^{(0.72 \times \sqrt{z_s} - 0.42)}$
$r^2 = 0.83$
$p = < 0.05$
n = 12

Figure 7.1 Relationship between chlorophyll concentration and Secchi disc depth and Secchi depth and euphotic depth. Chlorophyll and Secchi depth data were treated with $\log_{10}$ and square root transformations respectively (Table 3.4).

Table 7.1 Long-term physical, chemical and biological trends at km 91.7. Showing χ^2 and p values for inter-year and inter-period (data set in two 12 year periods; 75-86 and 87-98) tests and the inter-period medians. Significant tests are boxed.

	Mood median tests for					
	Inter-year χ^2	p	Inter-period χ^2	p	Period 1 median	Period 2 median
Discharge (mg L^{-1})	887.6	0.000	11.5	0.001	6.24	5.79
Temperature (°C)	43.8	0.006	2.4	0.123	11.00	12.00
Light (sunshine h)	59.5	0.000	4.1	0.042	3.10	3.40
NH_4-N (mg L^{-1})	88.9	0.000	44.7	0.000	0.18	0.08
NO_2-N (mg L^{-1})	57.6	0.000	5.9	0.015	0.10	0.09
NO_3-N (mg L^{-1})	82.3	0.000	0.1	0.721	9.39	9.23
TON (mg L^{-1})	84.2	0.000	0.2	0.641	9.50	9.34
TN (mg L^{-1})	79.4	0.000	1.8	0.183	9.84	9.25
TRP (mg L^{-1})	174.0	0.000	10.6	0.001	0.96	1.14
N:P (mg L^{-1})	44.6	0.000	3.72	0.054	9.80	8.20
SiO_2-Si (mg L^{-1})	32.7	0.018	1.0	0.329	6.07	5.90
Chlorophyll (µg L^{-1})	100.4	0.000	9.6	0.002	17.4	11.0
Chlorophyll (µg L^{-1}) minus 1975 and 1976	82.7	0.000	7.0	0.008	15.6	11.0

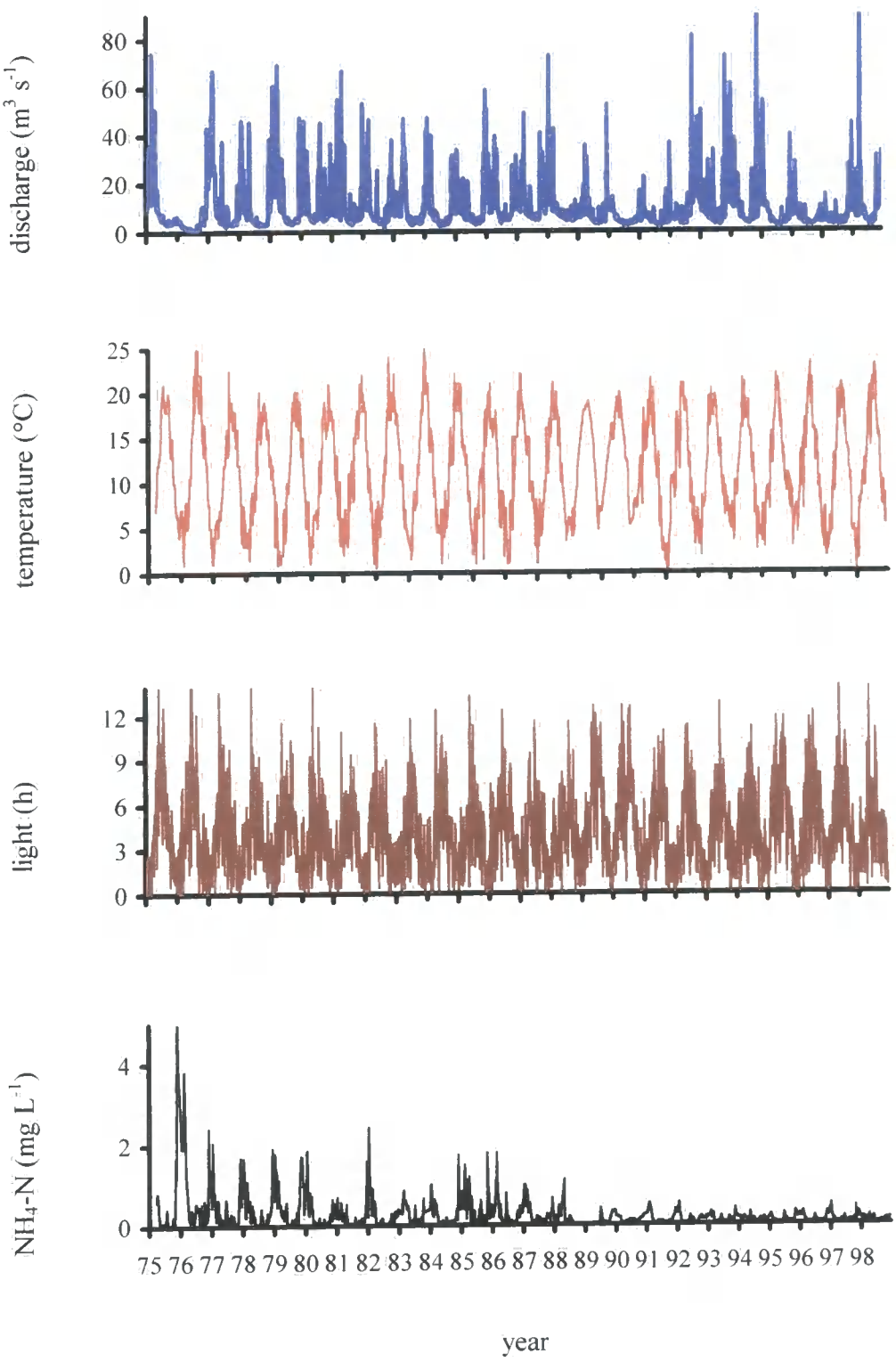


Figure 7.2 Time series for discharge (m^3 s^{-1}), temperature (°C), light (sunshine h and tenths) and NH_4-N (mg L^{-1}) at km 91.7 from 1975 to 1998. Discontinuities in the NH_4-N plot indicates missing data. Sample frequencies for discharge and light were daily. Median sample frequencies for NH_4-N and temperature were 7 d and 6 d respectively.

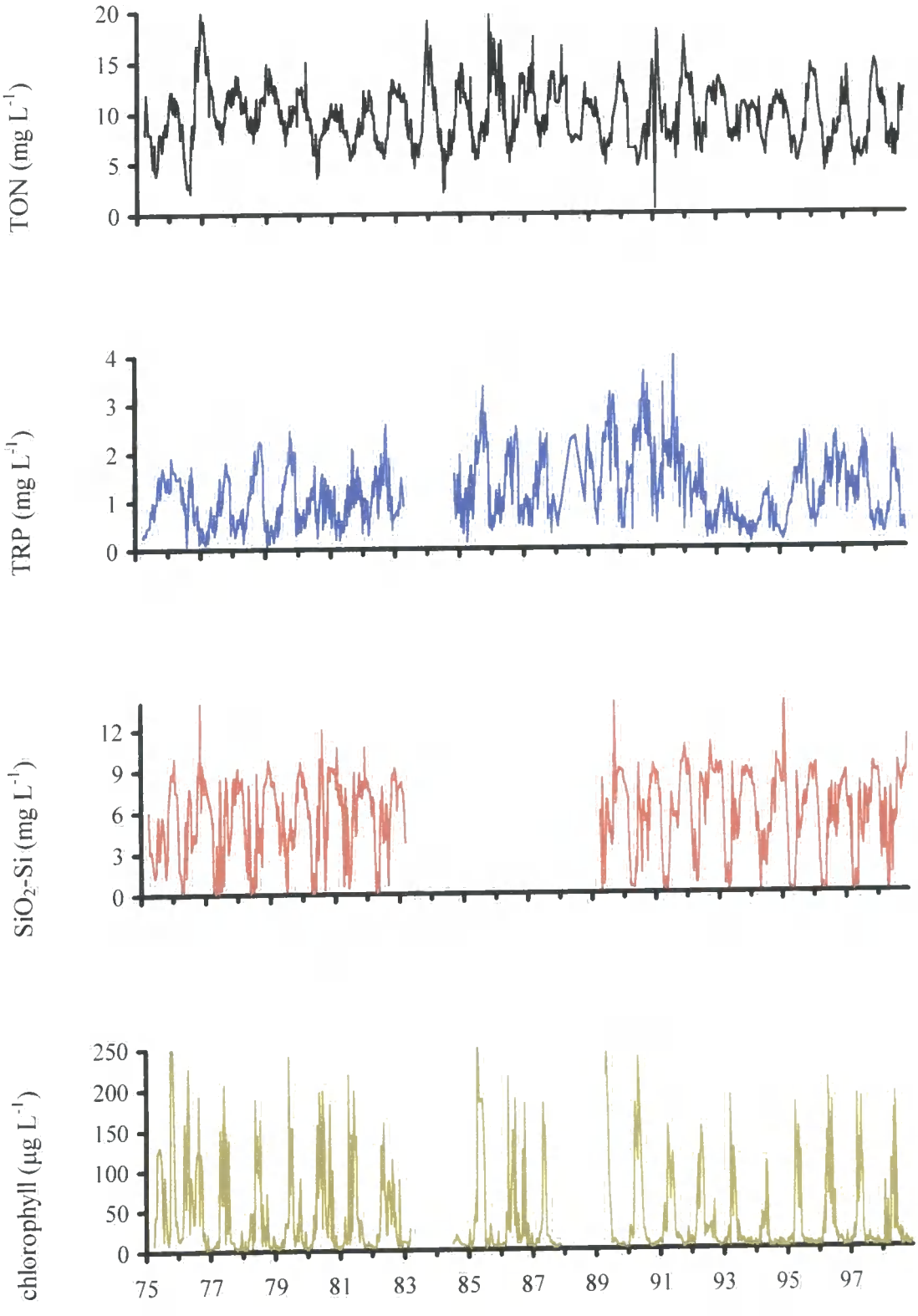


Figure 7.3 Time series for TON (mg L^{-1}), TRP (mg L^{-1}), silicate(mg L^{-1}) and chlorophyll (µg L^{-1}) at km 91.7 from 1975 to 1998. Discontinuities in the plots indicate missing data. Median sample frequencies were 7 d for all variables.

As discharge exhibited the greatest inter-year variability its relationship with other variables was explored using regression analysis (Table 7.2). Discharge correlates positively with N (except NO_2-N) and negatively with TRP, these opposing trends resulting in N:P having the most significant relationship. The silicate relationship was weak indicating the influence of other factors.

Table 7.2 Chemical variables regressed on discharge. Showing r^2, *p*, n and trend.

	r^2	*p*	n	Trend
NH_4-N	0.147	< 0.05	938	Positive
NO_2-N	0.005	NS	890	None
NO_3-N	0.336	< 0.05	889	Positive
TN	0.336	< 0.05	938	Positive
TRP	0.340	< 0.05	1030	Negative
N:P	0.527	< 0.05	890	Positive
SiO_2-Si	0.099	< 0.05	756	Positive

Nutrient concentrations at four main river and three tributary sites are summarised in Table 7.3 The results indicate general downstream increases in all nutrients with evidence of a slight decline at km 91.7. The influence of the major STW (Figure 2.1) on NH_4-N and P concentrations is evident at km 43.9 and in tributary 3 (Willow Brook). A large increase in NH_4-N and P occurs at km 43.9 and the average P concentration in tributary 3 is considerably higher than the other tributaries, these sites being respectively downstream of Billing and Corby STW. The downstream increase in TON concentrations reflect increasing catchment area, which is also evident in the differing catchment areas of the tributaries (Table 3.1). Comparison with the long-term data indicates that nutrient concentrations have generally declined in recent years, as identified in Table 7.1.

The 1994 to 1997 average NH_4-N and TON concentrations are respectively 0.19 and 1 mg L^{-1} lower than the long-term values. Table 7.3 also indicates that TON principally comprises NO_3-N and TP mainly comprises reactive P, with little difference occurring between TRP and FRP. On average, TON is 99% NO_3-N (min = 84%, max = 100%, n = 888) and TP 83% FRP (min = 45%, max = 100%, n = 51). TP has its lowest percentage of FRP during the spring and this possibly reflects high levels of particulate P, in the form of suspended algae.

Table 7.3 Nutrient concentrations (mg L^{-1}) at four main river and three tributary sites (1994 to 1997) and long-term at km 91.7 (1975 to 1998). Showing ammonium (NH_4-N), total oxidised nitrogen (TON), filterable reactive phosphorus (FRP), total reactive phosphorus (TRP) and total phosphorus (TP).

Site and period	Nutrient	Mean	Min	Max	n
1994 to 1997					
km 22.4	NH_4-N	0.10	< 0.03	0.62	74
(Duston Mill)	TON	7.71	3.40	14.00	74
	FRP	0.96	0.03	3.40	84
	TRP	0.82	0.10	2.40	74
	TP	0.98	0.15	2.46	117
Tributary 1	NH_4-N	0.11	< 0.03	0.80	47
(Brampton Branch)	TON	9.56	4.70	16.60	48
	FRP	0.35	0.06	1.60	84
	TRP	0.39	0.06	3.20	48
	TP	0.42	0.10	2.09	86
km 43.9	NH_4-N	0.23	< 0.03	0.81	48
(Wellingborough)	TON	9.90	4.90	15.50	48
	FRP	1.55	1.08	3.70	94
	TRP	1.57	0.33	3.60	48
	TP	1.67	0.15	4.28	97
Tributary 2	NH_4-N	0.11	< 0.03	0.82	48
(River Ise)	TON	7.01	1.80	15.00	48
	FRP	0.12	0.03	0.40	85
	TRP	0.12	0.03	0.40	48
	TP	0.18	0.04	1.28	85
km 64.6	NH_4-N	0.27	< 0.02	1.24	50
(Thrapston)	TON	10.21	5.80	14.90	49
	FRP	1.39	0.05	3.57	92
	TRP	1.44	0.25	3.10	49
	TP	1.55	0.13	3.72	97
Tributary 3	NH_4-N	0.10	< 0.03	0.50	50
(Willow Brook)	TON	6.50	0.80	13.60	50
	FRP	0.64	0.06	1.50	96
	TRP	0.71	0.09	1.74	48
	TP	0.76	0.08	2.21	116
km 91.7	NH_4-N	0.10	< 0.03	0.51	119
(Wansford)	TON	8.70	4.10	14.80	124
	FRP	1.07	0.04	2.68	108
	TRP	1.10	0.12	2.40	196
	TP	1.22	0.02	7.37	144
1975 to 1998					
km 91.7	NH_4-N	0.29	< 0.01	5.32	926
(Wansford)	NO_3-N	9.69	0.42	20.10	892
	NO_2-N	0.10	< 0.01	0.40	891
	TON	9.76	0.50	20.19	958
	TRP	1.17	0.07	4.39	1031

7.4 Long-term chlorophyll trends at km 91.7

The relationship between the long-term chlorophyll record and physical variables was investigated by regressing chlorophyll on discharge, temperature and light (Figures 7.4, 7.5 and 7.6). This analysis was undertaken using the whole chlorophyll data set and subsets, as shown.

Maximum chlorophyll concentrations mostly occur at a discharges below 10 $m^3 s^{-1}$, although chlorophyll concentrations greater than 10 $\mu g L^{-1}$ do occur at high discharge. Chlorophyll data from the first half of the year are influenced more by discharge than those from other times. Data from the latter part of the year having a less significant relationship (Figure 7.4). Discharge produced more significant relationships with chlorophyll when expressed as a four-day average, compared to daily values or averaging over longer or shorter periods.

Temperature correlates poorly with the whole chlorophyll data set, but maximum chlorophyll concentrations do not occur below 5°C. Chlorophyll concentration has a linear relationship with temperature, up to the spring chlorophyll peak, thereafter increasing temperature tends to result in a wider scatter and a general decrease in the chlorophyll concentration (Figure 7.5). Polynomial regression significantly improved the r^2 coefficient for the January to June data but made little difference to the January to spring chlorophyll peak. During July to December a wide range of chlorophyll values are evident, with low concentrations occurring throughout the temperature range.

Generally light correlated poorly with chlorophyll (Figure 7.6), but again, the most significant relationships are found with chlorophyll data from the first half of the year. The poor relationship may result from the insensitivity or unsuitability of the data, with light only being recorded during periods of bright sunshine. This is evident in the plots, with many low records despite the seven-day cumulative summation of light data. Nevertheless, low chlorophyll concentrations rarely occur at the greatest light values, especially in the former half of the year. Although high chlorophyll values do occur throughout the light range.

Multiple regression analysis of the combined January to June data sets produced an enhanced relationship [$r^2 = 0.65$, $p < 0.05$, n = 450; $\log_{10}$ [chl] = 0.877 + 0.185(T) - 0.006 (T^2) + 0.003(*I*: 7 d CuSum) - 0.554(Q: 4 d mean)], the influence of light being negligible. A more comprehensive assessment of the long-term chlorophyll data and

combinations of physical variables over different time periods can be found in Balbi (2000).

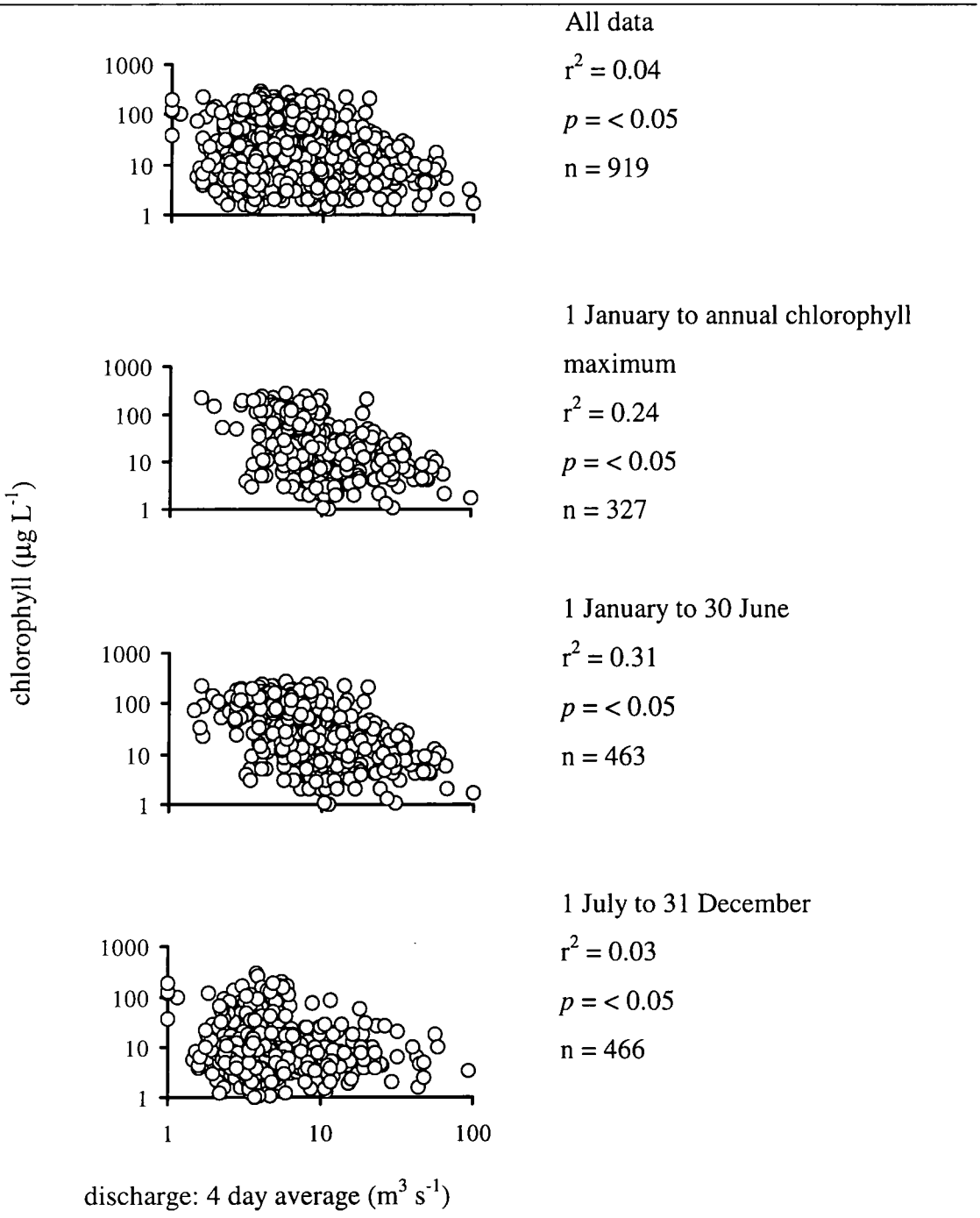


Figure 7.4 Chlorophyll plotted against discharge. Showing data range, correlation coefficients, probabilities and n. Data ranges are all data (top plot) and from restricted ranges, as indicated. For example, all data between 1 July and 31 December (bottom plot). See Table 3.7.

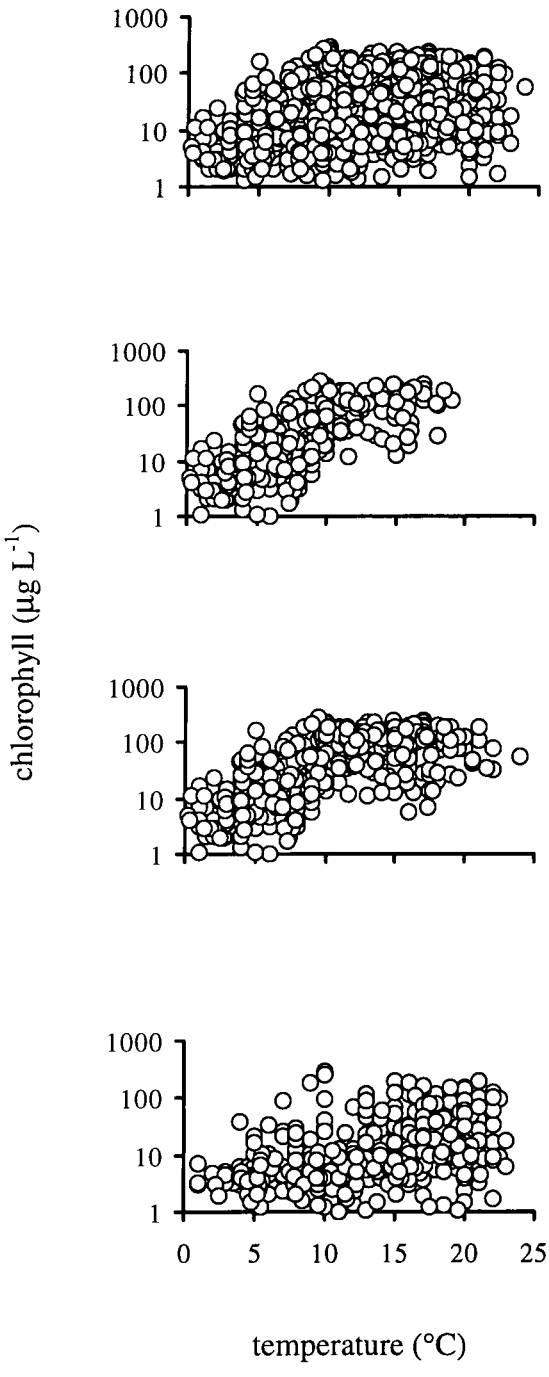


Figure 7.5 Chlorophyll plotted against temperature. Showing data range, correlation coefficients, probabilities and n. Data ranges are all data (top plot) and from restricted ranges, as indicated. For example, all data between 1 July and 31 December (bottom plot). See Table 3.7.

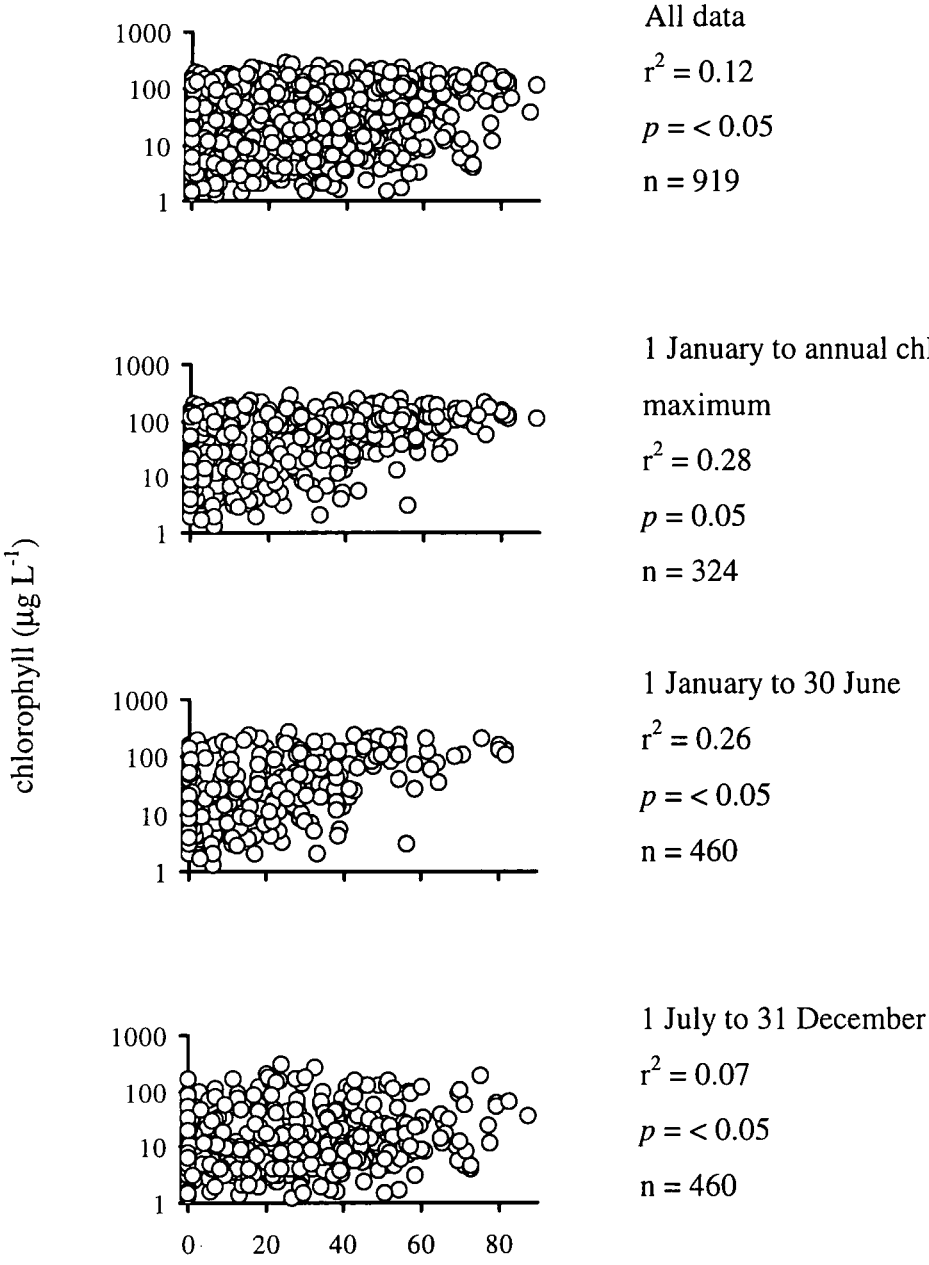


Figure 7.6 Chlorophyll plotted against sunlight hours and tenths. Showing data range, correlation coefficients, probabilities and n. Data ranges are all data (top plot) and from restricted ranges, as indicated. For example, all data between 1 July and 31 December (bottom plot). See Table 3.7.

The greater variability of chlorophyll data from the second half of the year warranted further investigation. Close scrutiny of the chlorophyll time series (Figure 7.3) indicates considerable variability of summer chlorophyll concentrations, with some years (e.g. early 1980s) having a relatively high concentration compared to others,

which had very low summer chlorophyll concentrations (e.g. 1993 to 1995). The influence of summers with low chlorophyll concentrations on the whole data set was investigated by establishing the 'low chlorophyll summer period' (Table 3.7) and removing these data from the whole chlorophyll data set. Low chlorophyll summers were most numerous in the latter part of the study, commencing from mid-June to late September and ranging from 40 to 194 days (Table 7.4).

Table 7.4 Years with 'Low chlorophyll summer periods' at km 91.7, between 1975 and 1996. (Too few data to include 1983 and 1988).

Year	Period start	Period end	Number of days	Chlorophyll Mean	Minimum	Maximum	Number of samples
1977	27 Sep	6 Dec	70	3.7	1.4	5.2	9
1984	13 Jul	31 Dec	171	8.6	4.0	18.2	8
1985	25 Jul	3 Sep	40	4.9	1.3	12.3	7
1987	29 Jul	31 Dec	155	4.1	1.0	9.1	10
1989	10 Jul	31 Dec	174	4.3	1.2	11.7	11
1990	15 Aug	31 Dec	138	4.5	1.5	10.0	9
1991	23 Sep	18 Nov	56	3.3	1.7	6.2	5
1993	21 Jul	31 Dec	163	5.2	1.9	11.2	22
1994	20 June	31 Dec	194	3.4	1.0	9.3	23
1995	17 Jul	31 Dec	167	4.8	3.0	8.9	26
1996	16 Aug	31 Dec	137	5.4	1.0	17.0	25
1997	03 Jul	31 Dec	181	6.6	1.6	18.0	33
1998	27 Aug	31 Dec	126	5.9	1.0	20.0	15

Removing low chlorophyll summer data greatly enhanced the relationship between the chlorophyll concentration and temperature and discharge, but made less difference to light. Thus indicating that low chlorophyll summer periods were independent of the main regulatory factors influencing the majority of data (more than 75% of the data).

Low chlorophyll summers were also evaluated by comparing physical variables from these periods with those from July to September from other years (Table 7.5). Temperature and light were not significantly different between the treatments, but discharge was significantly lower during the low chlorophyll periods. Higher summer discharge could explain higher chlorophyll concentrations in terms of providing the necessary turbulence to maintain diatoms in suspension. However, high discharge could also result in low chlorophyll summers as seen during 1994, which had an extended low chlorophyll summer period and an average summer discharge (July to September) of 5.4 $m^3 s^{-1}$.

Table 7.5 Mann-Whitney U tests comparing physical variables and chlorophyll concentration for 'low chlorophyll summers' and other years (July to September).

		'Low chlorophyll years'		'Other years'	
	p	median	n	median	n
Temperature (°C)	0.401	18.0	267	18.0	121
Discharge ($m^3 s^{-1}$)	0.000	3.2	1288	4.1	920
Light (h)	0.134	5.7	1288	5.3	920
Chlorophyll ($\mu g L^{-1}$)	0.000	10.0	166	37.2	89

The impact of physical variables upon the onset of low chlorophyll summer periods was investigated by comparing values from the month preceding each of these periods with values for June from 'other' years (Figure 7.7).

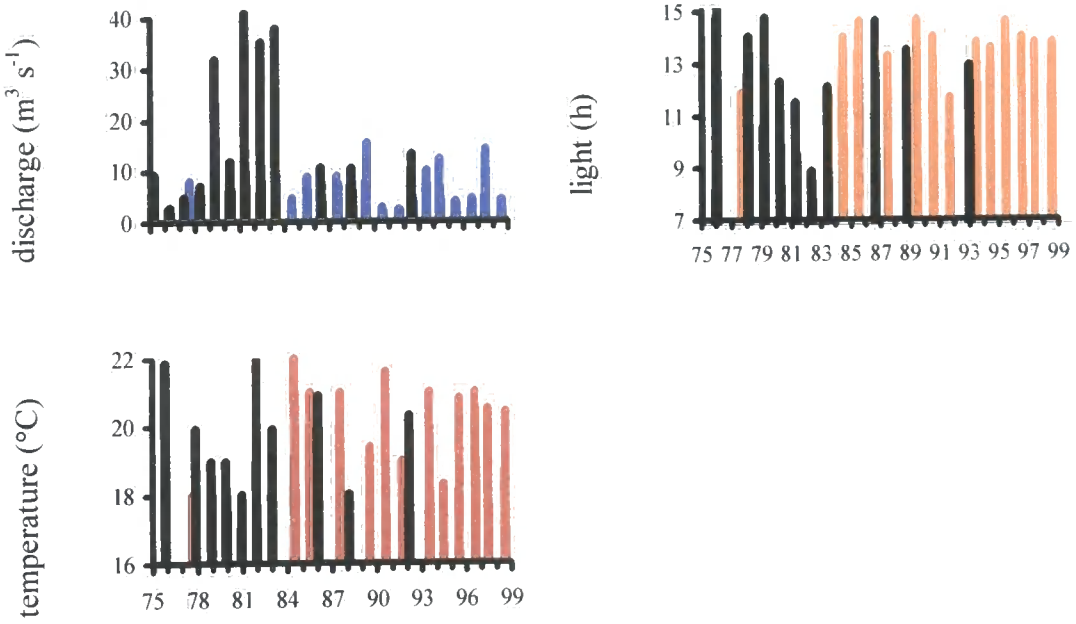


Figure 7.7 Physical variable from the month preceding the onset of low chlorophyll summer periods (coloured) and values for June of other years (black). Showing discharge ($m^3 s^{-1}$), light (sunshine hours and tenths) and temperature (°C).

Comparisons in Figure 7.7 are not strictly valid because the onset of low chlorophyll summer periods varied considerably (Table 7.4), but they do identify considerable differences especially during the early 1980s. 1980, 1981 and 1982 all had summers with extensive high chlorophyll concentrations and relative to other years these had high discharge, low light and temperature. Differences at other times are not so clear but interpretation could be hindered by using June data for comparison. Although a causal factor for the onset of 'low chlorophyll summer periods' is far from

conclusive, these periods are normally preceded by high temperature, high light and low discharge.

Physical variables do not appear to be wholly responsible for the data variability in the latter half of the year or the onset of low chlorophyll summer periods, so the relationship between chlorophyll and chemical variables was examined. This was undertaken for the whole chlorophyll data set and for the '1 January to the spring chlorophyll maximum', where nutrient limitation is most likely to have been evident (Figure 7.8). P does not appear to be limiting either during the whole or restricted data sets. In fact, P actually increases with increasing chlorophyll and this is presumably the result of chlorophyll peaks occurring during periods of reduced discharge. The N:P ratio also decreases with increasing chlorophyll concentration, as seen during the spring of 1996 (Table 7.6), further supporting the supposition that P was not limiting.

Nitrate concentrations do exhibit a negative relationship with chlorophyll, especially with the restricted data set. Although nitrate concentrations are generally greater than 5 mg L^{-1} even when phytoplankton are at their most abundant. Ammonium has the strongest negative relationship with chlorophyll of the N fractions examined. During the spring chlorophyll maxima ammonium concentrations were reduced to 20 $\mu g\ L^{-1}$ ($10^{-1.7}$) but with abundant nitrate at this time it is unlikely that N is limiting algal growth.

Silicate concentration has a strong negative relationship with chlorophyll, for the whole and restricted data sets. During the spring chlorophyll maxima silica concentrations are reduced to very low levels (below threshold at 30 $\mu g\ L^{-1}$) and could be limiting further increases in the population under these conditions, as indicated by the P:Si ratio (Table 7.6).

Table 7.6 N:P ratio, P:Si ratio (m mol L^{-1}) and Chlorophyll ($\mu g\ L^{-1}$) on three occasions during the spring of 1996.

Date	N:P ratio	P:Si ratio	Chlorophyll
3 April	35.5	44.3	65.0
9 May	23.3	38.8	123.9
21 May	16.5	26.7	156.0

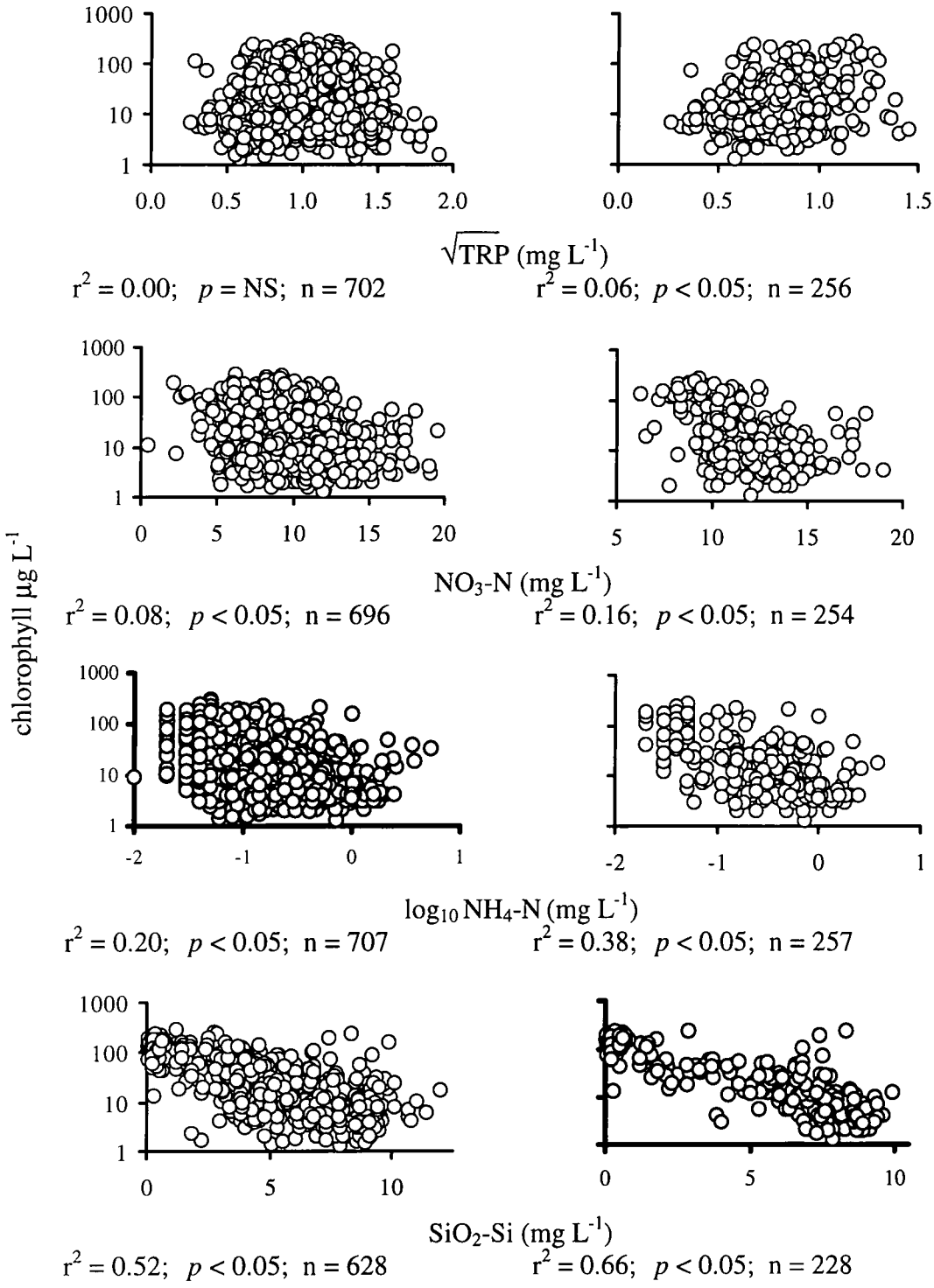


Figure 7.8 Chlorophyll plotted against nutrients and silicate for 'all data' and the '1 January to spring chlorophyll maximum' data sets. Also showing r^2, p and n. TRP and NH_4-N were square-root and $\log_{10}$ transformed respectively (Table 3.6).

The occurrence of such a strong relationship between silicate and chlorophyll indicates the presence of abundant planktonic diatoms. However, there are periods

where high chlorophyll and silicate concentrations occur simultaneously and low silicate and low chlorophyll concentrations occur together. These data respectively indicate times with abundant phytoplankton that do not have a requirement for silica and silica uptake from biota other than phytoplankton.

DIC concentrations, estimated from total alkalinity, are equally high throughout periods of high and low chlorophyll concentrations (Table 7.7). The difference in the DIC concentrations between the periods was not significant, although the greater abundance of algae during the spring could be responsible for the elevated pH values that occurred. The DIC concentrations during this study greatly exceed those which are likely to limit phytoplankton growth (Reynolds, 1993).

Table 7.7 $CaCO_3$ (mg L^{-1}), pH, temperature (°C) and DIC (m mol C L^{-1}) and Chlorophyll (µg L^{-1}) during spring and summer 1996. Mann Whitney U test = NS for DIC concentrations during spring (April & May) and summer (August & September).

Date	$CaCO_3$	pH	Temperature	DIC	Chlorophyll
25 April	190	8.60	13.0	3.8	181.0
9 May	220	9.00	11.5	4.2	138.0
21 May	201	8.90	12.5	3.9	148.0
16 August	197	8.27	18.5	3.9	14.0
28 August	177	8.15	18.5	3.5	6.7
12 September	201	8.17	15.0	4.0	6.0

7.5 Discussion

Several long-term trends are evident over the 24-year period, which reflect political, demographic and climatic influences. The most dramatic trend is that of ammonium (Figure 7.2), which declined significantly following the privatisation of the water industry in 1989 and was also influenced by the drought of the mid 1970s. Increases in P could mirror those of population, although investment following privatisation of the water industry and the implementation of the UWWT Directive resulted in reduced concentrations in recent years (Figure 7.3). Significant inter-year variation was evident in all variables examined and water chemistry is significantly influenced by discharge, which has the greatest inter-year variation. P is influenced negatively by increasing discharge and N positively. These trends reflect the origins of these nutrients, with P originating principally from point sources and N (except

ammonium) from diffuse inputs, the former being diluted and the latter leached concurrently with increasing rainfall and river flow.

Nutrient concentrations are generally high throughout the study section (Table 7.3), with considerable variations in the tributaries, and based on these concentrations the Nene can be classified as eutrophic (Environment Agency, 2000) to hypertrophic (Dokulil, 1995). This classification and the relationship between spring chlorophyll maxima and nutrients indicates that phytoplankton are unlikely to be nutrient limited in the Nene. A statement which is supported by the N:P ratios.

Ammonium concentrations are reduced to low levels during the spring chlorophyll peaks but this probably reflects preferential uptake over nitrate (Morris, 1974), which remains abundant throughout. P concentrations were influenced more by discharge than chlorophyll and the lowest TRP concentration recorded during the spring chlorophyll maxima (chlorophyll > 100 $\mu g\ L^{-1}$) was 0.33 $mg\ L^{-1}$, so it is unlikely that P is limiting algal growth.

Silicate concentrations are reduced to low levels during the spring chlorophyll maxima and are thought to limit phytoplankton abundance in other lowland systems (Swale, 1969; Skidmore et al., 1998). The strong negative relationship between silicate and chlorophyll indicates an abundance of planktonic diatoms, although a fuller analysis of the impact of reduced silica on phytoplankton development will be undertaken in the next chapter. Periods with high chlorophyll concentrations and high silicate probably reflect an abundance of green algae (which do not require silica for growth) and low silicate concentrations at times of low phytoplankton abundance possibly indicates silica uptake by benthic diatoms. Again, further investigations of these subjects will ensue in subsequent sections.

The influence and occurrence of low chlorophyll summer periods is of great significance to the elucidation and understanding of chlorophyll dynamics in the Nene. Summers with low chlorophyll concentrations are independent of the main influencing variables (discharge and temperature) at other times. The factors causing the onset of these periods is unclear but in some cases appears to be associated with high temperature and light and low discharge (Figure 7.7). Temperature and light appear to have increased in latter years (Table 7.1), as have the occurrence of low chlorophyll summer periods (Table 7.4). The summers of the early 1980s had relatively high chlorophyll concentrations and were associated with high discharge, low temperature

and light. A temperature range of approximately 5 to 15°C does appear to promote high chlorophyll (Figure 7.5) and inhibit suspended chlorophyll development in the Nene above 15 to 18°C. It is clear that 'low chlorophyll summer periods' are not the product of a few predictable processes and may result from a series of disparate events. Biological influences have not been considered here and grazing, filtration and parasitism may be important factors impacting on chlorophyll concentrations, especially in periods of low discharge which are likely to promote the development of planktonic grazers.

Many of the factors discussed here are expanded in the next chapters which will attempt to elucidate temporal patterns in the long-term chlorophyll data by detailed analysis of data colleted between 1993 and 1999.

7.6 Summary

1. Long-term data on physical, chemical and biological variables exhibit significant inter-year variation (especially discharge) and several of these variables show significant inter-period trends, some increasing (e.g. TRP) and others decreasing (e.g. NH_4-N) over the 24 year study period.

2. Nutrient concentrations are generally high throughout the study section and the Nene can be classified as eutrophic to hypertrophic. P originates mainly from point sources and N from diffuse inputs, with P correlating negatively and N positively with discharge. N and P are unlikely to be limiting phytoplankton abundance although silicate could be restricting further growth during periods of high phytoplankton abundance.

3. Chlorophyll concentrations exhibit considerable inter-year variation with some summers having relatively high concentrations compared to others years. The identification of low chlorophyll summer periods and the removal of these data from the main data set enhanced the relationships between chlorophyll and physical variables. Low chlorophyll summer periods appear to be independent of the controlling variables at other times and the reason for these periods is unclear but may be associated with high temperature and light and low discharge.

4. The relationship between silicate and chlorophyll indicates that diatoms are abundant in the plankton (particularly during the spring) and are the main constituent of the chlorophyll maxima. Chlorophyll maxima occur between a temperature of 8 and 18°C, and temperature increases above this level may be inhibitory to growth.

5. Many of the factors discussed in this chapter will be expanded upon in subsequent sections.

8 TEMPORAL TRENDS 1993 to 1998

8.1 Introduction

This chapter examines temporal chlorophyll and phytoplankton trends at km 91.7 (Wansford) throughout the study period and periodic high frequency investigations at km 92.5 (Wansford gauging station). This analysis builds and expands on the previous chapter and should facilitate a fuller understanding of long-term chlorophyll trends.

Chlorophyll sampling continued throughout the period and these data are included in the long-term analysis, in the previous Chapter. However, some additional analysis of the chlorophyll data is useful for the 1994 to 1997 period, as solar irradiance data were available for this time and these data could prove to be a better predictor of chlorophyll concentration than sunshine h, used for the long-term analysis.

The primary phytoplankton analysis is based on 188 taxa in 138 samples taken between 25/6/93 and 27/5/97 at varying frequencies (median = 8 d), but with a concentration of effort on periods of greatest phytoplankton abundance.

8.2 Chlorophyll

With the availability of solar radiation data (watts $m^2 h^{-1}$) for the period 1994 to 1997 the chlorophyll data for this period were re-evaluated substituting these data for the sunshine h used in the long-term analysis (Table 8.1). The substitution of solar-radiation data for sunshine h made little difference to the significance of the relationship between chlorophyll and light, with data from the first half of the year having least variability.

Table 8.1 Multiple-regression analyses for chlorophyll data (1994-1997) on the physical variables, discharge ($m^3 s^{-1}$), light (watts $m^2 d^{-1}$), temperature (°C) and temperature^2 (°C). Analysis was undertaken for all data, those data between 1 January and 31 June and those data between 1 January and the spring chlorophyll maximum (Table 3.7). The variables used in each analysis are indicated (X) and all analyses are significant at the 95% level.

Chlorophyll data range	Disch.	Light	Temp.	Temp.^{2}	r^2	p	n
All data		X	X	X	0.28	< 0.05	277
1 Jan to 31 Jun	X		X	X	0.50	< 0.05	141
1 Jan to spr. chl. max	X			X	0.40	< 0.05	86

8.3 Taxa groups

The top-twenty numerically most abundant taxa recorded at km 91.7 are listed in Table 8.2, and these represent more than 88% of all taxa recorded. Centric diatoms were the most abundant group (44%) followed by greens (excluding unidentifiable cells and picoplankton - 23%) and picoplankton (14%), these three groups constituted 75% of all taxa found. The objective of the initial analysis of taxa was to construct taxa groups with the purpose of easing analysis and understanding of temporal trends. The construction of these taxa groups was made with consideration to abundance/biovolume, periodicity and ecology.

Table 8.2 The twenty most numerically abundant taxa recorded at km 91.7 during the study. Taxa classified as a percentage of all cells recorded.

Taxa	Percentage abundance	cumulative
Centric diatom > 6 to ≤ 10µm ∅	20.7	20.7
Spherical cell ≤ 3µm ∅	11.0	31.7
Centric diatom ≤ 5µm ∅	9.4	41.1
Stephanodiscus hantzschii fo. *tenuis*	8.5	49.6
Chlorella sp.	6.0	55.6
Centric diatom > 11 to ≤ 20µm ∅	5.8	61.3
Rhodomonas lacustris var. *nannoplanktica*	4.2	65.5
Monoraphidium contortum	3.4	68.9
Rod cell ≤ 3µm GALD	3.3	72.2
Oscillatoria redekei	3.1	75.3
Flagellate ≤ 5µm GALD	2.4	77.7
Actinastrum hantzschii	2.2	79.9
Scenedesmus communis	2.2	82.1
Chlorella vulgaris	1.4	83.5
Spherical > 6 to ≤ 10µm ∅	1.0	84.5
Flagellate ≤ 6 to ≤ 10µm GALD	0.9	85.4
Oscillatoria agardhii	0.8	86.2
Dictyosphaerium pulchellum	0.7	86.9
Tetraedron caudatum	0.7	87.6
Tetrastrum staurogeniaeforme	0.7	88.3

Abundance and Biovolume

The initial analysis provides an idea of numerical abundance but biovolume requires consideration before further analysis can proceed. Biovolume is an important consideration because of the relative sizes of the taxa concerned, covering several orders of magnitude. For example, the two most abundant taxa recorded were centric diatom > 6 to ≤ 10µm ∅ and Spherical cell ≤ 3µm ∅ (Table 8.2), with the former taxa

being 1.9 times numerically more abundant than the latter. However, if the two taxa are compared volumetrically then centric diatoms are at least 53 time more abundant than spherical cells (based on a diatom cell with valve and girdle dimensions of 8 μm, a spherical cell with a 3μm ∅ and corrected for percentage abundance). This estimated difference in numerical and volumetric abundance results from a conservative estimate that the diatoms have a volume 28.5 times greater than a spherical cell. Likewise *Nitzschia acicularis* has been found (during this study) to have an average biovolume of 128 μm^3. *N. acicularis* is 17 times less abundant, numerically, then spherical cells ≤ 3μm ∅ but 1.9 times less abundant volumetrically.

Periodicity

For an initial appraisal of periodicity the phytoplankton taxa were divided into six main groups and plotted as percentage abundance (Figures 8.1).

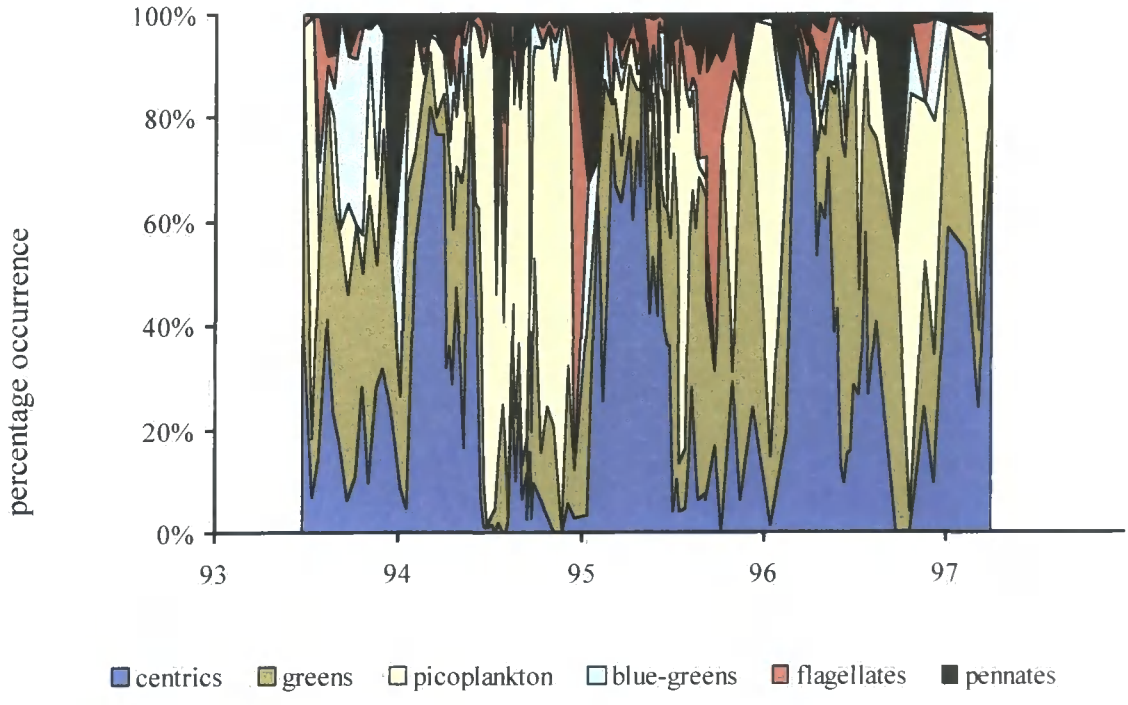


Figure 8.1 Percentage occurrence of numerically most abundant phytoplankton groups at km 91.7 (25/6/93 and 27/5/97).

Centric diatoms exhibit numerical dominance during the spring, achieving 90 % of taxa in 1996. Centric diatoms form a large proportion of taxa during the late summer also, but these periods are dominated principally by green algae, picoplankton and blue-green algae. Picoplankton were most abundant during the summer of 1994, when other

algae were infrequent. Blue-green algae were most numerous during the summer of 1993. Pennate diatoms generally have periods of abundance before and following the centric diatom peaks. Unidentified flagellates exhibit variable abundance and were particularly numerous during the summer of 1995.

Ecological considerations

Examination of the data revealed the occurrence of taxa that could be of particular value as environmental indicators. For example, the periodic occurrence of *Nitzschia fruticosa* and benthic diatoms could respectively indicate increased grazing pressure and light transparency. The colonial habit of *N. fruticosa* making it unpalatable to smaller grazers and benthic forms possibly being restricted by light during periods of high phytoplankton abundance.

Taxa groups

Considering abundance/biovolume, periodicity and ecology, and following some experimentation, 32 taxa groups were created to further investigate phytoplankton dynamics in the Nene (Table 8.3).

Table 8.3 Taxa groups and abundances, expressed as a percentage of all taxa. Short names (curved brackets) correspond to those used in the biplots (Figures 8.8, 8.9 and 8.10). Square bracketed taxa are included for comparative purposes only and are not included as groups in subsequent analysis.

Taxa	% abundance
[All blue-greens]	5.11
Oscillatoria redekei and *limnetica* (O. r. & l.)	3.57
Oscillatoria agardhii (O. aga.)	0.79
Other blue greens (Ot. BG)	0.74
Euglenophyceae (Eug.)	0.04
[Cryptophyceae]	4.62
Rhodomonas lacustris var. *nannoplanktica* (Rhod.)	4.15
Cryptomonas (Cryp.)	0.43
Dinophyceae (Dino.)	0.03
Chrysophyceae (Chry.)	0.06
Centric diatom ≤ 5μm ∅ (CD < 5)	9.40
Centric diatom > 6 to ≤ 10μm ∅ (CD 6-10)	20.71
Centric diatom > 11 to ≤ 20μm ∅ + *S. hantzschii* fo. *tenuis* (CD 11-20)	14.24
Filamentous centric diatoms (e.g. *Melosira varians*) (Mel. v.)	0.13
Benthic diatoms (*Amphora*, *Cymbella*, *Cocconeis*, *Cymatopleura*, *Gomphonema*, *Meridion*, *Rhoicosphenia*, *Surirella* Spp., *Navivula* spp. except *N. lanceolata*) (Benth.)	0.10

Navicula lanceolata (N. lan.)	0.08
Nitzschia acicularis (N. aci.)	0.61
Nitzschia fruticosa (N. fru.)	0.91
Asterionella formosa and *Diatoma elongatum* (A.f. & D.e.)	0.49
[All greens]	23.10
Scenedesmus spp. (Scen.)	6.69
Koliella longiseta (Kol.)	0.33
Actinastrum hantzschii (Act.)	2.30
Chlorella spp. (Chlor)	7.41
Monoraphidium contortum (Mon.)	3.40
Crucigenia (Cruc.)	1.13
Dictyosphaerium pulchellum (Dict.)	0.72
Tetrastrum staurogeniaeforme (Tet.)	0.66
Lagerheimia sp. (Lag)	0.43
Other greens (minus above and desmids) (O. Gr.)	3.05
Desmids (Des)	0.01
[Unidentified flagellates]	3.34
Flagellate ≤ 5μm GALD (F. <5)	2.37
Flagellate ≤ 6 to ≤ 10μm GALD (F. 6-10)	0.87
[Picoplankton]	14.29
Spherical cell ≤ 3μm ∅ (S. < 3)	10.97
Rod cell ≤ 3μm GALD (R. < 3)	3.32

8.4 Trends in phytoplankton

Time series

Temporal trends of phytoplankton groups were initially appraised by scrutiny of time series, some of which are shown in Figures 8.2, 8.3 and 8.4.

Centric diatoms exhibit greatest abundance during the spring with an indication of a shift from the smaller to larger forms as the spring progresses. Planktonic pennate diatoms also have spring peaks, which are dominated by *Nitzschia acicularis* and colonial forms (*Asterionella formosa* and *Diatoma elongatum*) to a lesser extent, whereas the colonial pennate *Nitzschia fruticosa* (not shown) became most abundant during the summers of 1995 and 1996. Those pennate diatoms classed as 'benthic' were generally low in abundance achieving less than 200 cells mL^{-1} at their most numerous. Benthic forms are mostly absent when centric diatoms are abundant, and most numerous following the decline of the centric peaks. During the spring of 1994 benthic forms were particularly numerous and their peak corresponds to a poor spring centric diatom peak.

Planktonic green algae were most numerous during May and June. Some green taxa, such as *Scenedesmus* spp. and *Monoraphidium contortum*, occurred every summer whereas other taxa, such as *Actinastrum hantzschii*, were far more numerous during 1996, than other years. Several taxa of green algae were most abundant during 1996, less so in 1995 and least numerous in 1994. The 'large' acicular green alga *Koliella longiseta* differed from other greens in its early annual occurrence and absence at other times.

Rhodomonas lacustris var. *nannoplanktica* was more ubiquitous than other taxa, being abundant over a wide period. *Oscillatoria redekei* and *O. limnetica* had a sporadic occurrence, being almost entirely absent during 1995 and having their peaks at various times during 1994 and 1996. Unidentified flagellates and picoplankton exhibit some similarities in their temporal occurrence but no obvious patterns are evident.

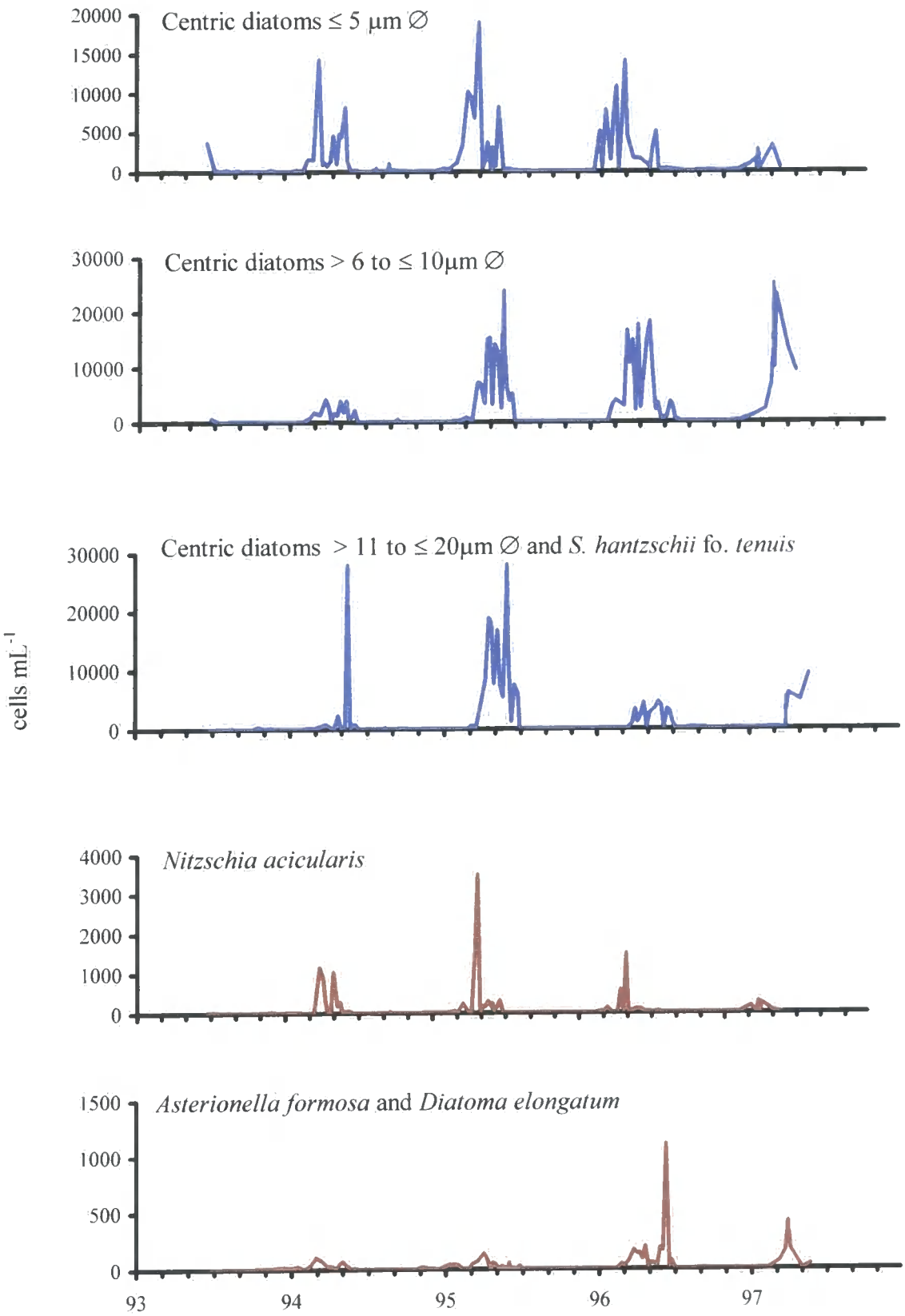


Figure 8.2 Time series of centric and pennate diatoms at km 91.7 (25/6/93 to 27/5/97).

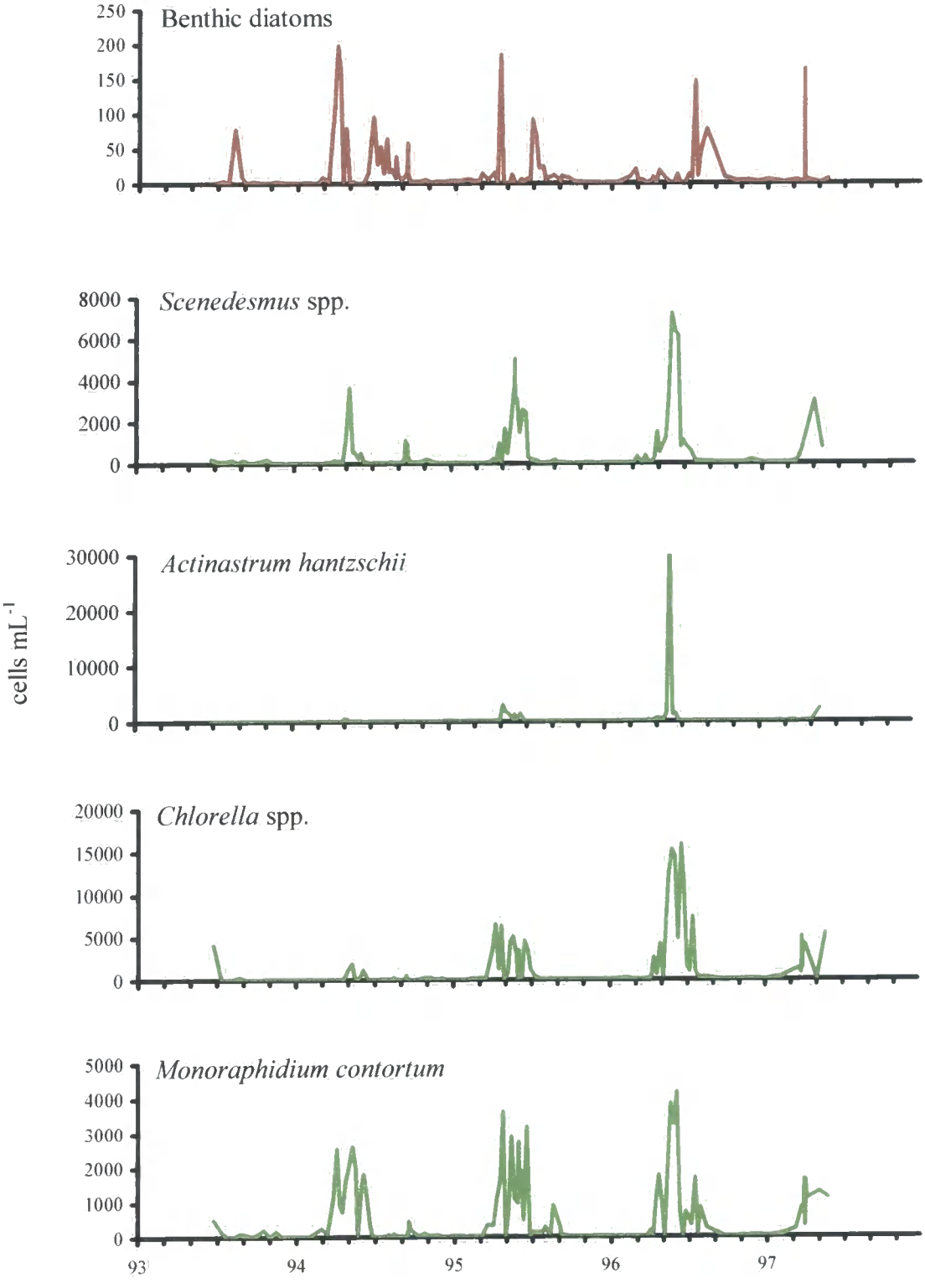


Figure 8.3 Time series of pennate diatoms and green algae at km 91.7 (25/6/93 to 27/5/97).

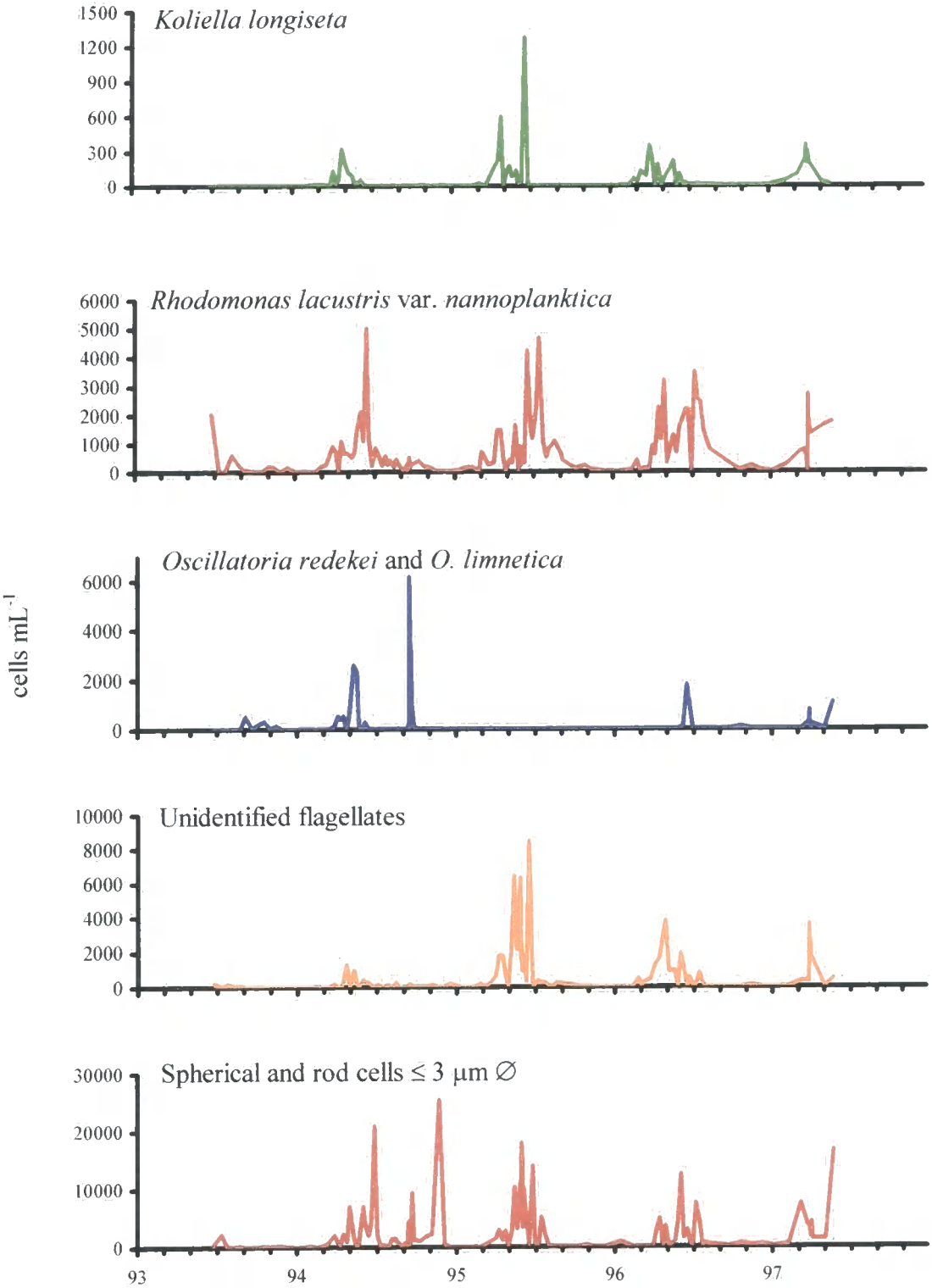


Figure 8.4 Time series of *Koliella*, *Rhodomonas*, blue-green algae, flagellates and picoplankton at km 91.7 (25/6/93 to 27/5/97).

Scatter plots

The relationship between selected phytoplankton taxa and discharge, temperature and light are shown in Figures 8.5, 8.6 and 8.7. The scatter plots indicate the optimum conditions for the individual taxa. Discharge and light were expressed as either daily values or values averaged over preceding days (as indicated *fd*). With the exception of centric diatoms the graphs consist of all data recorded for the taxa shown. Centric diatom plots are shown for all data and for date from the period 1 January to 30 June and colour coded for the three dominant groups (Table 8.3).

In many cases the optimal taxa response to one of the physical variables is clear, but in other cases these is considerable scatter (e.g. *Oscillatoria redekei* and *O. limnetica* - Figure 8.6). In the former situation response lines could be readily applied to the plots by hand or in the example of *N. acicularis* (Figure 8.7) fitted mathematically. In this case a second order polynomial function has been fitted around the periphery of the scatter to describe the optimal behaviour of the taxa to changing physical conditions. Furthermore, the functions can be differentiated and the first derivative used to establish the gradient of the curve and the point at which maximum growth occurs. In the case of temperature the maximum abundance of *N. acicularis* occurs at 11.3°C.

Following this method the differing responses of the taxa to their physical environments can be examined. Some taxa have their optimal growth at low temperature and light (e.g. *N. acicularis* and centric diatoms) relative to others which have their optimal growth under higher light and temperature conditions (e.g. *Scenedesmus* spp.). The observed patterns of optimal conditions reflect the periodicity of taxa in the time series (Figure 8.2, 8.3 and 8.4), with the diatoms occurring earlier in the year than most of the green taxa. The scatter plots also suggest that some species are more ubiquitous than others, indicated by the rate of response. For example, *Rhodomonas* has a much shallower response curve to temperature and light than *Actinastrum.*

Oscillatoria redekei and *O. limnetica* species show a wide scatter and this may reflect their infrequent occurrence in the plankton or their lentic origin. Other taxa that show a wide scatter are the picoplankton and unidentified flagellates and this could result from the mixed nature of these groupings.

The influence of discharge will now be considered. Although most taxa exhibit their optimal growth at a distinct flow regime, interpretation has to carried out with the knowledge that the minimum recorded discharge between 1993 and 1997 was 1.4 $m^3 s^{-1}$. Therefore extrapolation of taxa dynamics below this flow rate cannot be undertaken. Nevertheless, those taxa which are most dominant in the spring have a higher discharge maxima than summer taxa. For example, *N. acicularis* were most abundant at a discharge of 6.3 $m^3 s^{-1}$ whereas *N. fruticosa* were most numerous at 3.0 $m^3 s^{-1}$.

The centric diatoms size classes exhibit a differential response to physical variables. Centric diatom ≤ 5µm ∅ and centric diatoms > 6 to ≤ 10µm ∅ have a similar response to discharge, temperature and light, being most abundant at high flow (approx. 7 $m^3 s^{-1}$) and low temperature (approx. 11°C for ≤ 5µm ∅ and 12°C for > 6 to ≤ 10µm ∅) and having a similarly wide light range (approx. 2334 to 5600 watts $m^2 d^{-1}$). The centric diatoms of the size class > 11 to ≤ 20µm ∅, which consist principally of *S. hantzschii* fo. *tenuis*, occurs at a lower discharge (approx. 4.8 $m^3 s^{-1}$), higher temperature (approx. 14.5°C) and narrower light response (approx. 4171 watts $m^2 d^{-1}$) than the other two centric diatom taxa.

Comparison of the centric diatom plots for the full year and first half of the year indicate a greater spread of taxa in the latter part of the year and much of the scatter of low abundance is for the size class > 11 to ≤ 20µm ∅. Centric diatoms ≤ 5µm ∅ can persist in the latter part of the year at an abundance of about 100 cell mL^{-1}, as seen during the summers of 1994 and 1995.

Some of the relationships with discharge indicate an element of 'system flushing', where a small number of samples correlate positively with increasing discharge. An example of flushing can be seen in *Oscillatoria redekei* and *O. limnetica* (Figure 8.6), where autumn counts exceeded 100 cells mL^{-1} at a daily discharge greater than 70 $m^3 s^{-1}$.

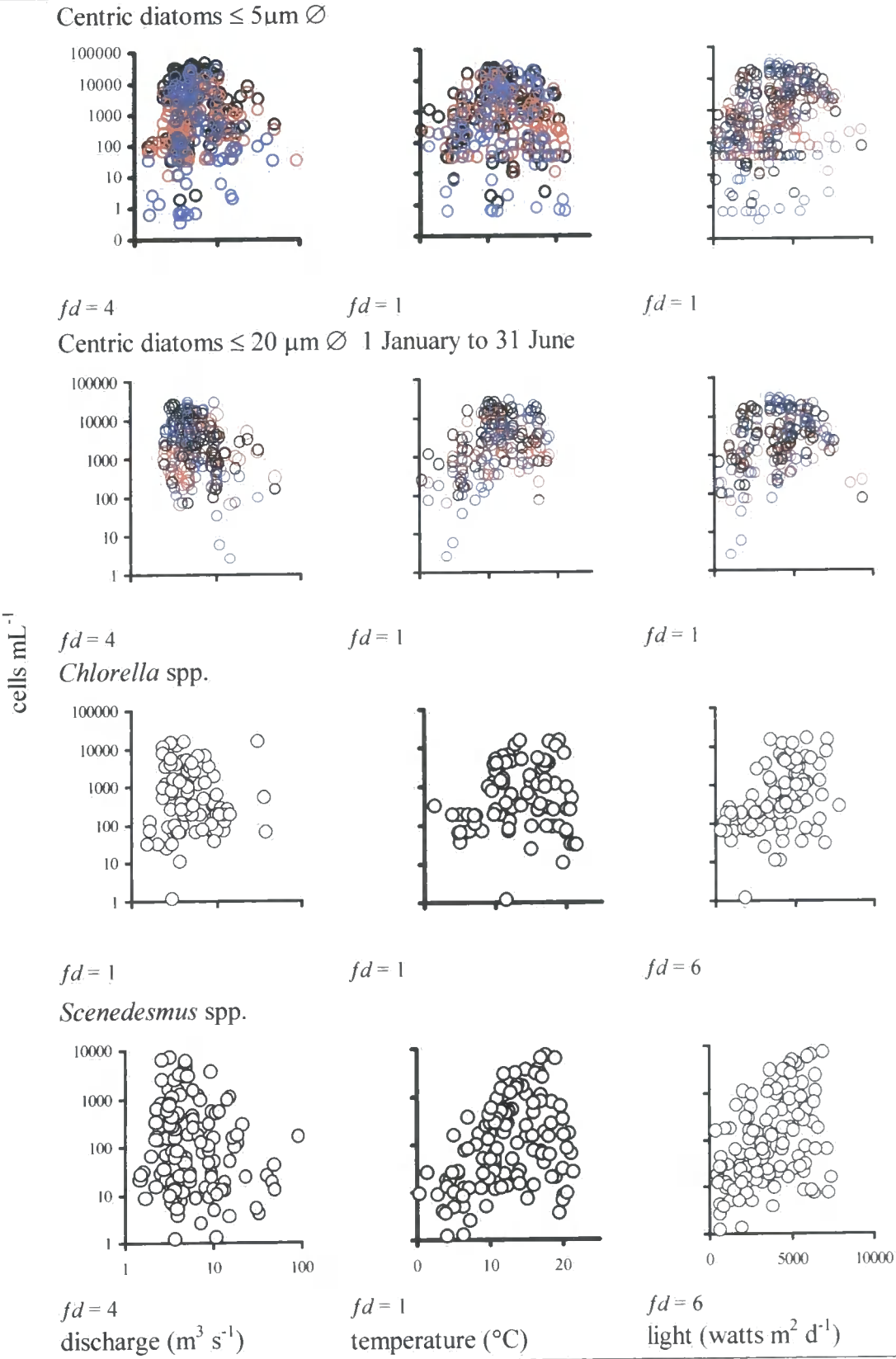


Figure 8.5 Centric diatoms groups, *Chlorella* spp. and *Scenedesmus* spp. plotted against discharge, temperature and light. Centric diatom groups are colour coded: red, ≤ 5µm ∅; black, > 6 to ≤ 10µm ∅ and blue, > 11 to ≤ 20µm ∅ + *S. hantzschii* fo. *tenuis*. *fd* = indicates the number of days over which the data were averaged to produce the minimum of scatter.

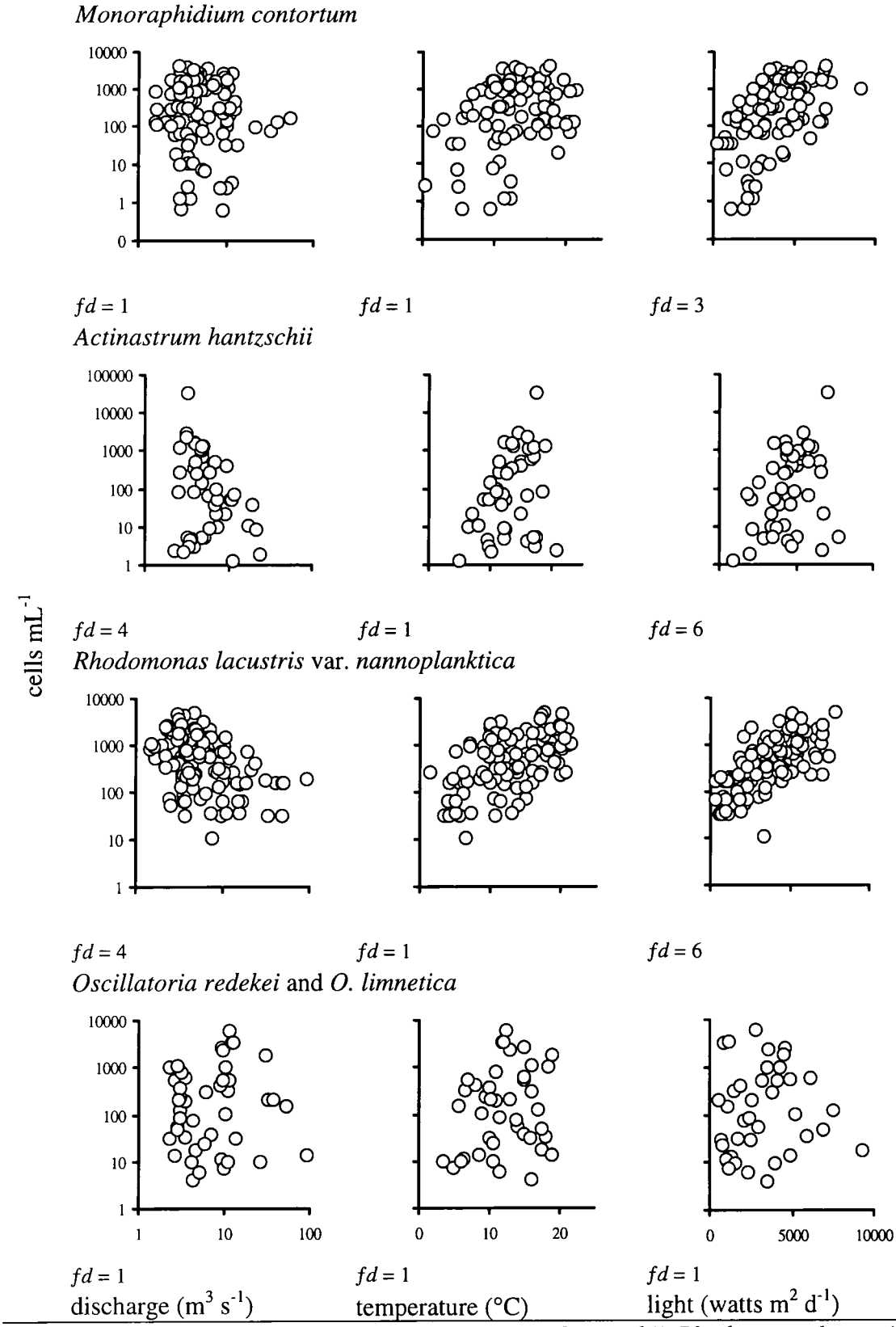


Figure 8.6 *Monoraphidium contortum*, *Actinastrum hantzschii*, *Rhodomonas lacustris* var. *nannoplanktica*, *Oscillatoria redekei* and *O. limnetica* plotted against discharge, temperature and light. *fd* = indicates the number of days over which the data were averaged to produce the minimum of scatter.

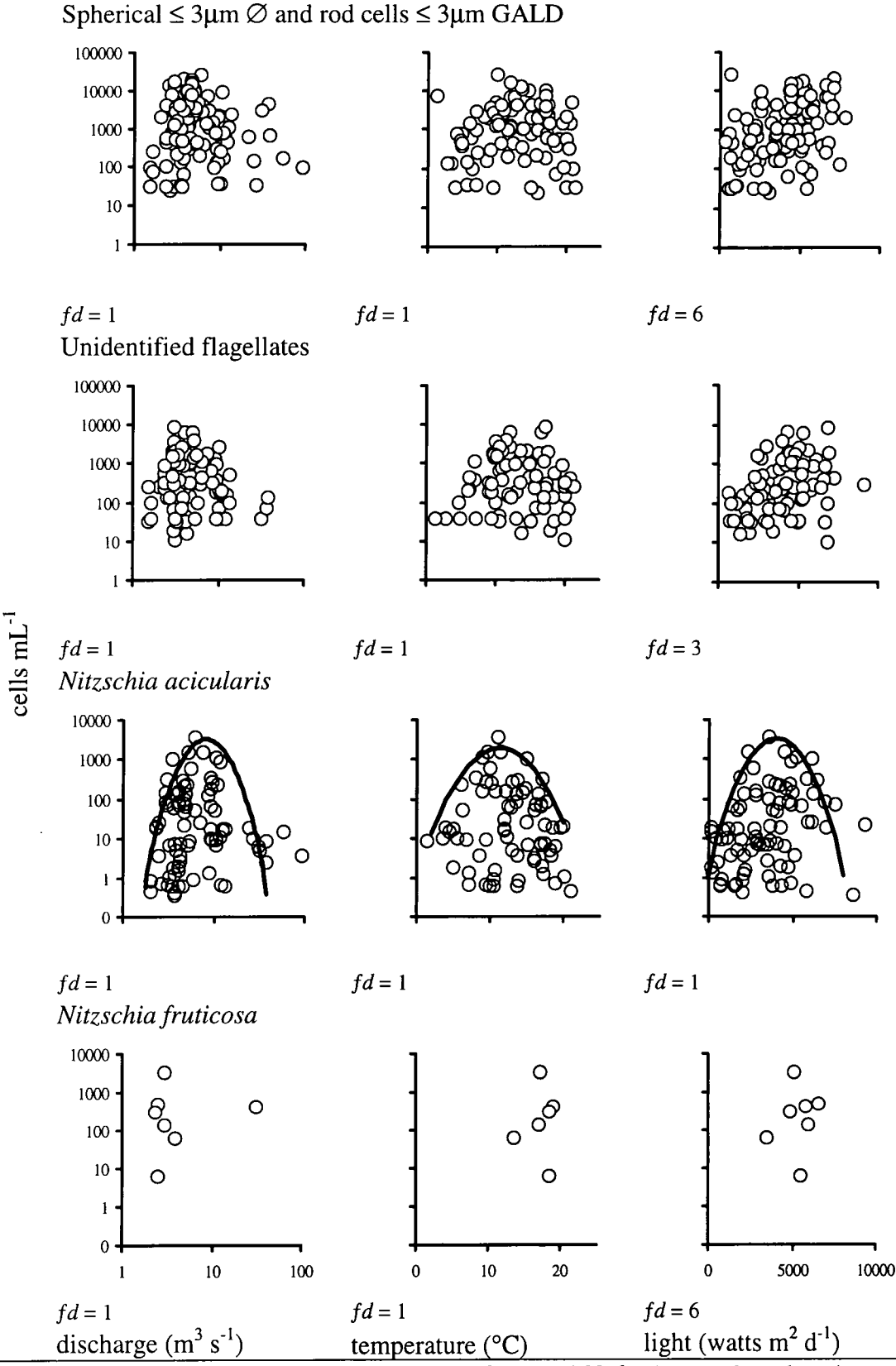


Figure 8.7 Picoplankton, flagellates, *N. acicularis* and *N. fruticosa* plotted against discharge, temperature and light. *fd* = indicates the number of days over which the data were averaged to produce the minimum of scatter. *N. acicularis* plots have fitted polynomial functions that depict the optimal trend (see '*Scatter plots*' in Section 8.4).

Biplots

The results of the CCA analysis are shown in three pairs of Biplots (Figure 8.8, 8.9 and 8.10) and were undertaken for all data and for the 1 January to 30 June and 1 July to 31 December subsets. The results of the initial stepwise analyses, to evaluate the significance of environmental variables, are shown in Table 8.4. The same variables were used for the full year and 1 January to 30 June analyses, with averaged light and discharge being preferentially selected over daily values, and N and P were non-significant. The analysis of the 1 July to 31 December data resulted in the selection of two variables, light and flow, with averaged data again being selected in preference to daily values.

Table 8.4 Evaluation of the significance of environmental variables for inclusion in the CCA models. Only those variable with a p value < 0.05 were included in the final analyses. Labels in brackets are identifiers used in the biplots (Figure 8.8, 8.9 and 8.10).

Variable	All data	1 Jan. to 30 Jun.	1 Jul. to 31 Dec.
Daily discharge ($m^3 s^{-1}$)	NS	NS	NS
4 day average discharge ($m^3 s^{-1}$) (Flo04)	0.005	0.005	0.005
Daily temperature (°C) (Tmp)	0.005	0.005	NS
Daily light (watts $m^2 d^{-1}$)	NS	NS	NS
6 day average light (watts $m^2 d^{-1}$) (Lgt06)	0.005	0.005	0.005
SiO_2-Si (mg L^{-1}) (SiO2)	0.005	0.005	NS
FRP (mg L^{-1})	NS	NS	NS
NH_4-N (mg L^{-1})	NS	NS	NS
NO_3-N (mg L^{-1})	NS	NS	NS

Each pair of biplots show the first two ordination axes along with environmental variables for the taxa groups and samples. The interpretation of the CCA biplots is not intuitive so a brief explanation is included. CCA is a combination of ordination and multiple regression, with taxa or samples indicated by points and environmental variables by arrows. The taxa points indicate the relative location of the two-dimensional niche of that taxa in the ordination diagram. The arrows for the environmental variables run from the origin of the diagram, and the arrow's coordinates are the correlation of the variable with the axes. The strength of the environmental correlations are indicated by the arrow's length (ter Braak and Verdonschot, 1995).

For example, in Figure 8.8 *Nitzschia lanceolata* (N. lan.) correlates positively with flow (ca. +1.0) and negatively with temperature and light (ca. -1.0), whereas *Nitzschia fruticosa* (N. fru.) exhibits the opposite trend. In summary, the pairs of

biplots indicate the influence of environmental variables on the taxa groups and under what conditions they prevail. The relative positioning of the samples, to the taxa groups and environmental variables, completes the interpretation. The large number of samples prohibited individual labelling and the approximate timing of the samples has been generally identified on the plots.

The results support the assumptions made from the time series and scatter-plots and adds further information not available in the aforementioned assessments. The analysis using all of the data and the 1 January to 30 June period are mostly similar. Spring samples correlate positively with high discharge and negatively with light and temperature, whereas samples from early summer have the opposite response (Figures 8.8 and 8.9).

A gradient of spring taxa correlating with discharge is evident. At high discharge, benthic diatoms such as *Navicula lanceolata* (N. lan.) and the colonial-planktonic pennate diatoms *Asterionella formosa* and *Diatoma elongatum* (A.f & D.e.) are most abundant. A lower discharge tends to favour the centric diatoms (CD), *Nitzschia acicularis* (N. aci.) and *Koliella* (Kol.). The larger centric diatoms (CD 10-20) correlate negatively with the silicate concentration more so than the other centric groups.

Summer samples correlate positively with increasing temperature and light and negatively with increasing discharge. This period favours the greens *Dictyospherium* (Dict.) and *Actinastrum* (Act.) and most positively the colonial pennate diatom *Nitzschia fruticosa*. Other green algae, like *Scenedesmus* (Scen.) and *Chlorella* (Chlor.) appear to be less influenced by light than temperature and occur at a lower temperature than *Actinastrum* and *Dictyosphaerium*.

Dissimilarities between the results for all data and 1 January to 30 June period are also indicative of temporal change in the Nene. For example, those taxa classed as 'benthic' (benth.) are located differently within the biplots, for the two periods. The analysis for all data indicates an association between the 'benthic' forms and the summer, but in the 1 January to 30 June analysis (when this period is unavailable) benthic diatoms occur at high discharge and their presence is presumably the result of scouring. Likewise, the occurrence of *Rhodomonas* (Rhod.) under differing physical conditions is indicative of its ubiquitous occurrence.

The analysis for the 1 July to 30 December period supports much of the above (Figure 8.10). However, the reduced number of significant physical variables, and the

short arrows produced, indicates that much of the variability is due to factors other than those considered here, as concluded previously.

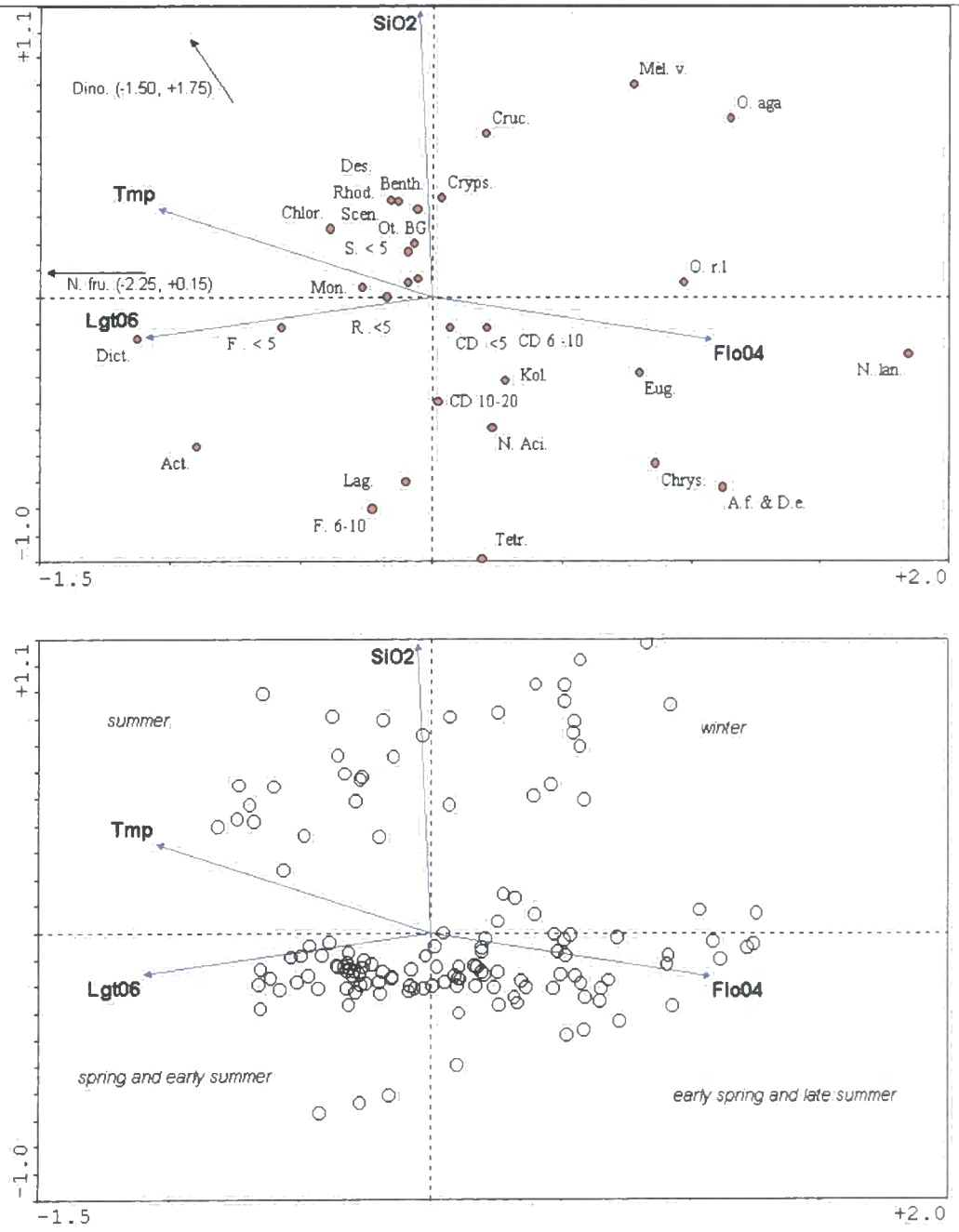


Figure 8.8 Biplots of the CCA analysis for all data. Environmental and taxa data above and environmental and sample data below. The cumulative percentage of variance explained for taxa-environment relationship is 48% and 70% for axes one and two respectively. The taxonomic groups Dinoflagellates and *Nitzschia fruticosa* were outliers and were removed in the final analysis (relative position indicated by black arrows). Seasonal distribution of samples shown in italics. Abbreviations for taxa and environmental variables are shown in Table 8.3 and Table 8.4.

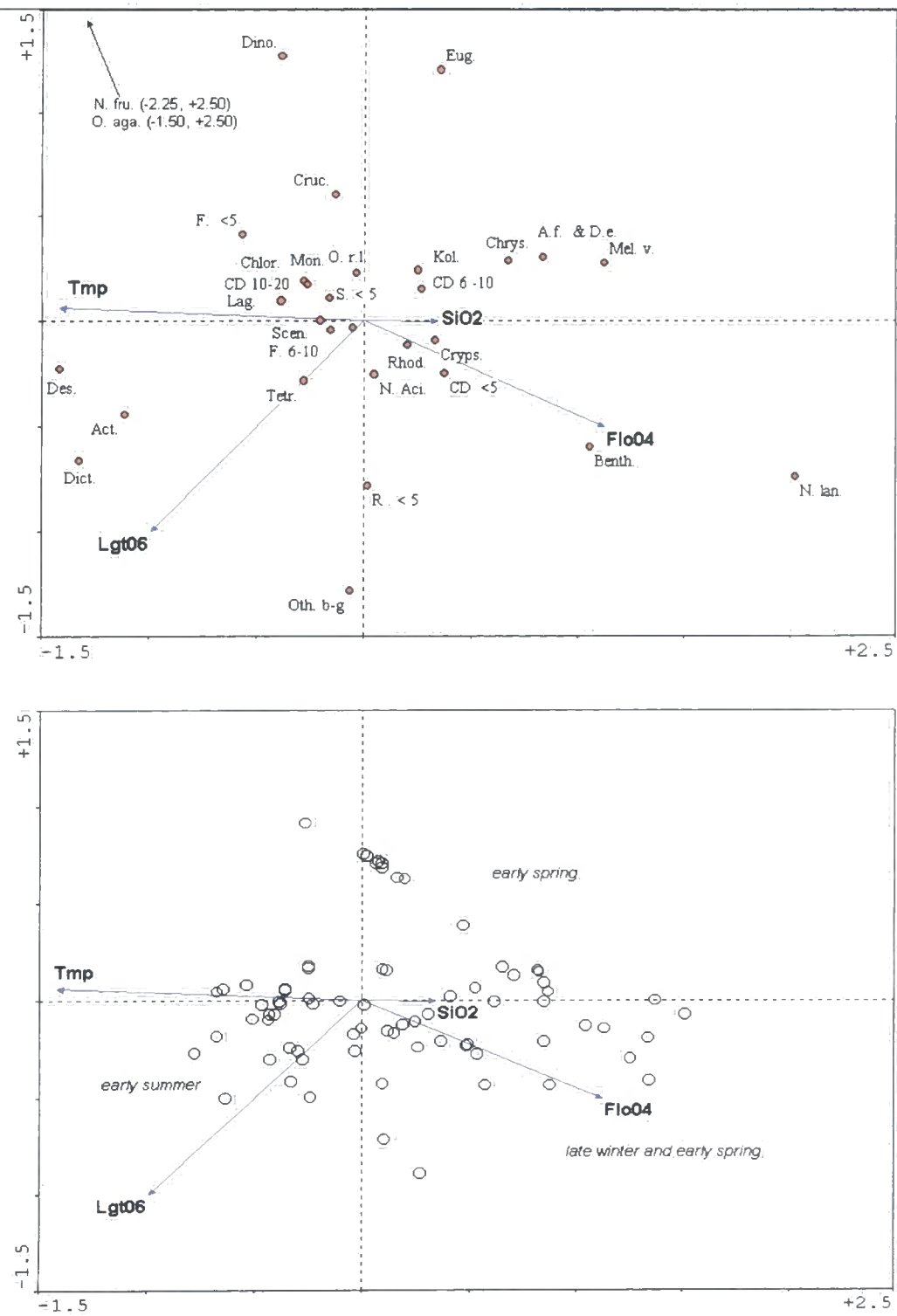


Figure 8.9 Biplots of the CCA analysis for 1 January to 30 June data. Environmental and taxa data above and environmental and sample data below. The cumulative percentage of variance explained for taxa-environment relationship is 53% and 72% for axes one and two respectively. The taxonomic groups *O. agardhii* and *Nitzschia fruticosa* were outliers and were removed in the final analysis (relative position indicated by black arrow). Seasonal distribution of samples shown in italics. Abbreviations for taxa and environmental variables are shown in Table 8.3 and Table 8.4.

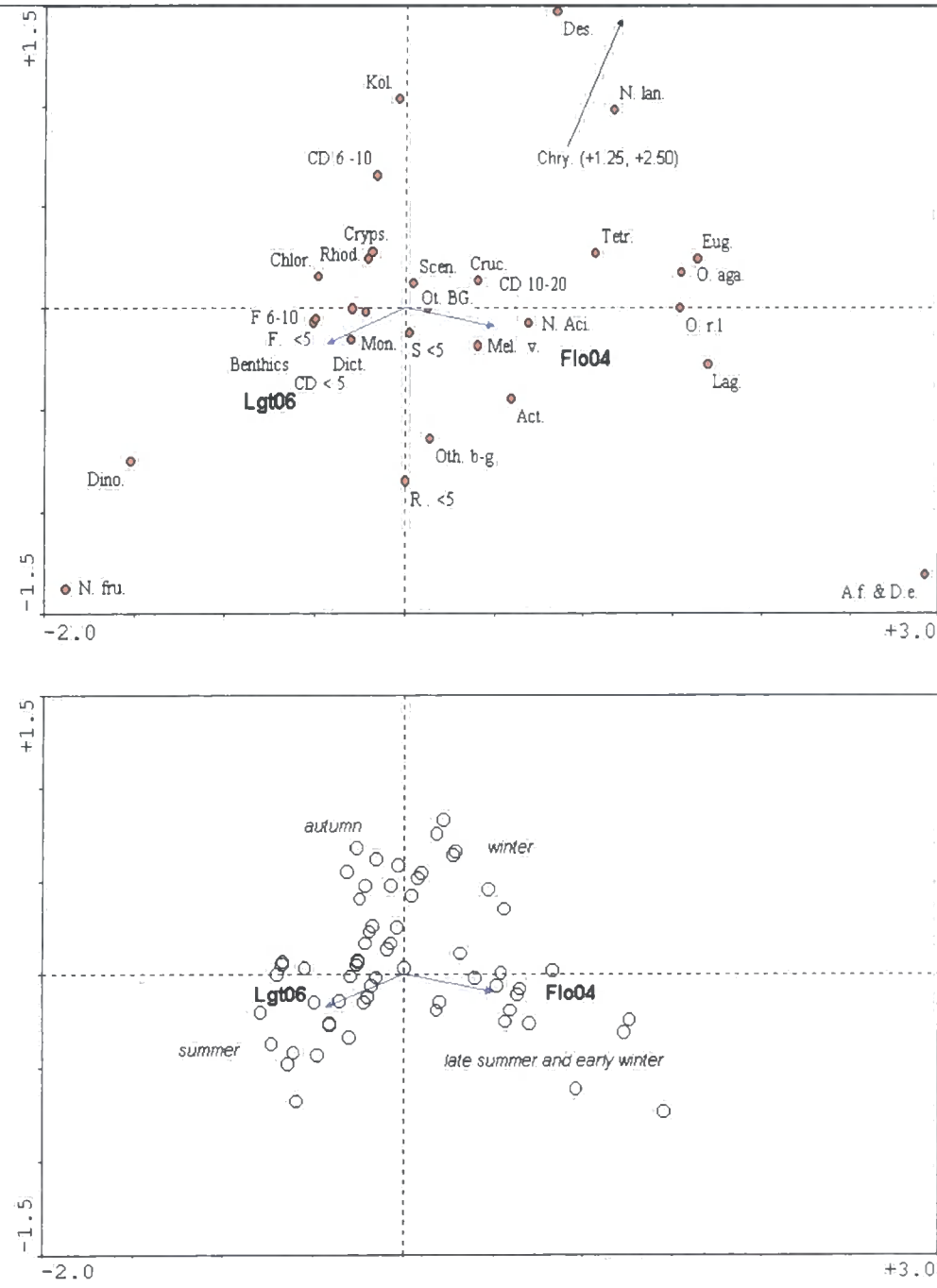


Figure 8.10 Biplots of the CCA analysis for 1 July to 31 December data. Environmental and taxa data above and environmental and sample data below. The cumulative percentage of variance explained for taxa-environment relationship is 85% and 100% for axes one and two respectively. The taxonomic group Chrysophyceae was an outliers and was removed in the final analysis (relative position indicated by black arrows). Seasonal distribution of samples shown in italics. Abbreviations for taxa and environmental variables are shown in Table 8.3 and Table 8.4.

Silica

The silicate concentration is reduced to a low level during periods of diatom abundance and this is reflected in all three centric diatom groups and *Nitzschia acicularis* (Figure 8.11). Swale (1964) calculated the silica requirements for *Stephanodiscus hantzschii* in culture and these calculations allow the silica requirements for further increases in the centric diatoms populations to be estimated. At the lowest silicate concentration (0.2 mg L^{-1}) the larger centric diatoms range from 1097 to 16710 cells mL^{-1}. These concentrations have a silica requirement of 0.04 and 0.64 mg L^{-1} respectively for a further cell division.

The greatest and second greatest abundance of centric diatoms (24888 cells mL^{-1} on 1 April 1997 and 23174 cells mL^{-1} on 8 April 1997 respectively) occurred in the intermediate size class, at respective SiO_2-Si concentrations of 0.9 and 0.5 mg L^{-1}. The population size at the start of this period had a silica requirement of approximately 0.96 mg L^{-1} to facilitate a further division. There was enough silica present for most of the population to complete a further division, although by the end of the period the SiO_2-Si concentration was significantly reduced without a concurrent increase in diatom abundance. It appears there was enough silica to maintain the population (against loss factors) but not enough to facilitate further enlargement. It is therefore likely that the magnitude of centric diatom populations was limited by the availability of silica.

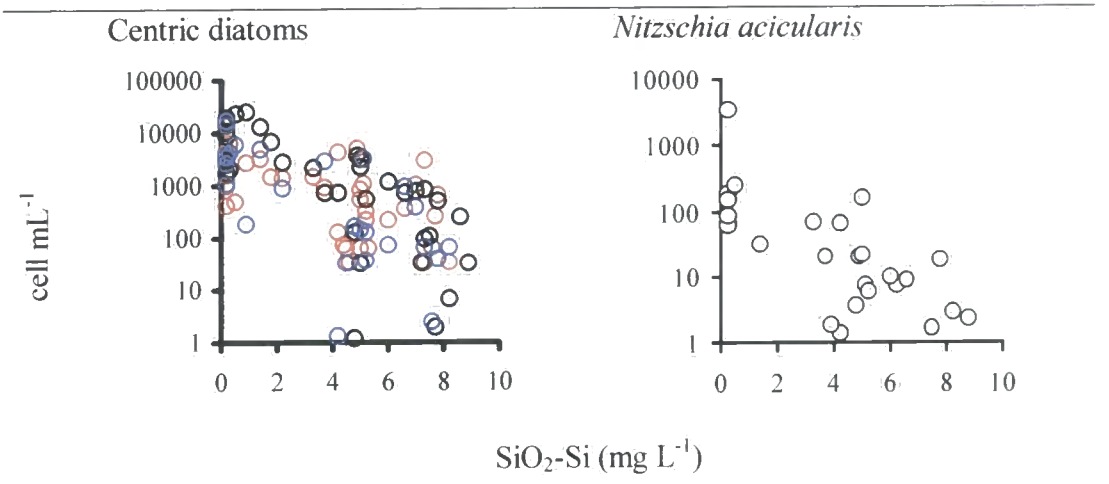


Figure 8.11 Relationship between centric diatoms and *Nitzschia acicularis* and SiO_2-Si concentration. Centric diatom groups: red, ≤ 5µm ∅; black, > 6 to ≤ 10µm ∅ and blue, > 11 to ≤ 20µm ∅ + *S. hantzschii* fo. *tenuis*.

8.5 Short-term trends

Four surveys of short-term temporal change in chlorophyll and phytoplankton were undertaken. A 24-h pilot study during April 1996 and three more extensive studies were carried out during early and mid April 1997 and early May 1997. Throughout the 1997 surveys discharge, light and temperature were recorded at 30-min, 10-min and 15-min intervals respectively and chlorophyll and phytoplankton at varying intervals between 1 and 4 h.

Temporal change in chlorophyll and phytoplankton were investigated using regression analysis, with discharge, temperature and light as predictors. Temperature and discharge were averaged over varying periods preceding the sample and light was integrated in the same way.

24-h survey April 1996

During the 24-h sampling period, trends in temperature and light varied predictably, with an evident lag in temperature (Figure 8.12). Chlorophyll does exhibit a temporal trend but no obvious relationship with depth. The diatom data also has a temporal trend and two of the samples have a significant difference for depth (Mann Whitney U tests: 14:00, $p = 0.010$; 18:00 $p = 0.013$), with a significantly greater abundance occurring at the surface. Chlorophyll and cell numbers correlate positively with temperature, with surface concentrations of diatoms producing the strongest relationships and surface chlorophyll concentrations the poorest. The survey was undertaken during a period of gradually declining flow (5/4/96 = 6.06 $m^3 s^{-1}$; 4/4/96 = 5.96 $m^3 s^{-1}$) and the SiO_2-Si concentration was 1.2 mg L^{-1} on the 3/4/96.

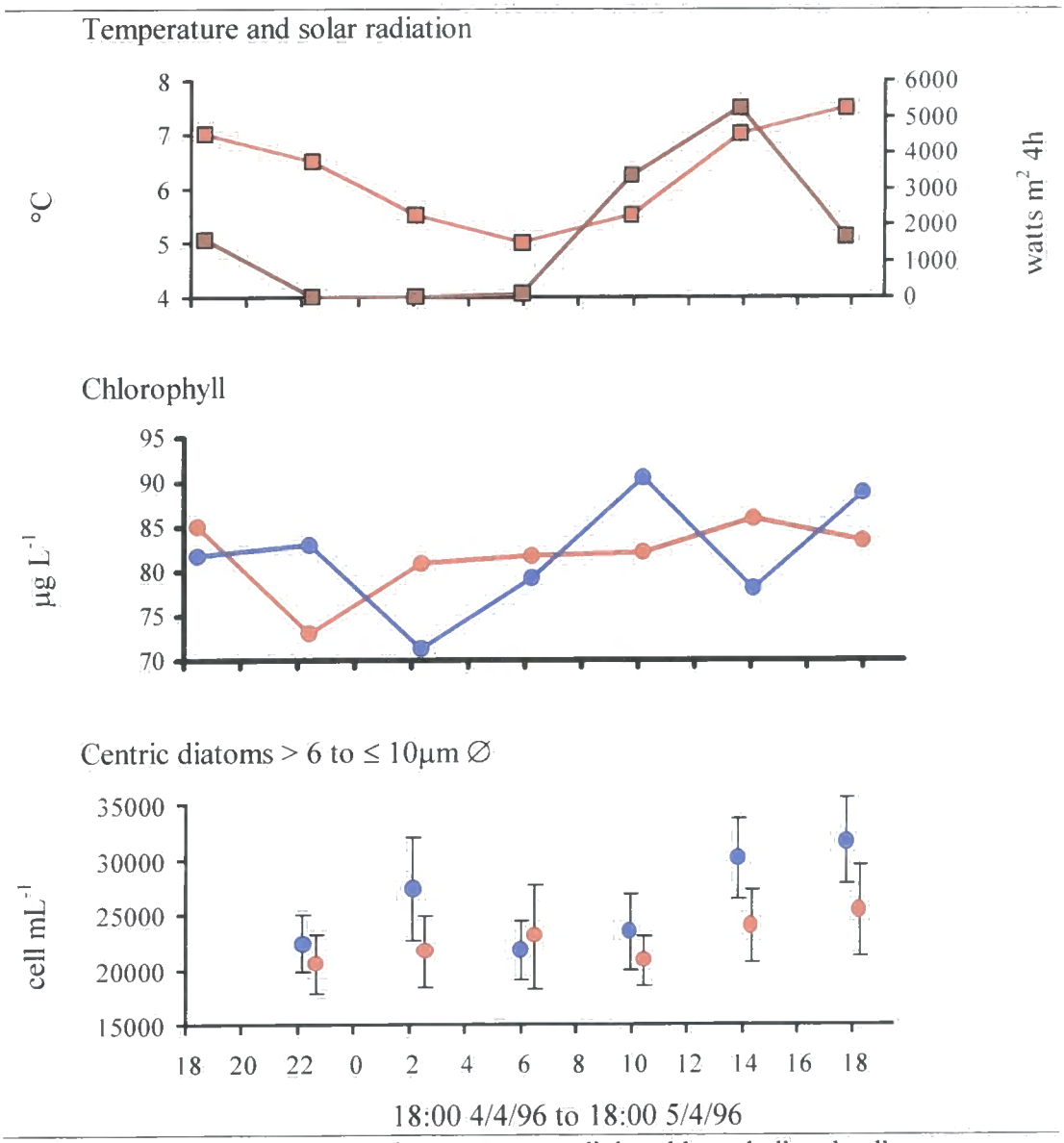


Figure 8.12 Temporal change in temperature, light, chlorophyll and cell count at km 91.7 over a 24-h period at two depths. Upper: spot temperature (red) and 4-h integrated light (dark red). Middle: chlorophyll. Lower: Centric diatoms > 6 to ≤ 10µm ∅. Counted using 75 SF, CI fitted using student t. Blue = 0 m and red = 1 m. Measurements and samples were taken every 4-h between 4/4/96 18:00 and 5/4/96 18:00. Phytoplankton samples have been offset by 15 min for clarity.

96-h survey early-April 1997

The results of the 96-h survey are shown in Figures 8.13 and 8.14. Discharge was relatively consistent throughout the sample period, but temperature and light varied considerably.

Chlorophyll correlated significantly with increasing temperature (4 h; $r^2 = 0.23$; $p < 0.05$; n = 25). Centric diatoms > 6 to ≤ 10µm ∅ correlated most significantly with discharge and temperature (2 h; $r^2 = 0.54$; $p < 0.05$; n = 25) and their abundance increased significantly through most days. With few exceptions, 4-h differences were

insignificant and most significant change occurred over several sample intervals. Cell counts ranged from 16192 to 30023 and averaged 24569 cell mL^{-1}.

Centric diatoms > 10 to ≤ 20 μm ∅ had a similar pattern of occurrence as the smaller diatoms, with cell counts generally increasing throughout the survey period. Although the larger diatoms were much less abundant and correlated significantly with light (24 h; r^2 values = 0.15; $p < 0.05$; n = 27). These centric diatoms did not exhibit as much diurnal variation in abundance as the smaller taxa and had greatest variation during the warmer period, where a significant increase is evident.

Nitzschia acicularis counts correlated most significantly with discharge and light (24 h; $r^2 = 0.34$; n = 27). These diatoms were most abundant early in the survey showing a general decline with time. *Koliella longiseta* numbers were relatively consistent throughout most of the survey with decline latterly. This taxa did not correlate significantly with any of the variable tested but responded most significantly to changes in temperature.

The 96-h period was put into longer-term perspective by comparing daily chlorophyll and phytoplankton values (08:30) with those occurring several days before and after the survey (Figures 8.15 and 8.16). The physical variables used for this evaluation were temperature, daily and 4-day average discharge and daily, 3 and 6-d average light. This overview indicates that the survey was carried out during a period of generally declining discharge and increasing temperature with variable solar radiation.

The chlorophyll data indicate a trend of general increase and correlated most significantly with discharge (4 d discharge; $r^2 = 0.91$; $p < 0.05$; n = 7). Similarly, the centric diatoms exhibited an increase in abundance over time, with the smaller taxa responding to temperature and flow and the larger taxa with flow alone (Centric diatoms > 6 to ≤ 10 μm ∅: 4 d discharge; $r^2 = 0.96$; $p < 0.05$; n = 7. Centric diatom > 10 to ≤ 20 μm ∅: 4 d discharge; $r^2 = 0.90$; $p < 0.05$; n = 7).

Nitzschia acicularis and *Koliella longiseta* responded most significantly to temperature and light respectively, but the results were not statistically significant.

SiO_2-Si was not monitored throughout the survey, but the concentrations were 1.8, 0.9 and 0.5 mg L^{-1} on the 26 May, 1 April and 8 April (08:30) respectively.

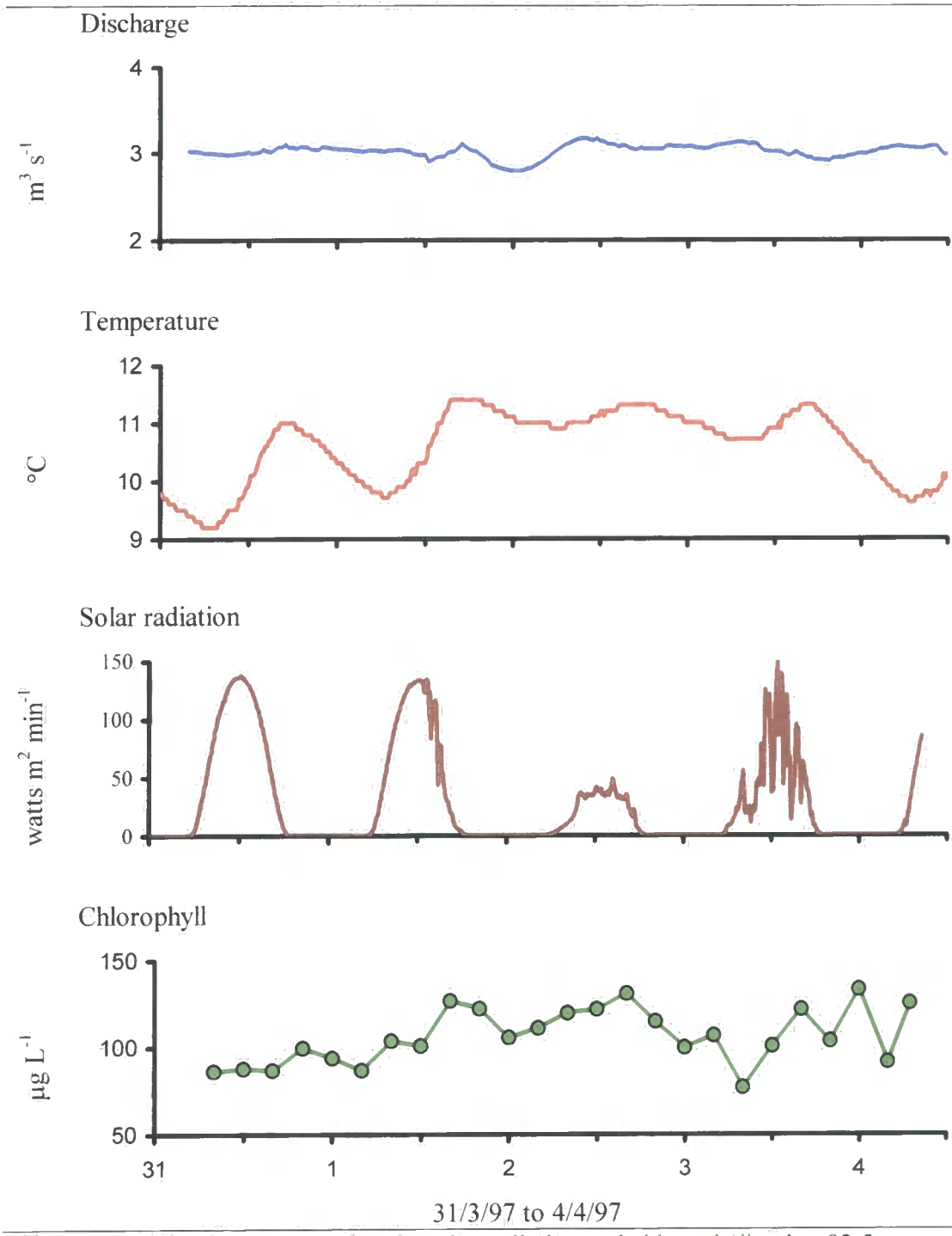


Figure 8.13 Discharge, temperature, solar radiation and chlorophyll at km 92.5 (31/3/97 to 4/4/87). Discharge and temperature are based on 30-min readings, light on 5-min readings and chlorophyll was sampled every 4 h.

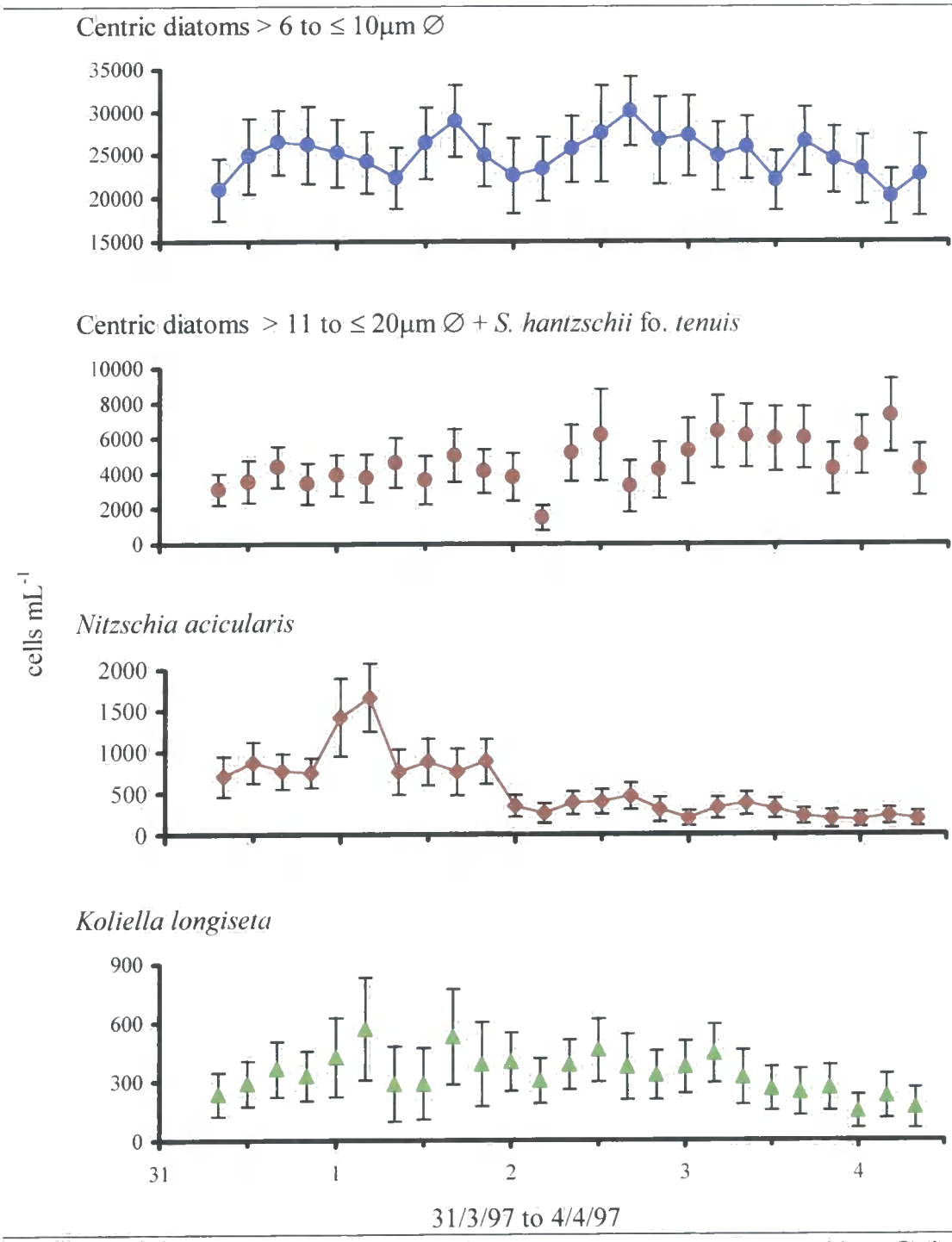


Figure 8.14 Centric diatoms > 6 to ≤ 10µm ∅, Centric diatoms > 11 to ≤ 20µm ∅ (inc. *S. hantzschii* fo. *tenuis*), *Nitzschia acicularis* and *Koliella longiseta* at km 92.5 (31/3/97 to 4/4/87). Samples were collected every 4 h.

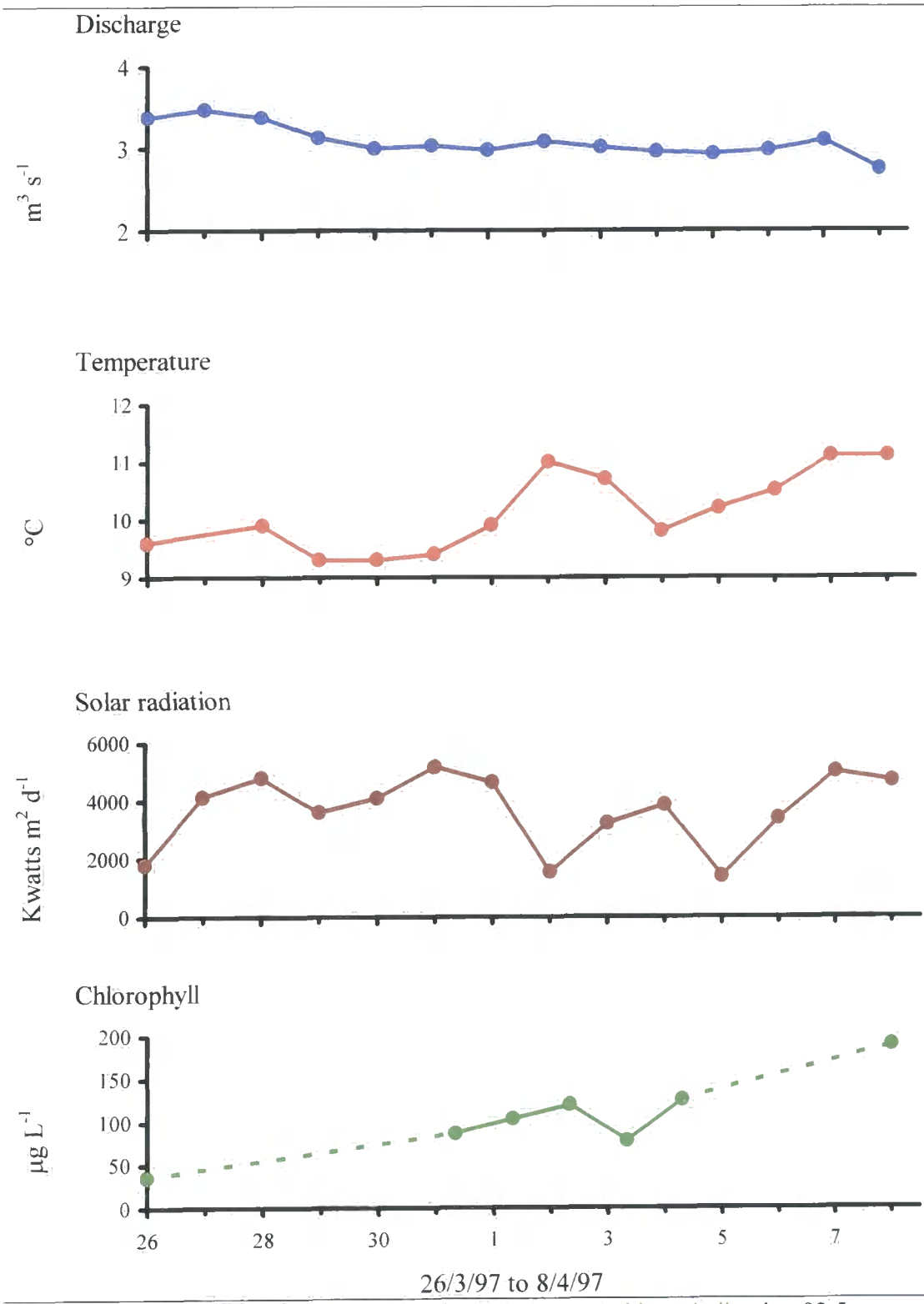


Figure 8.15 Discharge, temperature, solar radiation and chlorophyll at km 92.5 (26/3/97 to 8/4/87). Discharge is a daily average, light a daily integral and temperature and chlorophyll values for 08:30.

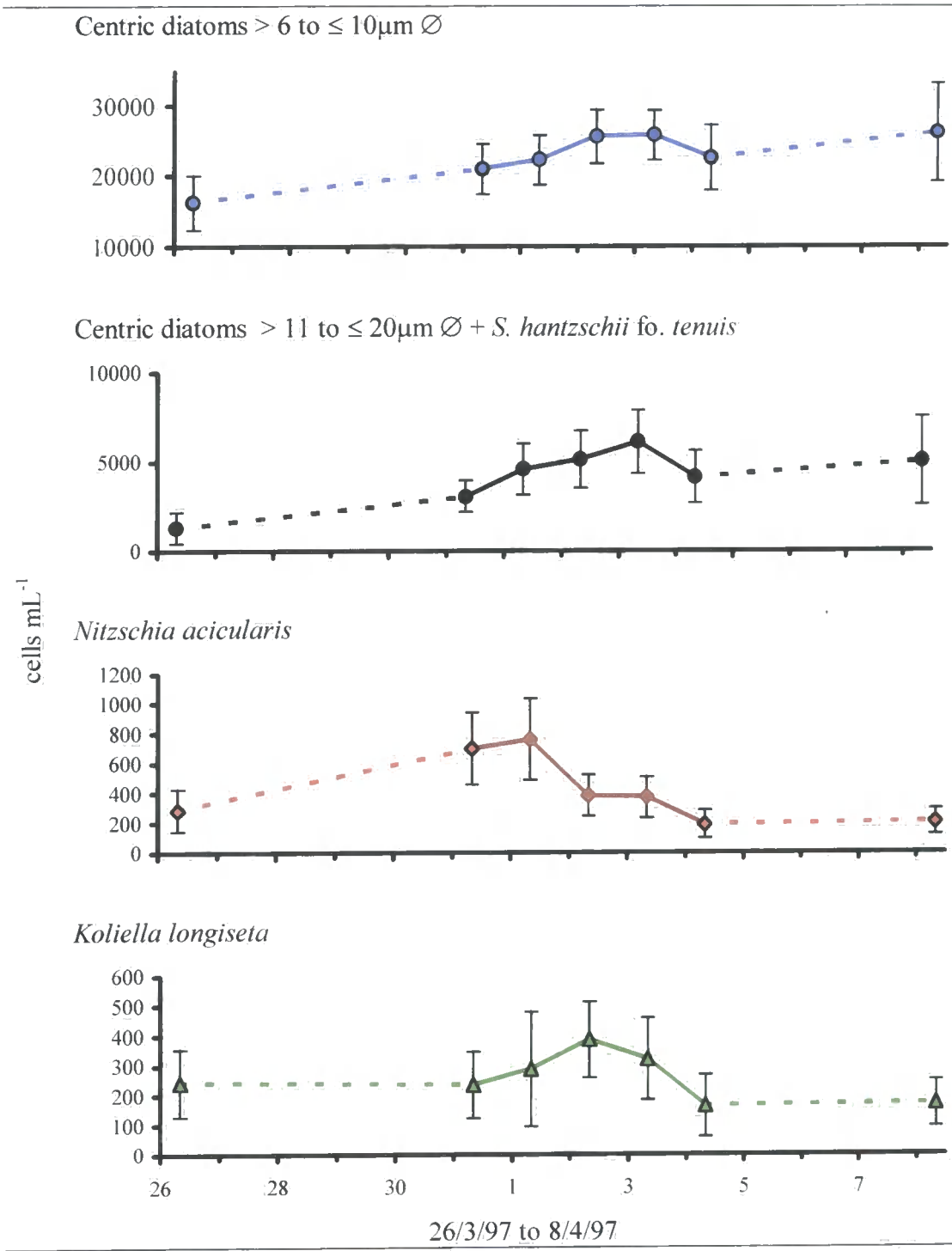


Figure 8.16 Centric diatoms > 6 to ≤ 10µm ∅, Centric diatoms > 11 to ≤ 20µm ∅ (inc. *S. hantzschii* fo. *tenuis*), *Nitzschia acicularis* and *Koliella longiseta* at km 92.5 (26/3/97 to 8/4/87). Samples were collected at 08:30.

96-h survey mid-April 1997

The results of the mid-April survey are shown in Figures 8.17 and 8.18. Compared to the previous survey this one was undertaken during a period of lower, but more variable, discharge and higher temperature. Chemical determinands were also measured at 4-h intervals for the first 48 h of the survey (SiO_2-Si, NH_4-N, TON, TRP and pH).

During this survey the abundance of centric diatoms > 6 to ≤ 10 μm ∅ were significantly lower than during early April, but the numbers of larger centric diatoms were similar to the previous survey. The SiO_2-Si concentration was below the detectable limits (< 0.1 mg L^{-1}) through much of the survey, although it increased overnight, 14/15 April, and following increased discharge on the 16 April. The combined abundance of centric diatoms during the 14 and 15 April was approximately 11500 cell mL^{-1} and would require a silica concentration of 0.4 ml L^{-1} to facilitate a further division of the population (Swale, 1963). As the silica concentration at this time was < 0.1 mg L^{-1} it is likely that diatom abundance was silica limited, an assumption which is supported by increases in diatom numbers occurring when the SiO2-Si concentration increased to 0.5 mg L^{-1}.

The abundance of the green alga *Monoraphidium contortum* was similar to the early-April survey. *M. contortum* did not exhibit any marked increases in abundance throughout the survey, perhaps indicating the diatom increases were due to increased silica rather than some other variable which would have also been reflected in the abundance of this species.

During the first 48 h of the survey TRP and TON concentrations were 1.3 mg L^{-1} and 8.9 mg L^{-1} respectively. Ammonium was reduced to less than 0.03 mg L^{-1} and pH varied from 8.5 to 7.6 concurrently with changes in chlorophyll, being most alkaline during periods of greatest chlorophyll concentration.

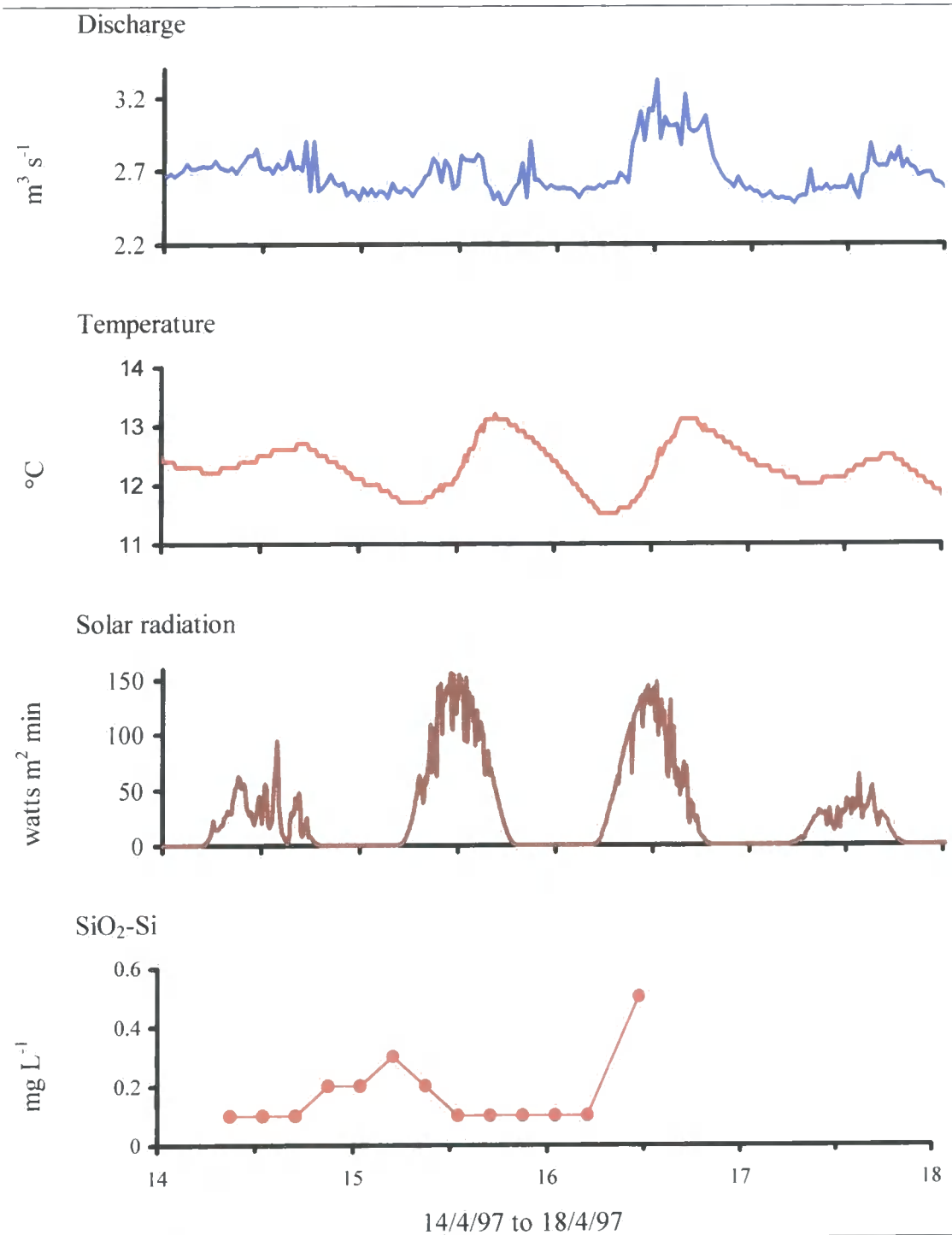


Figure 8.17 Discharge, temperature, solar radiation and silica at km 92.5 (14/4/07 to 18/4/97). Discharge and temperature are based on 30 min readings, light on 5-min readings and silica was sampled every 4 h (for the restricted period shown and lowest values are below threshold).

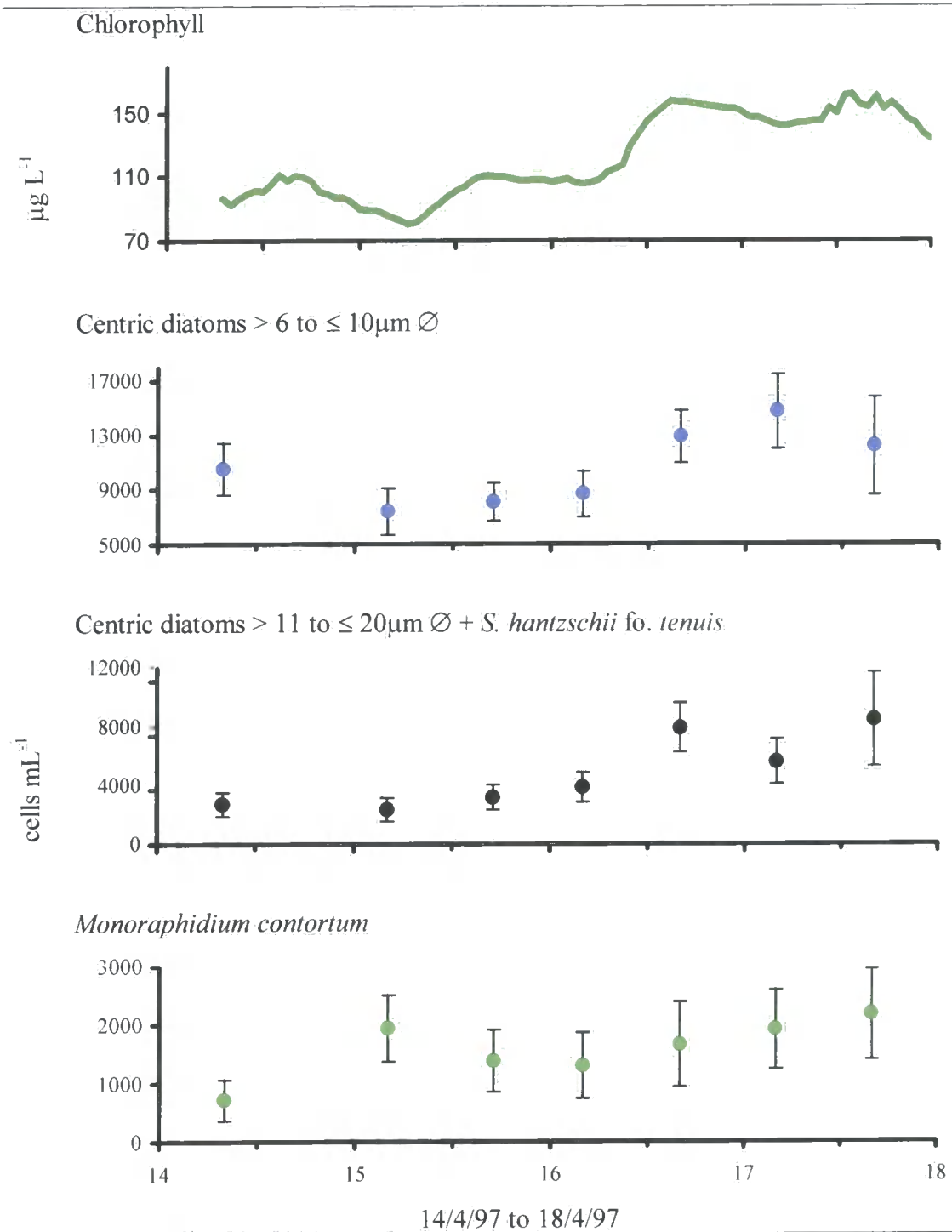


Figure 8.18 Chlorophyll, Centric diatoms > 6 to ≤ 10µm ∅, Centric diatom > 11 to ≤ 20µm ∅ and *S. hantzschii* fo. *tenuis* and *Monoraphidium contortum* at km 92.5 (14/4/97 to 18/4/87). Samples were taken every h and phytoplankton samples were selected to reflect changes in chlorophyll concentration.

96-h survey early-May 1997

The short-term survey of mid-May 1997 covered a period of considerable physical diversity. During this survey temperature decreased over four degrees, discharge more than doubled and there was a large pulse mid-way through the period (Figure 8.19).

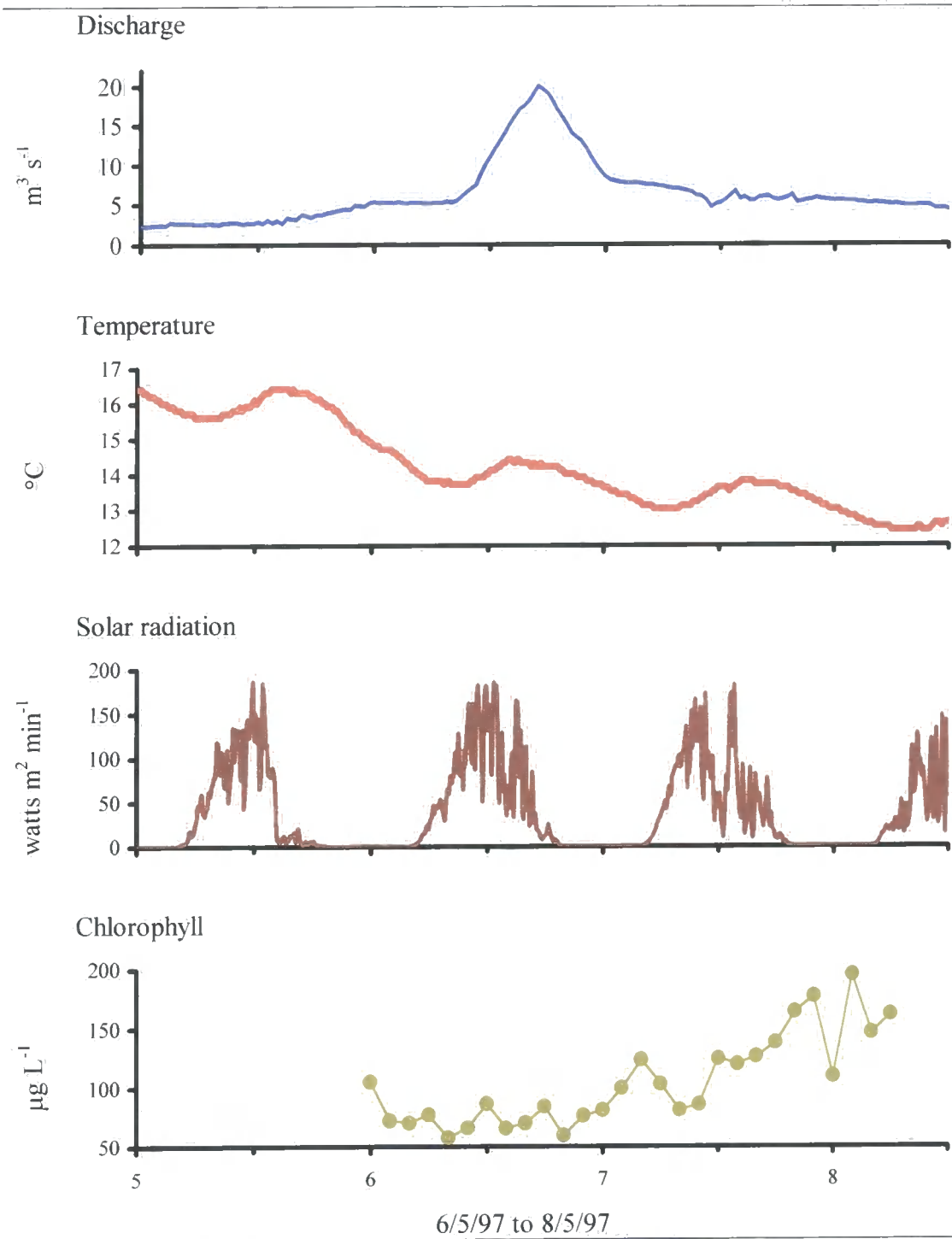


Figure 8.19 Discharge, temperature, solar radiation and chlorophyll at km 92.5 (6/5/97 to 8/5/87). Discharge and temperature are based on 30 min readings, light on 5 min readings and chlorophyll was sampled every 2 h.

This period of change coincided with an increase in chlorophyll, which correlated significantly with decreasing temperature ($r^2 = 0.37$; $p < 0.05$; n = 28). The abundance of four taxa from the former and latter part of this period were estimated and a

significant increase in centric diatoms established (Figure 8.20). The green algae *Scenedesmus acuminatus* and *S. communis* decreased in abundance, and in cells per unit, through the period of physical change but not significantly so.

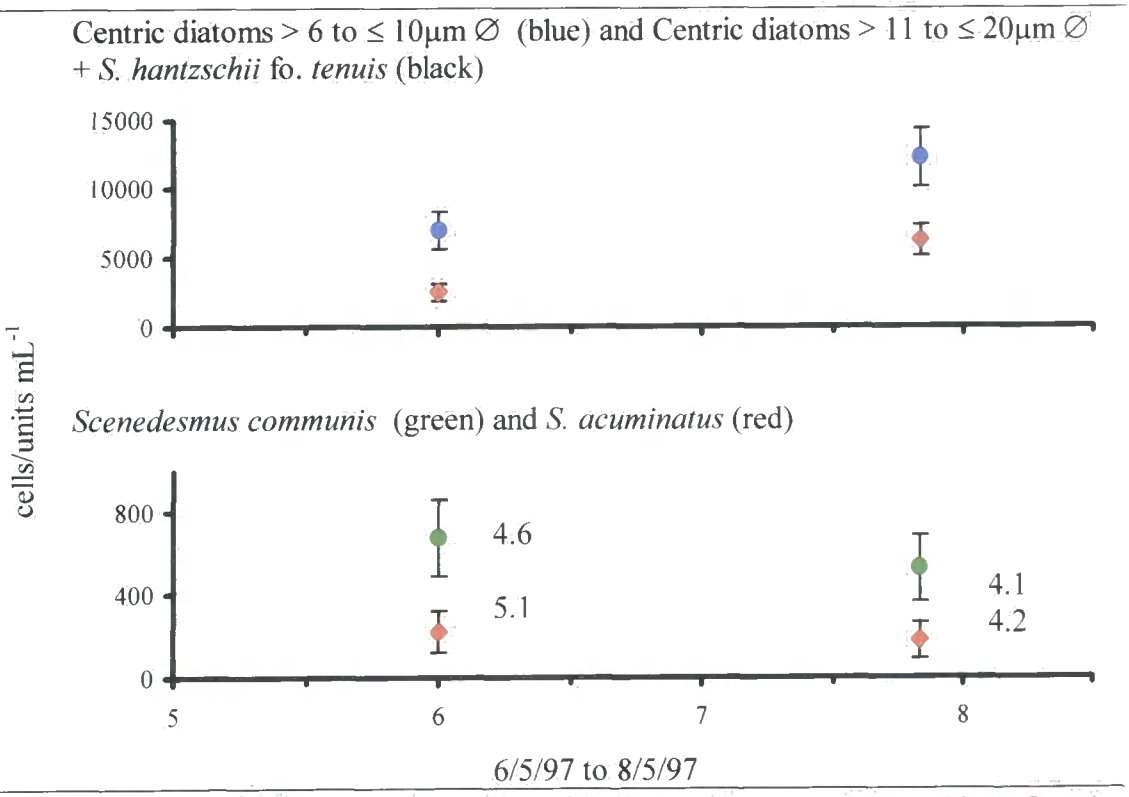


Figure 8.20 Centric diatoms > 6 to ≤ 10µm ∅, Centric diatoms > 11 to ≤ 20µm ∅ and *S. hantzschii* fo. *tenuis*, *Scenedesmus communis* and *S. acuminatus* at km 92.5 (6/5/97 to 8/5/87). Samples counted 6/5/97 00:00 and 7/5/97 20:00 and were chosen to reflect change. Numbers in lower plot are average cells per unit.

8.6 1994 flood event

A flood event that occurred during the September 1994 provided an opportunity to investigate the influence of increasing discharge on phytoplankton composition (Figure 8.21). The summer of 1994 (1 July to 13 September), preceding the September spate, had an extended period of low chlorophyll (average = 3.6 µg L^{-1}, maximum = 6.3 L^{-1}) and a relatively consistent discharge (average = 3.5 m^3 s^{-1}, maximum = 4.8 m^3 s^{-1}). Very heavy precipitation on the 14/9/94 (daily rainfall = 73 mm, Wittering) resulted in a three fold increase in discharge over the subsequent three days (maximum daily discharge = 38 m^3 s^{-1}, on 16/9/94).

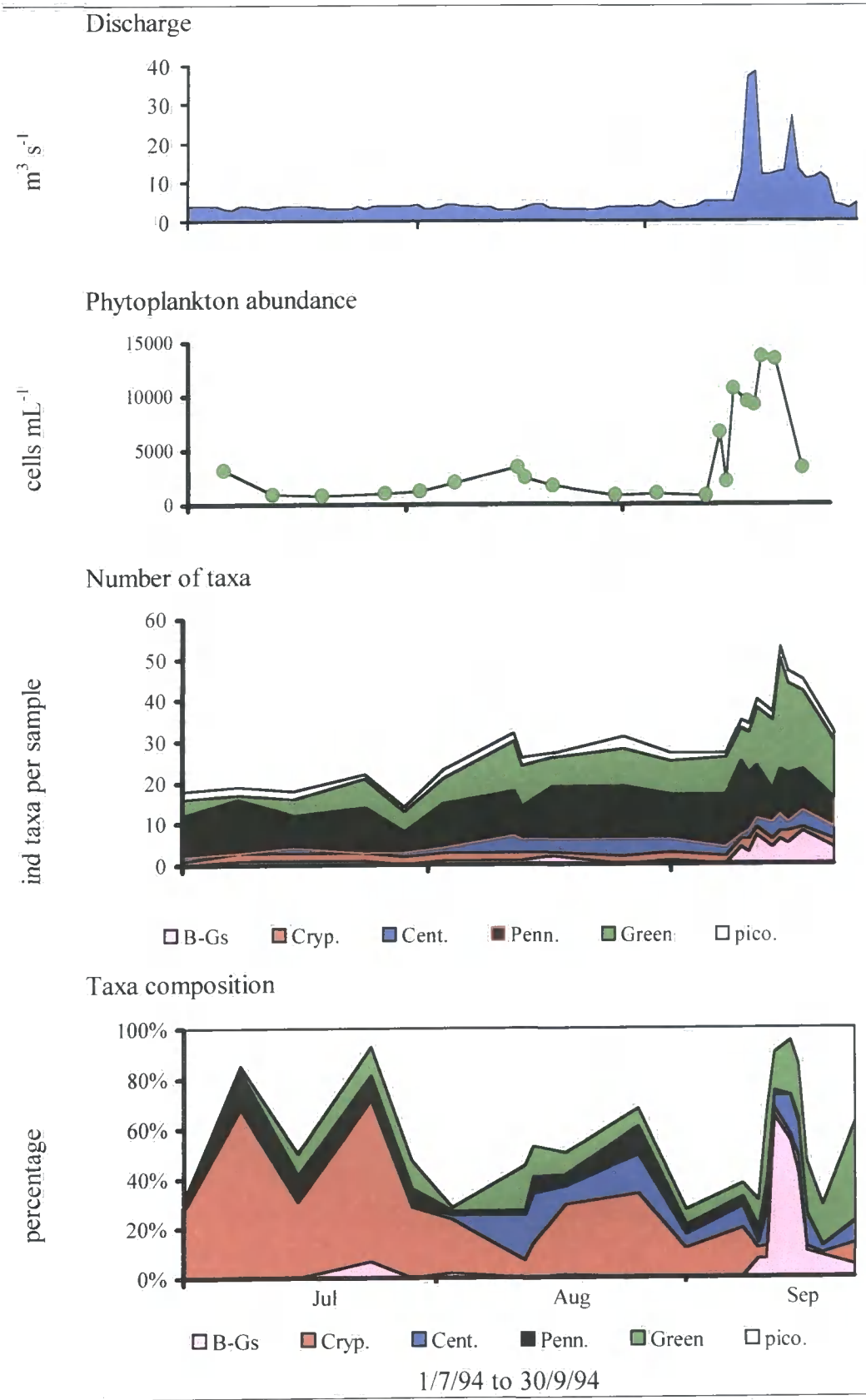


Figure 8.21 Temporal changes in discharge and phytoplankton preceding and during spate flows 1994. Taxa groups: blue-green algae (B-Gs), cryptophytes (Cryp), centric diatoms (Cent.), pennate diatoms (Penn.), green algae (Green) and picoplankton (pico.).

The phytoplankton composition was explored by constructing taxa groups, based on the methods described above. The phytoplankton preceding the September spate consisted mainly of the *Rhodomonas lacustris* var. *nannoplanktica,* picoplankton and pennate diatoms. During this period total phytoplankton abundance remained less than 5000 cells mL^{-1}, with between 20 and 30 individual taxa (Figure 8.21). The abundance and number of some taxa tended to increase (e.g. centric diatoms) throughout the study whereas other taxa remained relatively constant (e.g. pennate diatoms and picoplankton). The abundance of centric diatoms increased from early August. This trend corresponds to decreasing temperature, with average monthly values of 21, 18 and 14°C for July, August and September respectively.

The spate flows during September resulted in a significant change in the phytoplankton taxa. Blue-green algae, which were very scarce preceding the spate, became dominant over several days. The blue-green algal peak consisted mainly of *Oscillatoria agardhii, O. limnetica* and *O. redekei* the former of which exceeded 6000 cells mL^{-1} and was not found in the plankton of the Nene before the flood. Other blue green algae found during the spate, which are not normally found in the plankton, were *Aphanizomenon flos-aquae, Merismopedia* and *Microcystis* spp.

Scenedesmus acuminatus and *S. communis*, which were the most abundant green algae preceding the September spate also increased in abundance throughout the spate. Likewise, Picoplankton, were the most numerically abundant taxa throughout most of the survey, they increased in abundance with increasing discharge and remained abundant following the initial spate.

Perhaps surprisingly, the number of pennate diatom taxa did not increase markedly with spate flow and remained relatively constant throughout.

8.7 Picoplankton survey

The results of the picoplankton survey and fluorescence analysis are shown in Figure 8.22. Picoplankton were most abundant at times when the larger phytoplankton were less numerous, as seen in the routine counts. Blue-green picoplankton were more abundant than greens, and of the blue-green picoplankton spherical cells 1 μm ∅ were most numerous. The green picoplankton had a more sporadic occurrence, with spherical cells ≤ 1 μm ∅ being most abundant in the winter. The most abundant green

picoplankton group were spherical cells 1 μm ∅ and these had their period of greatest abundance shortly before the spring blue-green picoplankton peak.

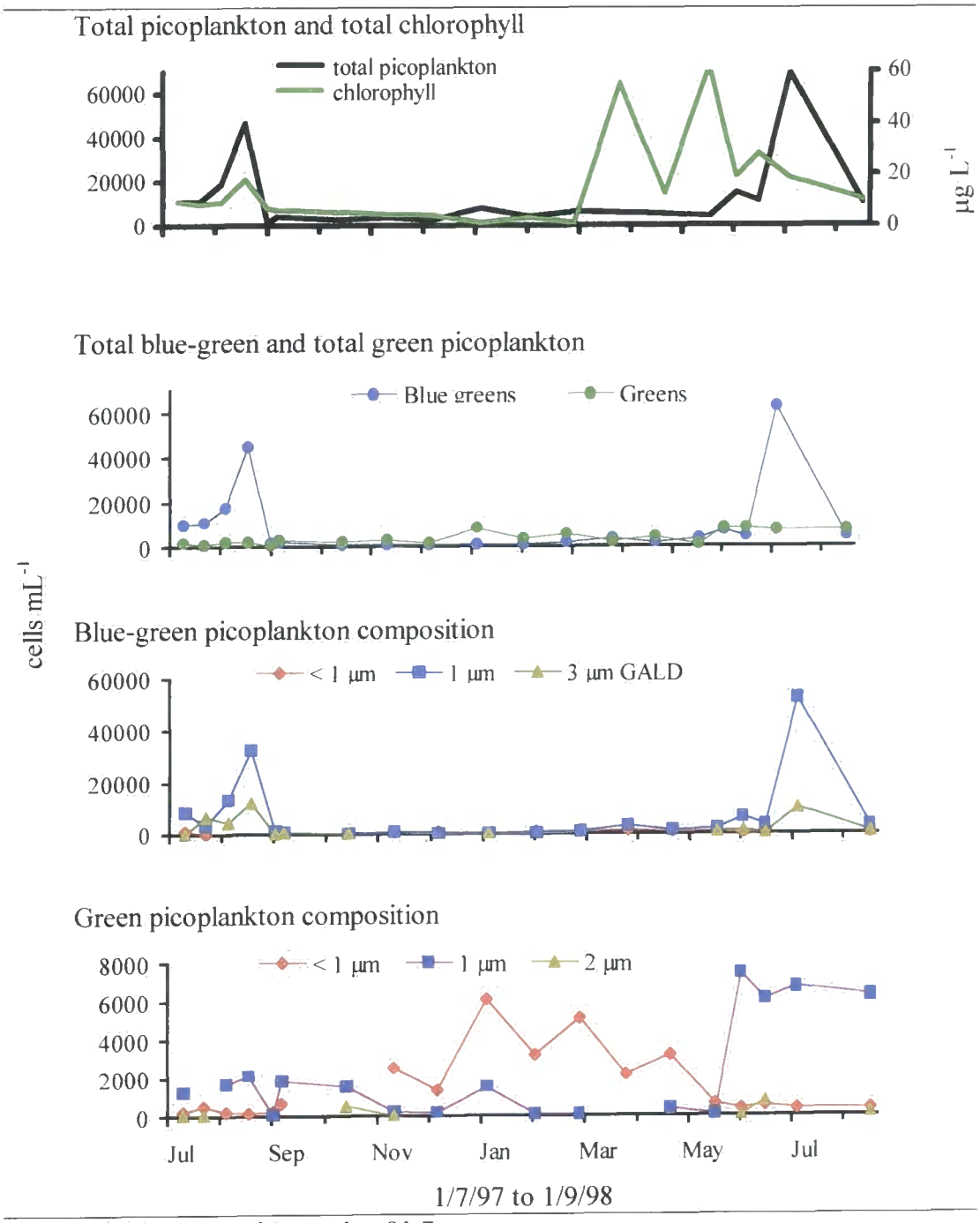


Figure 8.22 Picoplankton at km 91.7.

8.8 Sample frequency

The influence of varying sample frequencies on the 1996 chlorophyll data set at km 91.7 is shown in Figure 8.23. Altering the sample frequency from 7 to 14 d results

in the annual recorded chlorophyll maximum being reduced from 210 to less than 140 μg L^{-1}. Further increasing the sample frequency to 28 d reduced the annual recorded chlorophyll maximum to 89 μg L^{-1}. However, the influence of sample frequency has little effect over most of the year.

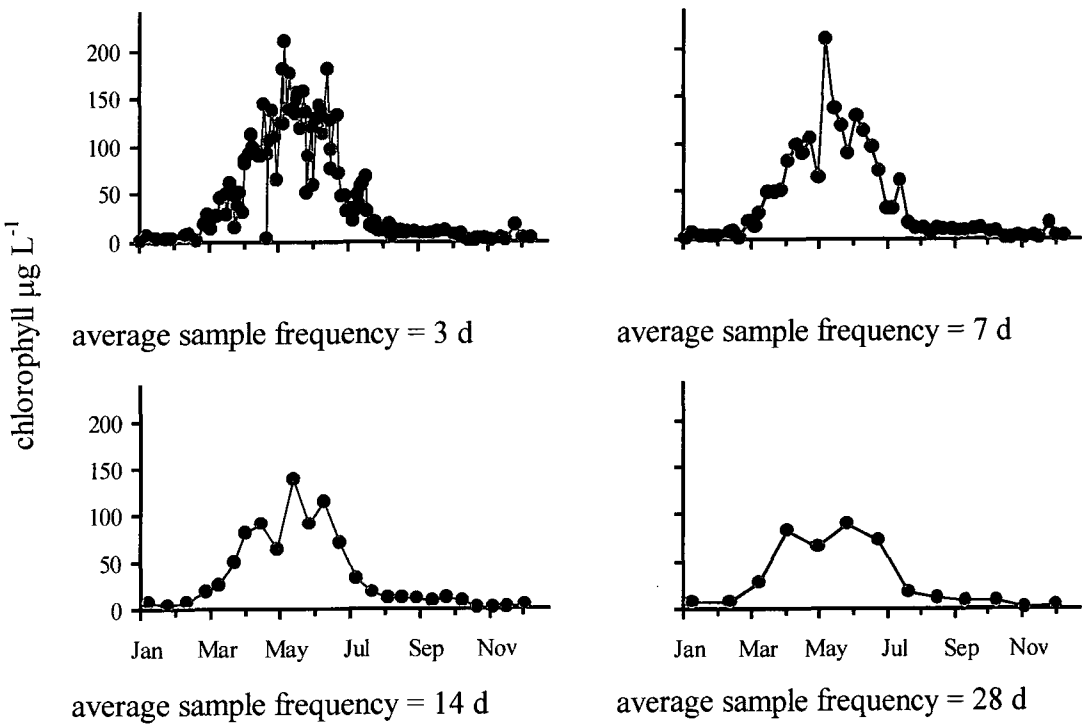


Figure 8.23 The impact of varying sample frequencies on 1996 chlorophyll data.

8.9 Discussion

Analysis of temporal trends provides a detailed insight into the behaviour of chlorophyll and phytoplankton, and provides an opportunity for further interpretation of the long-term chlorophyll data.

Further analysis of the chlorophyll data, substituting light expressed as 'sunshine' h with integrated irradiance, made little difference to the strength of the resulting relationships. This supports the findings of the previous chapter and identifies light as a poor predictor of chlorophyll concentration, particularly in the first half of the year.

The construction of taxa groups reduced a large data set (188 taxa) to a manageable size (32 taxa). Taxa grouping is a compromise between ease of interpretation and loss of information, but if undertaken with care should result in a greater benefit than loss. Taxa grouping was possible because the twenty most

abundant taxa constituted 88% of all taxa recorded and centric diatoms (excluding *Melosira varians* and *Skeletonema* spp.) 44% of all taxa.

As already mentioned, the danger of grouping taxa is loss of information. For example, six *Scenedesmus* spp were recorded during the counts and these were grouped into a single category for further analysis. However, of the six taxa *Scenedesmus* species *S. communis* and *S. acuminatus* made up 60% and 23% respectively. The response of these two taxa to discharge, temperature and light was very similar, so grouping was thought justifiable. If taxa were inappropriately grouped then this would be evident in the scatter plots (Figures 8.5, 8.6 and 8.7) and this is the most likely explanation for the wide variation seen in the scatter plots of picoplankton against physical variables.

Picoplankton were most abundant when the larger taxa were scarce, as seen in several lake studies (Happey-Wood, 1988). The detailed study of picoplankton indicates varying periodicities and abundances of the taxa, with blue-green forms being most numerous. The separation of the two main picoplankton groups (spherical and rod cells ≤ 3 µm ∅) in the CCA biplots supports this, with the rod cells being associated with greater light and discharge values (early spring and autumn) and the spherical cells with temperature (summer). Although being numerically abundant the picoplankton constitute a much smaller proportion of the total phytoplankton when expressed as a volume.

The three tiered approach to taxa analysis (time-series, scatter plots and CCA biplots) are complementary. The analysis clearly identifies periodicities and abundances in response to changes in discharge, temperature and light. The physical environment is most favourable to some taxa during periods of high discharge, low temperature and light (Spring taxa: e.g. *Nitzschia acicularis* and *Koliella longiseta*). Whereas, other taxa are most abundant during periods of low discharge, high temperature and light (Summer taxa: e.g. *Actinastrum hantzschii* and *Dictyosphaerium pulchellum*). A few taxa appear to have a universal distribution, being able to proliferate throughout much of the year (e.g. *Rhodomonas lacustris* var. *nannoplanktica*). These categories are not absolute and gradations of taxa occurrence are evident, as seen in the centric diatom size classes.

The results indicate a shift from smaller to larger centric diatoms (*Stephanodiscus hantzschii* to *S. hantzschii* fo. *tenuis*), which are concurrent with decreasing discharge,

increasing temperature and light. This trend was evident in the time-series, scatter plots, biplots and short-term high-frequency samples. The biplots also identified the larger centric diatoms as being associated with low silicate concentration, more so than the other size classes.

Although there is a paucity of silicate data it has still been possible to demonstrate silica limitation. The results here follow the findings of Swale (1963) and population increases are restricted when silica concentration is low. Silica limitation has been demonstrated in lakes (Lund et al., 1963) but rivers are further complicated by varying replenishment rates. Figures 8.17 and 8.18 clearly show how increased discharge can result in a concurrent increase in SiO_2-Si and sequentially facilitate an increase in diatom abundance.

This phenomenon could explain some of the temporal trends seen in the Nene. The low temperature optimum for centric diatoms could be the result of selection pressure for their proliferation when silica is most abundant, and this will often be early in the year when temperature and light are limited. The temperature optimum for centric diatoms > 6 to ≤ 10 μm (*Stephanodiscus hantzschii*) found during this study is considerably lower than other British rivers (Swale, 1969).

Few detailed studies of diurnal trends in phytoplankton have been undertaken. This study clearly shows considerable diurnal change in chlorophyll and phytoplankton abundance. During his study of phytoplankton and chlorophyll in the Danube at Göd, Kiss (1996) attributed daily increases, which often resulted in two peaks per day, to the rapid division of centric diatoms. In the Danube poor agreement was found between cell numbers and chlorophyll, which was thought to result from overnight decreases in pigment concentration. This is contrary to the finding here where concurrent changes in chlorophyll and phytoplankton abundance can be seen.

The influence of N and P on phytoplankton abundance in the Nene appears to be negligible, as concluded from the long-term data. However, ammonium concentrations are reduced to very low levels during phytoplankton peaks and the aforementioned conclusion assumes that nitrate is readily utilised by phytoplankton in the absence of ammonium.

The analysis of the 1994 September spate revealed some patterns of abundance not previously evident. The increase in discharge brought about a large increase in phytoplankton abundance and number of taxa, which lasted for several days. This

pattern is contrary to other studies that have linked increases in flow with a decline in phytoplankton abundance (Lack, 1971). Naturally, sustained increases in flow will eventually result in reduced phytoplankton abundance, but in the Nene there is an element of flushing which results in concurrent high flow and phytoplankton abundance. This phenomena will contribute to the overall variability in the relationship between phytoplankton and physical variables and produce some of the scatter seen in previous plots (Chapter 7).

Increases in discharge brought about by sustained precipitation will have a two-fold flushing effect; Flushing of standing waters and the flushing of the river itself. The former of these situations is evident in Figure 8.21. The sudden increase in blue-green algae and the occurrence of *Microcystis* and *Aphanizomenon*, which are normally associated with lentic systems, was probably the result of flushing from lakes, and their sudden appearance possibly indicates the lakes were relatively close by. Lakes along tributary 3 (Willow Brook) are a likely source of these algae (see Section 9.4).

The continued occurrence of centric diatoms, cryptomonads and particularly green algae after the spate indicates that these were being flushed from the river itself, or more distant lakes than those where the initial increase in blue-green algae originated. The low abundance of pennate diatoms is of interest, as this group would be expected to be scoured from the benthos throughout the spate. A plausible explanation for low numbers of benthic diatoms is that they were removed from the system relatively suddenly and this was not detected by the daily sample programme. This could have been the case, especially if many of the benthic forms were attached to macrophytes, which would have been flushed from the system with the onset of increased discharge.

The short-term samples and the frequency adjustments made to the 1996 chlorophyll data provide an insight into sample programme design. The most efficient use of sampling effort would be to concentrate on periods when plankton are most abundant. The difference in the maximum chlorophyll concentration recorded for 1996 between the 7 and 14-d samples is dramatic, although the overall trend is unchanged. However, 1996 had a high chlorophyll concentration compared to other years. During 1994 the chlorophyll peak was greatly restricted compared to 1996 (Figure 7.3) and 14-d sampling would have resulted in an increased chance of missing the peaks. The short-term analysis for chlorophyll and phytoplankton indicate how these can vary

considerably through the day. Therefore care must be taken when considering daily chlorophyll data when samples are taken at different times of the day.

In the Nene a sufficient sampling programme would include samples for phytoplankton and chlorophyll taken at weekly intervals between 1 March and 31 October and at two-weekly intervals at other times (excepting any extraordinary events, like extreme drought). It is also preferable if silicate samples are taken along with the weekly samples and preferably these would be analysed to a lower level of detection. Nutrient data collected as part of the EA routine monitoring programme is probably sufficient, although this would need to be reviewed periodically.

Many of the results collected during this work provide a foundation for dynamic modelling of phytoplankton in the Nene. The scatter-plots and examples of fitted lines provide the mathematics for relating species abundance to changes in the physical environment, as does the short-term trend data. Although lines might have to be fitted more precisely to some data, than the example given. The findings also highlight the importance of calibrating a Nene phytoplankton model with actual data from this river and how inappropriate data taken from the literature would be. However, any model of the Nene would also need to consider other biological and physical loss factors (Chapter 10).

This and the previous chapter have explored temporal trends at km 91.7. The next chapter will now put these observations into a wider context.

8.10 Summary

1 The substitution of solar radiation data for sunshine h made little difference to the significance of light as a predictor for chlorophyll concentration.

2 188 taxa were recorded in the Nene at km 91.7. The 20 most abundant of these taxa constituted 88% and centric diatoms (minus *Melosira varians* and *Skeletonema* spp.) being 44% of the total.

3 Taxa groups (32) were constructed and used to explore their relationship with physical and chemical variables, using time-series, scatter plots and CCA biplots.

4 Taxa occurrence and abundance was linked to physical variables and silicate concentrations, which were validated using long and short-term data.

5 There is evidence that the magnitude of centric diatom populations are limited by the availability of silica. This conclusion is based on the concurrent abundance of centric diatoms and silicate concentrations.

6 Picoplankton consisted mainly of blue-green algae. Green algae picoplankton had a different periodicity to the blue-greens and tended to be most abundant during periods when other algae were scarce.

7 Spate flows during September 1994 provided additional information on temporal trends. Phytoplankton abundance increased significantly with the onset of spate flows. A marked increase in blue-green algae was probably the result of flushing from lentic systems. Phytoplankton that were normally present in the river persisted over several days of high flow.

8 A sampling programme is suggested that consists of weekly samples between 1 April and 30 September and fortnightly at other times.

9 SPATIAL DISTRIBUTIONS

9.1 Introduction

The main objective of this chapter is to place the temporal trends presented in the preceding chapters into a wider perspective. The previous two chapters explored temporal trends in chlorophyll and phytoplankton at km 91.7 (Wansford). This chapter expands on the previous two by looking at large and small-scale spatial trends. These studies include longitudinal trends in chlorophyll and phytoplankton (upstream of km 91.7) and an investigation into localised trends, including a comparison between a bay and the main river and the influence of lentic systems on river phytoplankton composition.

9.2 Longitudinal trends

Chlorophyll

Spatial trends in chlorophyll concentration are shown in Figure 9.1, which uses all available data from 1 January 1993 to 31 December 1997. The chlorophyll concentration increases markedly in the three most upstream sites and mostly so between km 22.4 (Duston Mill) and km 34.0 (Great Billing). The data exhibit marked seasonality at all sites downstream of km 34.0, which is consistent with km 91.7.

Each data set was standardised to produce an evenly spaced series and investigated using time series analysis (Table 9.1). During the analysis each series was decomposed into a linear trend, seasonal component and error. The trend data for each pair of sites, working in a downstream direction, were then compared using Mann-Whitney U tests.

A significant change in chlorophyll concentration occurred in most comparisons, but mostly so at the first three sites. Other changes, although significant, were small and no downstream trend was apparent downstream of km 64.6.

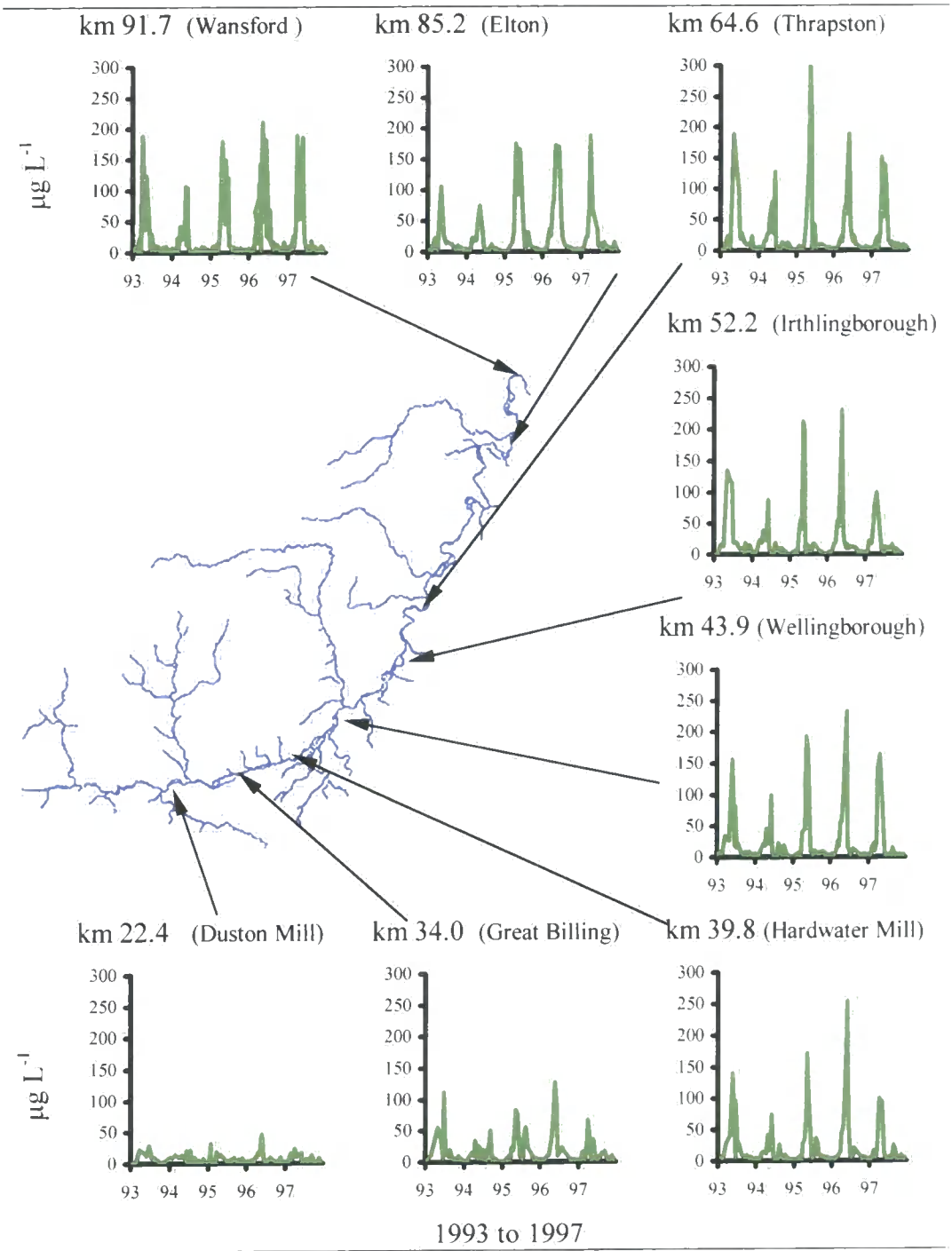


Figure 9.1 Spatial chlorophyll concentrations at eight main river sample points (1993 to 1997).

Table 9.1 Analyses of spatial chlorophyll data. Showing n, average frequency of days between samples (Ave. Freq.), overall median of data, median of fitted trend and *p* values for Mann-Whitney U Tests. Tests are for paired samples working downstream (e.g. First *p* is a for comparison between km 22.4 and km 34.0).

			Median chlorophyll (µg L^{-1})		
Site	n	Ave. Freq.	Overall	Trend	*p*
km 22.4 (Duston Mill)	90	14	4.0	6.69	------
km 34.0 (Great Billing)	87	15	7.2	17.19	0.000
km 39.8 (Hardwater Mill)	88	15	5.4	21.87	0.000
km 43.9 (Wellingborough)	82	16	5.0	23.66	0.000
km 52.2 (Irthlingborough)	82	16	9.0	23.05	0.020
km 64.6 (Thrapston)	82	16	8.1	28.41	0.000
km 85.2 (Elton)	81	16	8.0	29.60	NS
km 91.7 (Wansford)	91	14	8.0	27.57	0.000

Chlorophyll development in the upper part of the navigation section was investigated by calculating retention time at three sites (km 34.0, km 39.8 and km 43.9). Chlorophyll concentration (January to June and restricted to 5°C to 15°C) correlates significantly with retention time ($r^2 = 0.38$; $p < 0.05$; n = 80), approximately doubling with daily increases in estimated retention (Figure 9.2).

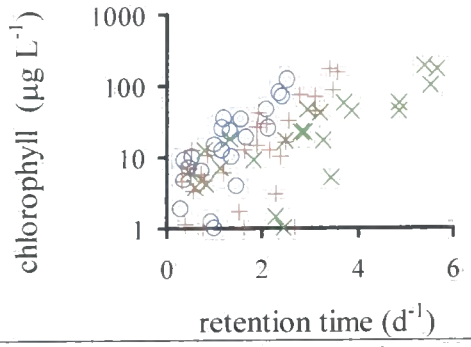


Figure 9.2 Chlorophyll concentration plotted against retention time (1 January to 30 June, restricted to between 5°C and 15°C). o = km 34.0; + = km 39.8; × = km 43.9.

Figure 9.1 also indicates that the chlorophyll maxima at downstream sites occur earlier and are of less magnitude than upstream sites, especially during dryer years. The situation was clarified by listing chlorophyll maxima and their date of occurrence (Table 9.2).

Table 9.2 Spring chlorophyll maxima (μg L^{-1}) and date when they occurred (brackets) for sites and years.

Site	1993	1994	1995	1996	1997
km 34.0	112 (25/6)	35 (26/4)	77 (31/5)	127 (22/5)	67 (8/4)
km 39.8	139 (27/5)	73 (7/6)	170 (16/5)	253 (5/6)	99 (8/4)
km 43.9	155 (27/5)	99 (7/6)	192 (16/5)	232 (5/6)	167 (23/4)
km 52.2	135 (11/5)	86 (7/6)	212 (16/5)	231 (22/5)	99 (23/4)
km 64.6	198 (11/5)	78 (12/5)	307 (16/5)	188 (22/5)	151 (8/4)
km 85.2	105 (10/5)	74 (12/5)	175 (20/4)	172 (8/5)	187 (8/4)
km 91.7	189 (5/4)	106 (12/5)	179 (20/4)	209 (8/5)	189 (8/4)

The phenomenon of spatially differential chlorophyll maxima was further investigated by plotting chlorophyll peaks at downstream and intermediate locations along with contemporaneous velocity and temperature data (Figure 9.3).

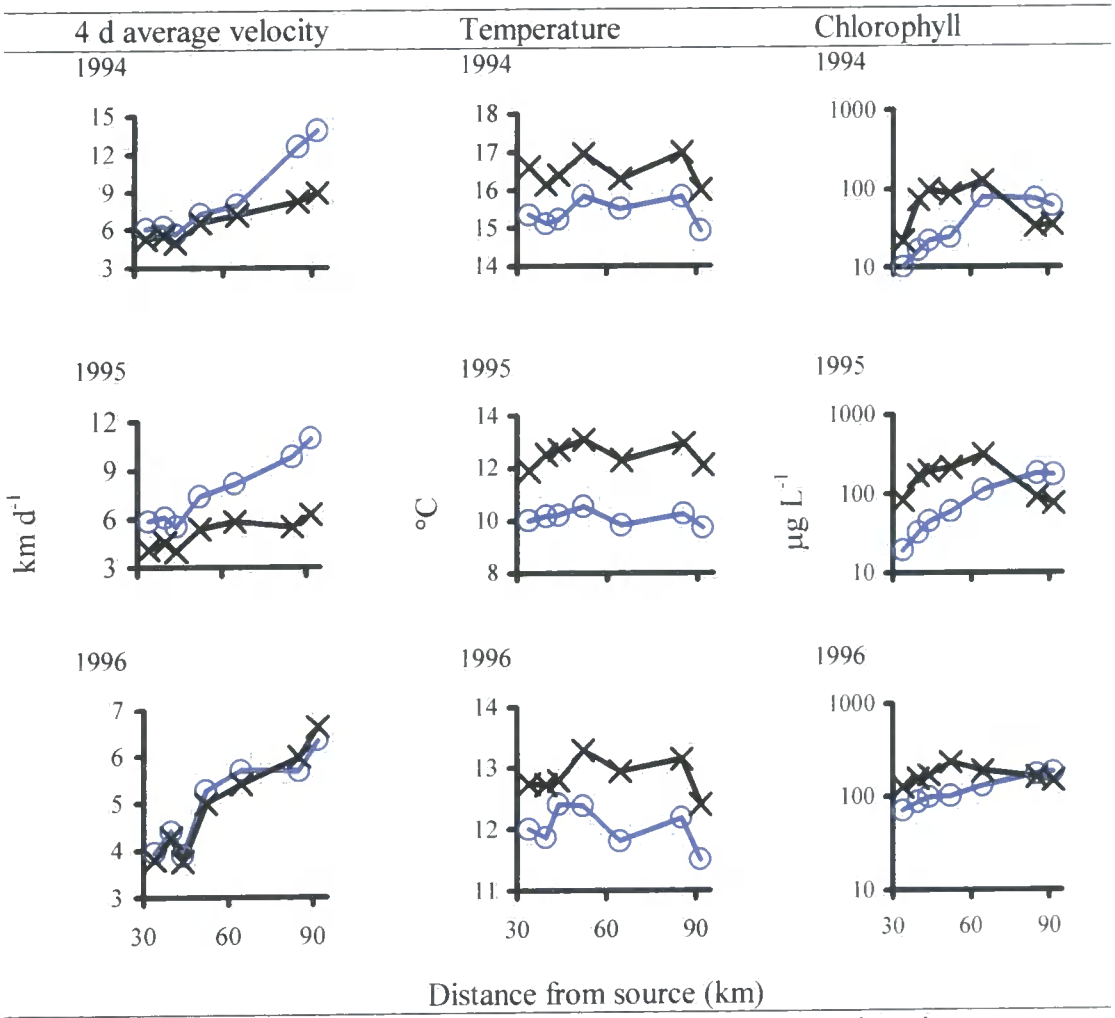


Figure 9.3 Spring chlorophyll maxima at downstream and intermediate sites, velocities and temperature (1994 to 1996) from km 34.0 to km 91.7 (o = downstream chlorophyll maxima; × = chlorophyll maxima at intermediate sites). Dates of maxima as Table 9.2.

Multiple-regression analysis identified temperature, velocity and downstream distance from source as significant predictors of downstream spring chlorophyll concentration ($r^2 = 0.99$; $p < 0.05$; n = 21), whereas spring chlorophyll peaks at intermediate sites were best predicted by temperature alone ($r^2 = 0.34$; $p < 0.05$; n = 21). It is important to consider, at this point, that the longitudinal temperature record is likely to be biased by sampling method. Samples were mostly collected in an upstream direction and increases (or decreases) in daily temperature would be better represented in samples taken later in the day.

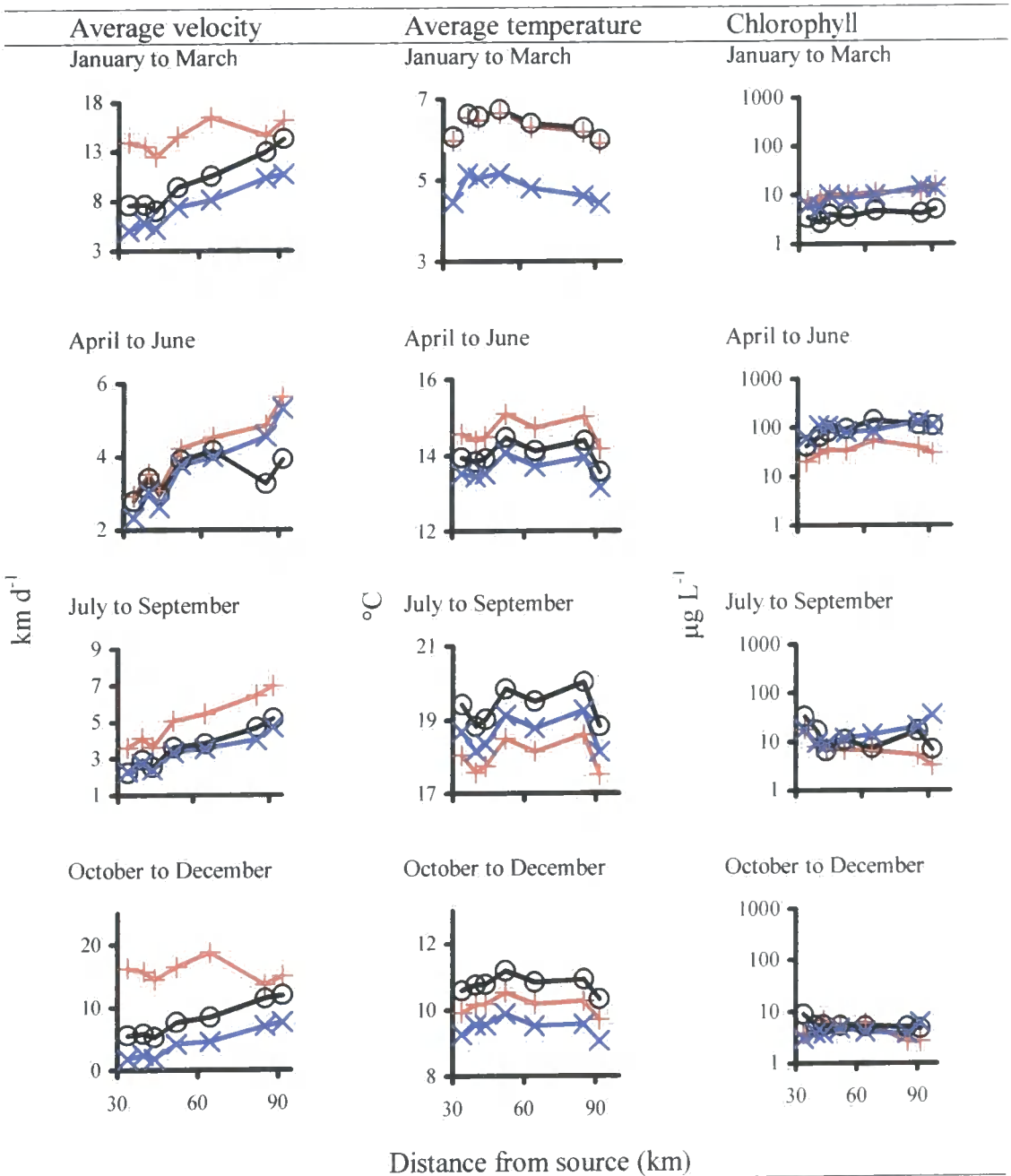


Figure 9.4 Three-monthly average velocities, temperature and chlorophyll concentrations from km 34.0 to km 91.7 (+ = 1994; o = 1995; × = 1996).

Three-monthly average velocity, temperature and chlorophyll concentration are shown in Figure 9.4. Velocities generally increase in a downstream direction, whereas temperatures decrease during the colder seasons and increase slightly during the spring and summer. Chlorophyll concentrations were low throughout the January to March and October to December periods, increasing in a downstream direction during the former period and declining in the latter. Chlorophyll concentrations were consistently greatest during the April to June period and most variable between July and September.

Multiple-regression analysis for combined period data, using velocity, temperature and downstream distance from source as predictors for chlorophyll concentration, identified significant relationships for the January to March and April to June periods. Temperature and velocity were the most significant predictors of chlorophyll concentration for the January to March period ($r^2 = 0.62$; $p < 0.05$; n = 21), and temperature and distance from source between April and June ($r^2 = 0.37$; $p < 0.05$; n = 21). Velocity best predicted the average chlorophyll concentration between July and September ($r^2 = 0.22$; $p < 0.05$; n = 21) whilst none of the predictors were significant for the October to December period.

The influence of temperature and velocity on spring chlorophyll concentrations was similar at four locations (km 34.0, km 43.9, km 64.6 and km 91.7) and exhibited similar characteristics of those seen in the long-term data for km 91.7 (Figure 9.5).

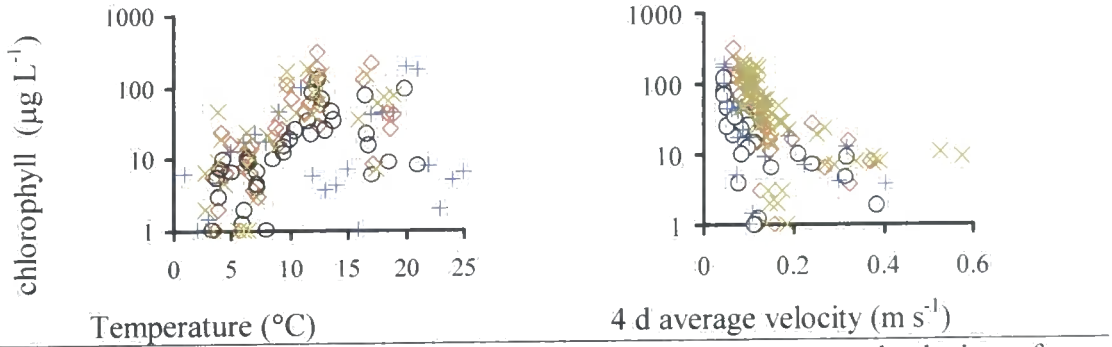


Figure 9.5 Chlorophyll concentration plotted against temperature and velocity at four main river sites (both restricted to 1 January to 30 June; temperature restricted to 5 to 15°C). o = km 34.0; + = km 43.9; ◊ = km 64.6; × = km 91.7.

Phytoplankton

The spatial distributions of phytoplankton taxa at four Nene locations are shown in Figures 9.6, 9.7 and 9.8.

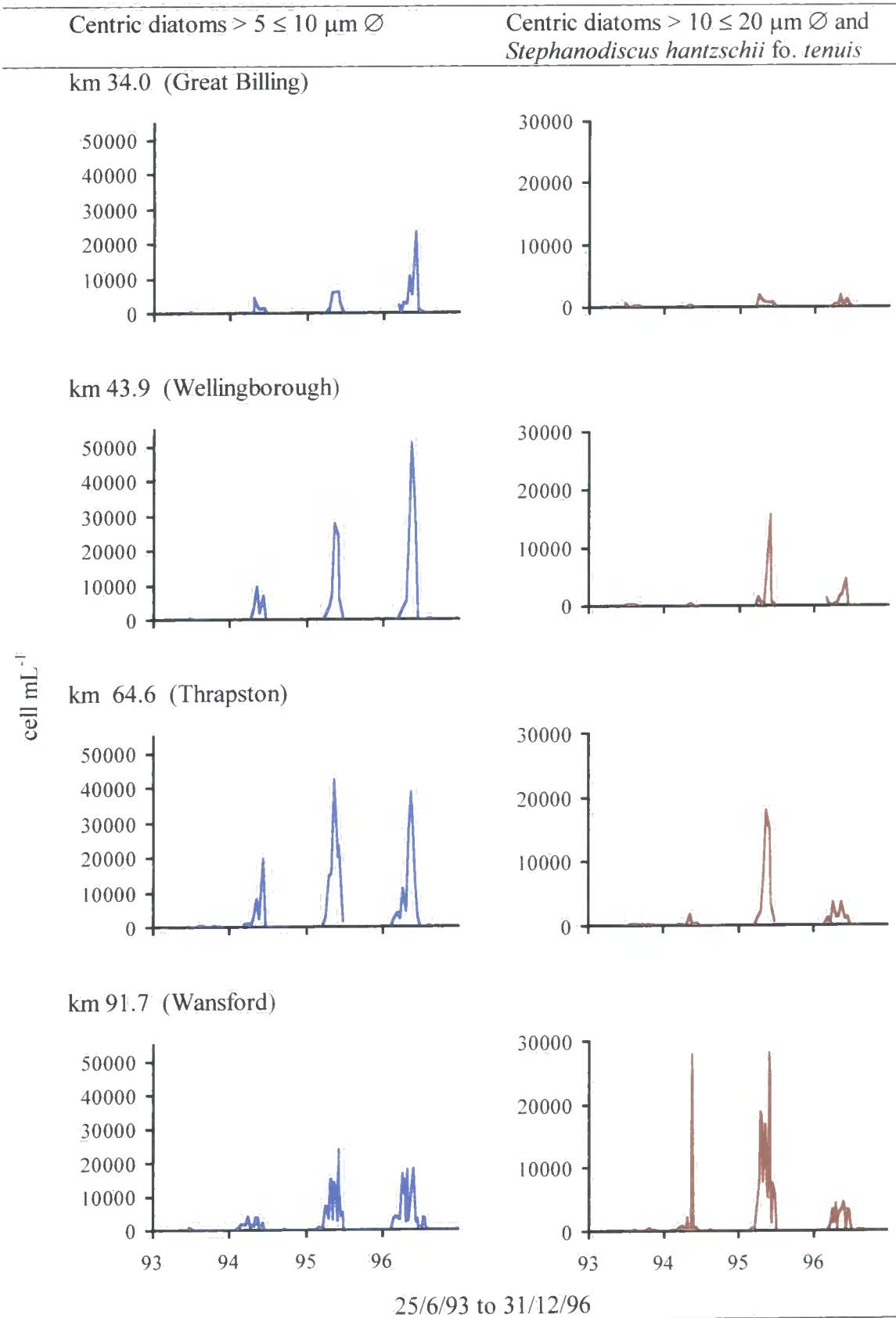


Figure 9.6 Time series for Centric diatoms > 5 ≤ 10 µm ∅ and Centric diatoms > 10 ≤ 20 µm ∅ (inc. *Stephanodiscus hantzschii* fo. *tenuis*) at four Nene sites. Sites other than km 91.7 were selectively analysed during periods when chlorophyll > 10 µg L^{-1}.

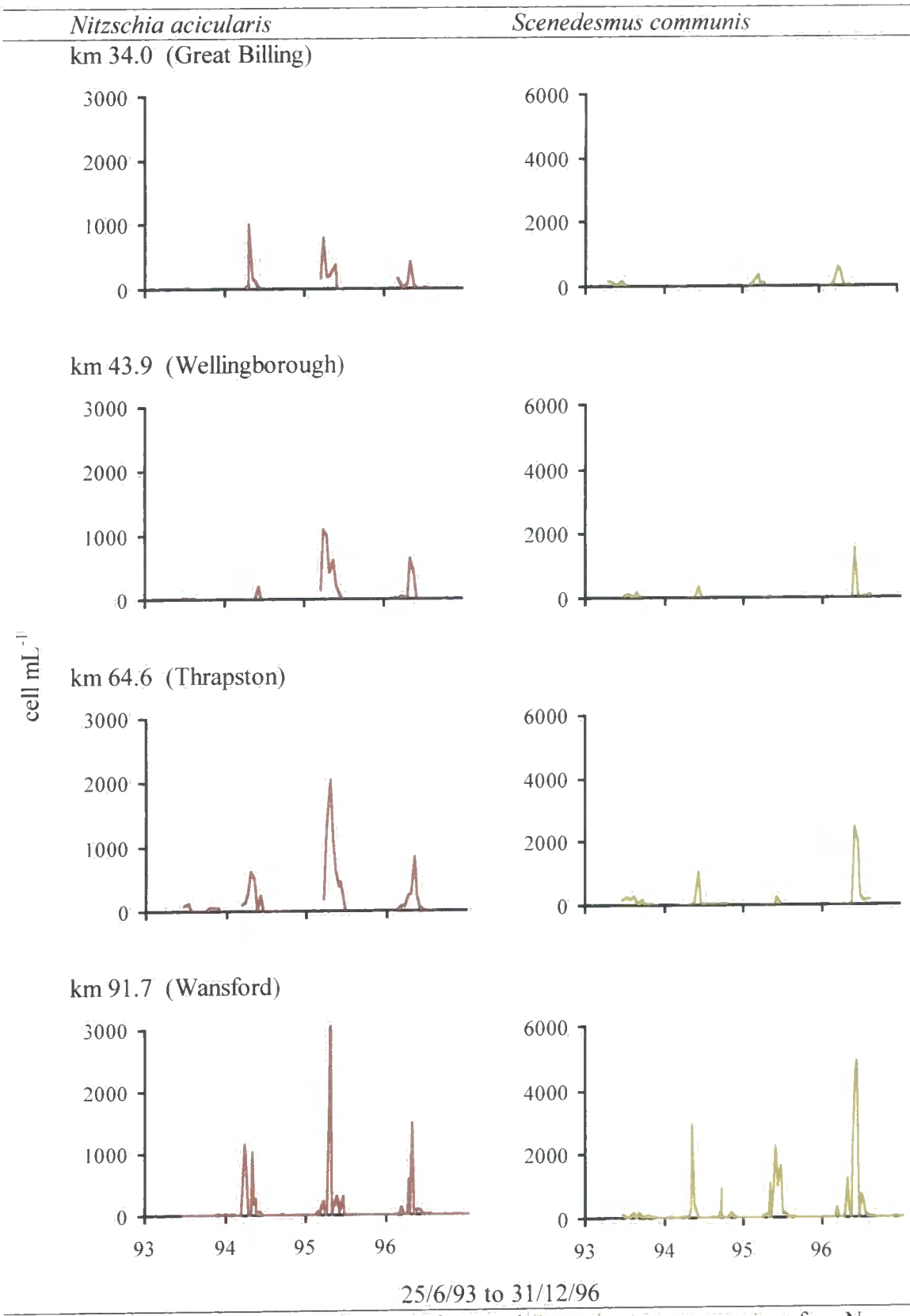


Figure 9.7 Time series of *Nitzschia acicularis* and *Scenedesmus communis* at four Nene sites. Sites other than km 91.7 were selectively analysed during periods when chlorophyll > 10 µg L^{-1}.

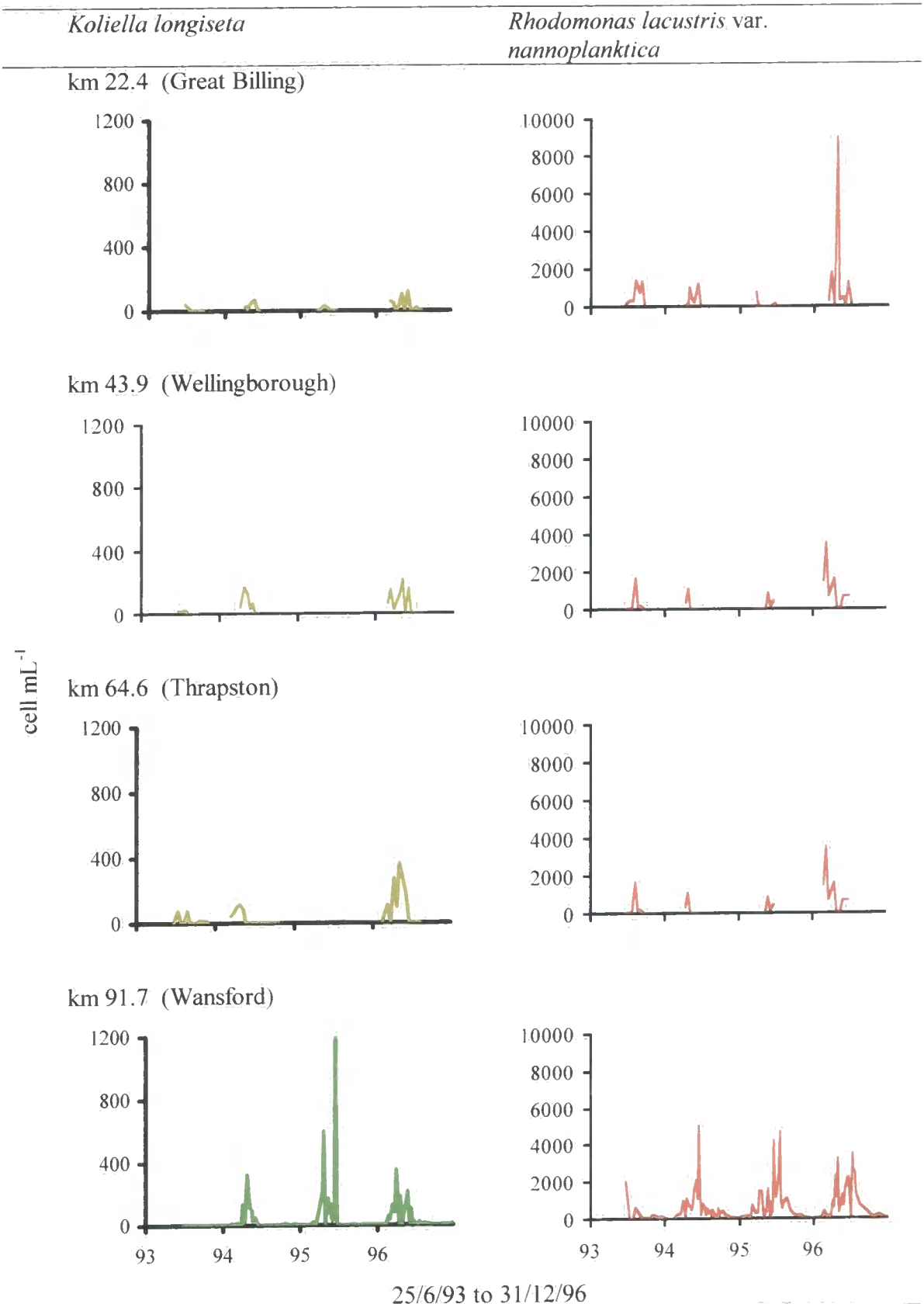


Figure 9.8 Time series of *Koliella longiseta* and *Rhodomonas lacustris* var. *nannoplanktica* at four Nene sites. Sites other than km 91.7 were selectively analysed during periods when chlorophyll > 10 µg L^{-1} (except *Koliella* at Thrapston and Wellingborough during 1995 which was not counted).

The timing and magnitude of periods with abundant centric diatoms > 6 to ≤ 10µm ∅ was similar to the chlorophyll, with km 91.7 having its peak earlier and of less magnitude than upstream sites. This finding is not surprising as these diatoms are the most abundant taxa during spring peaks. What is surprising though, and not obvious in the chlorophyll plots is the magnitude of difference, with km 43.9 and km 64.6 having twice the abundance of km 91.7 (Mann-Whitney U Tests: $p = 0.000$ for 16/5/95 and 22/5/96, km 91.7 compared with km 64.6 and km 43.9). Additionally, the much greater abundance of larger centric diatoms at the downstream site during 1994 and 1995 could be indicative of silica limitation here, as this taxa mainly comprises *Stephanodiscus hantzschii* fo. *tenuis*, the silica deficient form of *S. hantzschii*.

Scenedesmus communis exhibited a downstream increase in abundance, whereas other species had a more sporadic pattern of occurrence. *Nitzschia acicularis* exhibited signs of silica limitation (as described in Chapter 4) at all sites, although this alga was most abundant at km 91.7.

Centric diatoms of the size range > 6 to ≤ 10µm ∅ from the four locations exhibit a similar response to temperature, with peak abundance between 10 and 15°C, thereafter numbers tending to decline (Figure 9.9).

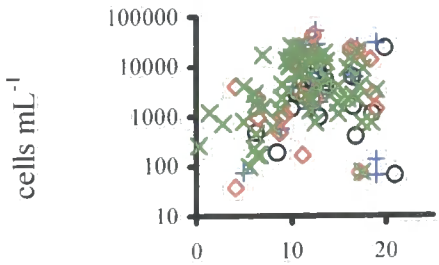


Figure 9.9 Centric diatoms > 6 to ≤ 10µm ∅ plotted against temperature at four Nene sites (restricted to 1 January to 30 June). o = km 91.7; + = km 43.9; ◊ = km 64.6; × = km 91.7.

9.3 Spatial distributions at km 91.7

Bay/main-river comparison

During 1995 additional samples were taken in a small bay adjoining the main river at km 91.7 (Figure 3.2). Samples were taken for chlorophyll and phytoplankton, and temperature differences were noted. The aim of the survey was to assess differential growth patterns between the two locations in light of the possible influence of 'dead zones' (Wallis et al., 1989).

Comparisons of temperature and chlorophyll concentration for the main-river and bay are shown in Figure 9.10. Temperature varied little between the two sites. Whereas chlorophyll exhibits an elevated concentration in the bay during the early part of the period (April to late May), followed be the opposite trend (late May to mid-June) and the remaining period had equivalent concentrations. It may be important to note that the apparent switch between the most abundant chlorophyll in the bay and main river (late May) coincided with a water temperature of approximately 15°C.

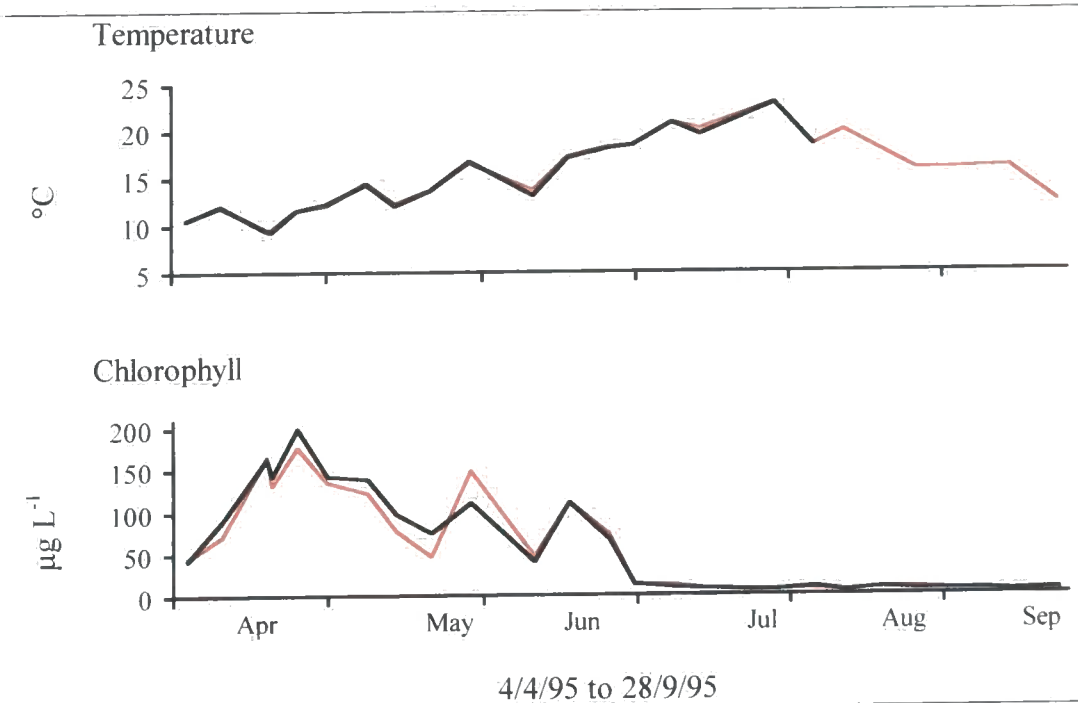


Figure 9.10 Temperature and chlorophyll comparison between bay (black) and main river (red) sample sites at km 91.7.

Evaluation of the phytoplankton dynamics at the two locations helped to explain the pattern seen in the chlorophyll series (Figure 9.11). The initial period with a higher chlorophyll concentration in the bay was dominated by centric diatoms > 6 to ≤ 10µm

∅, which were significantly more abundant in the bay than in the main river (26/4/95: Mann-Whitney U Test: $p = 0.018$).

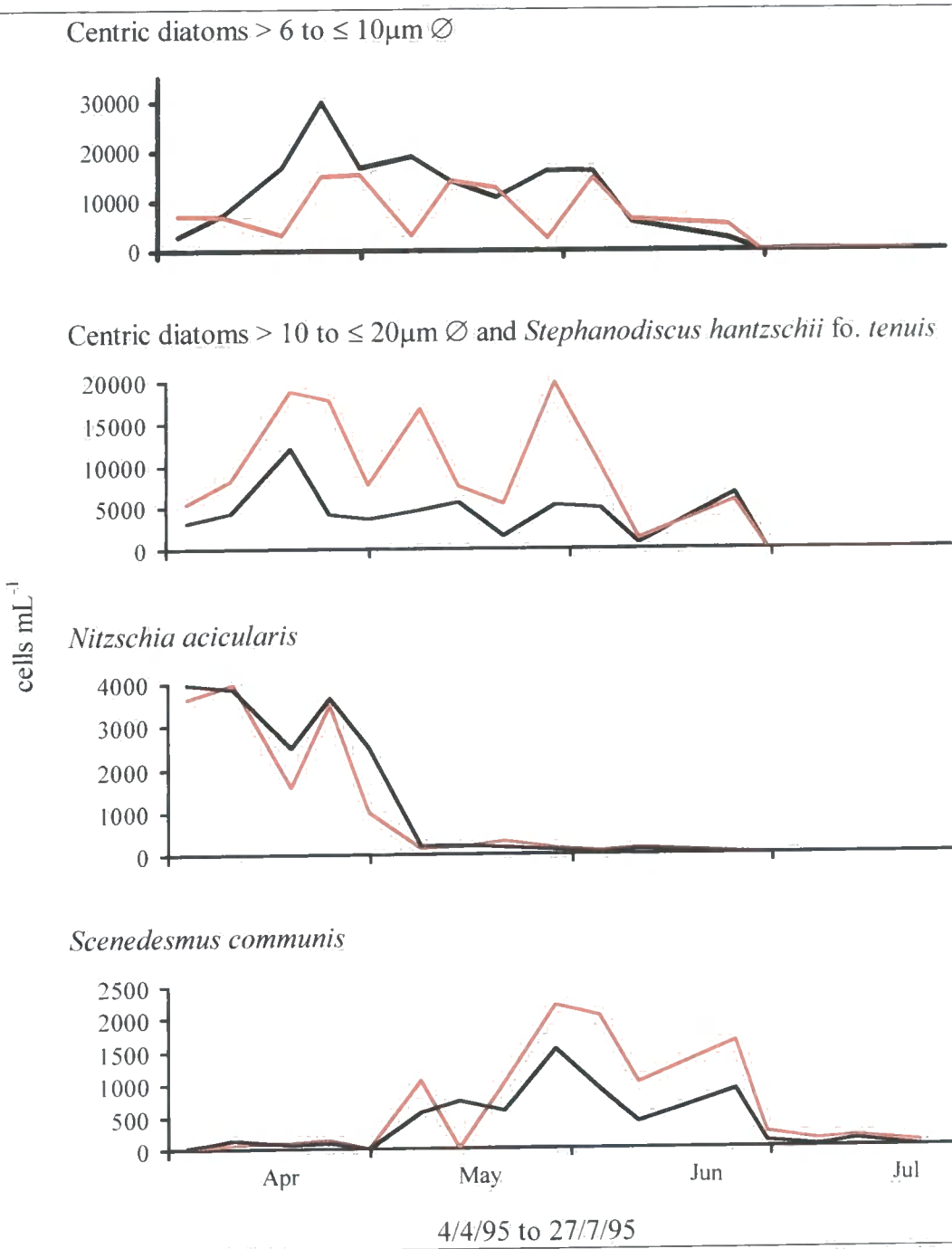


Figure 9.11 Comparison of phytoplankton abundance between bay (black) and main river (red) sample sites at km 91.7.

Differences in abundance between phytoplankton in the main river and bay were further investigated by carrying out SF counts on selected species in samples collected on the 11/4/95, 23/5/95 and 3/7/95 (Table 9.3). On the 11/4/95 *Nitzschia acicularis*, CD

≤ 5 μm ∅, *Koliella longiseta* and *Cryptomonas* sp. were significantly more abundant in the bay than in the main river. On the 23/5/95 *N. acicularis* was most abundant in the main river, which was the same for *Scenedesmus communis* on the 3/7/95. Of those taxa that exhibited a significant difference between the locations, the ones with greatest abundance in the bay occurred in the early sample and those that occurred later had the opposite trend.

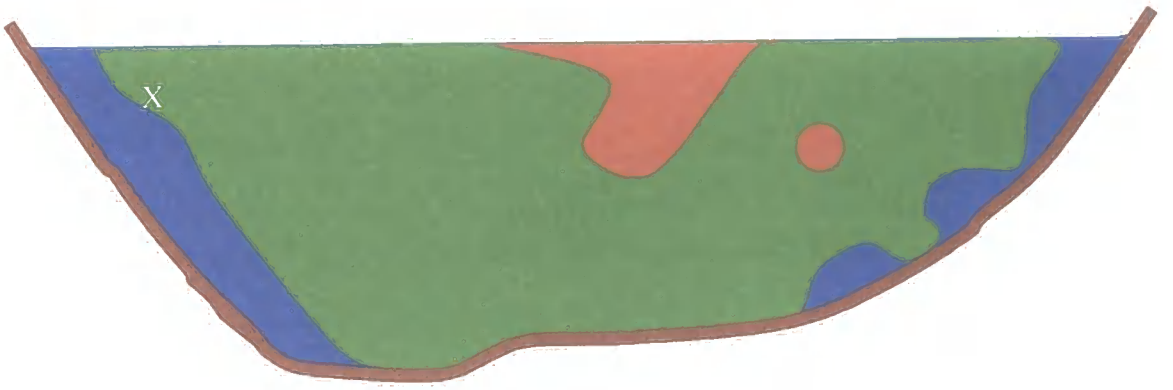
Additionally, on the 11/4/95 all six taxa were more abundant in the bay than the main river, whereas on the 23/5/95 four taxa were more abundant in the bay and three in the main river. Using χ^2 tests (H_o: the bay is having no influence on the distribution of taxa with greatest abundance) the bay was identified as having a significant influence during April (χ^2 = 6, df = 1) but not so in May (insufficient taxa in the July sample to test).

Table 9.3 Taxa abundance comparisons between the bay and main river on three occasions, using Mann-Whitney U tests (SF counting method). Of the tests which were significant some had greatest abundance in the bay (b) and others in the main river (m).

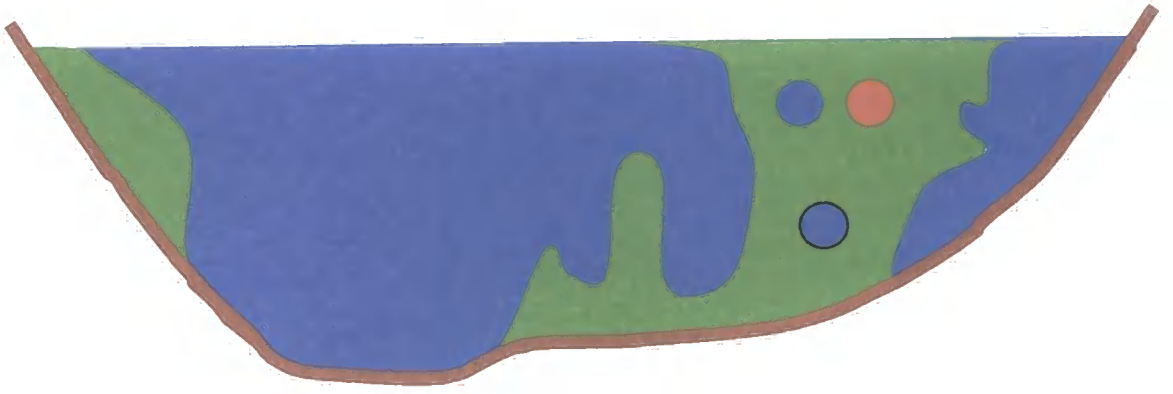
	11/4/95	23/5/95	3/7/95
Centric diatoms ≤ 5μm ∅	0.0002 (b)	NS	----------
Centric diatoms > 6 to ≤ 10μm ∅	NS	NS	----------
Centric diatoms > 11 to ≤ 20μm ∅	NS	NS	----------
Nitzschia acicularis	0.0005 (b)	0.0117 (m)	----------
Koliella longiseta	0.0325 (b)	NS	----------
Cryptomonas sp.	0.0211 (b)	----------	NS
Scenedesmus communis	----------	NS	0.0016 (m)
Actinastrun hantzschii	----------	NS	----------

River profiles and spot samples

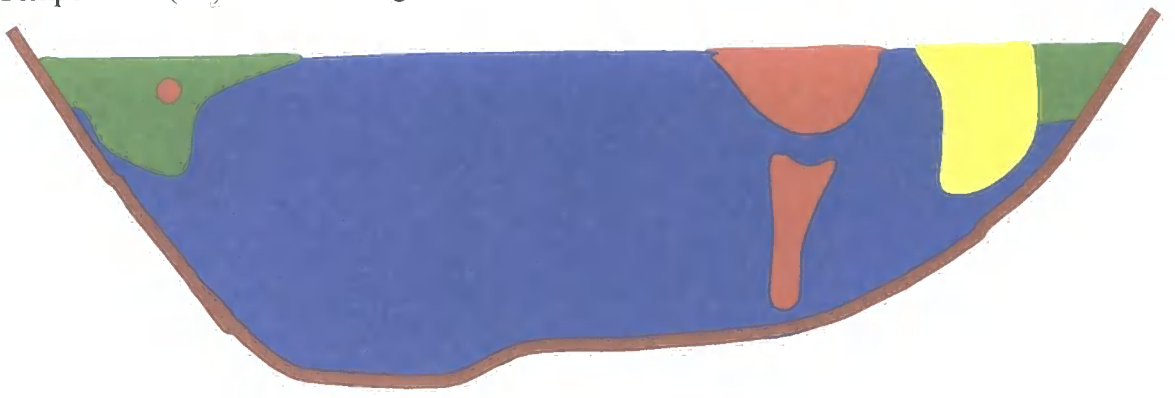
On one occasion during 1997 two river profiles were explored for differences in velocity, temperature and chlorophyll concentration. These surveys were undertaken at km 91.7 and km 92.5, along with surface chlorophyll evaluations. River profiles are shown in Figure 9.12 and Figure 9.13 and the data is summarised in Table 9.4.



Velocity (mm s^{-1}): blue = 11 to 20, green = 41 to 60, red = 61 to 80

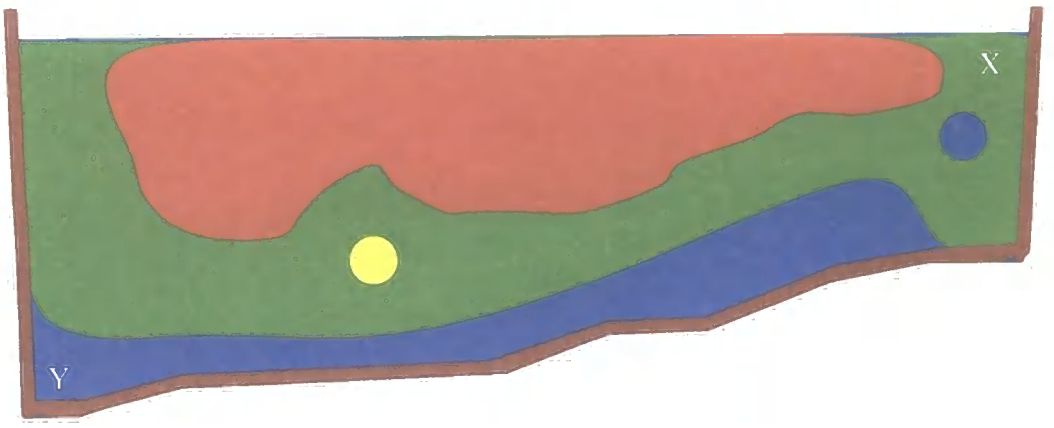


Temperature (°C): blue= 11.5, green = 11.6, red = 11.7

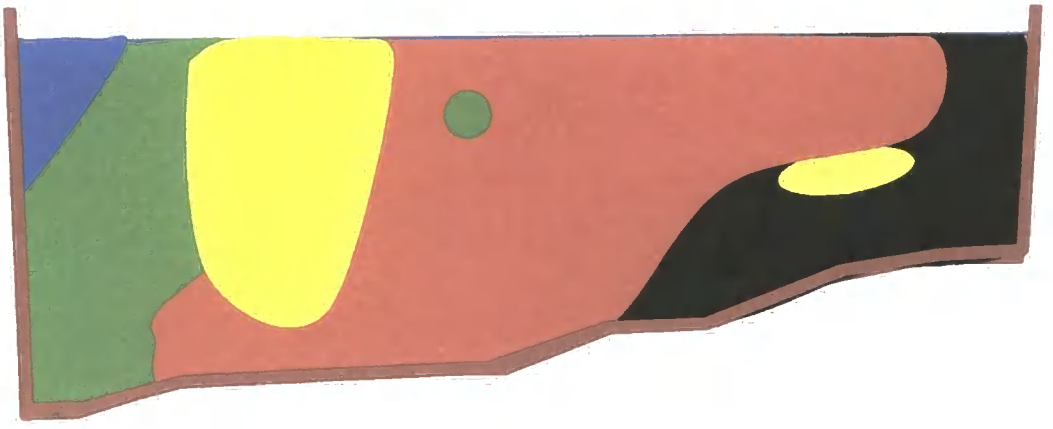


Chlorophyll (μg L^{-1}): blue = 131 to 150, green = 151 to 170, red = 171 to 190, yellow = 191 to 220

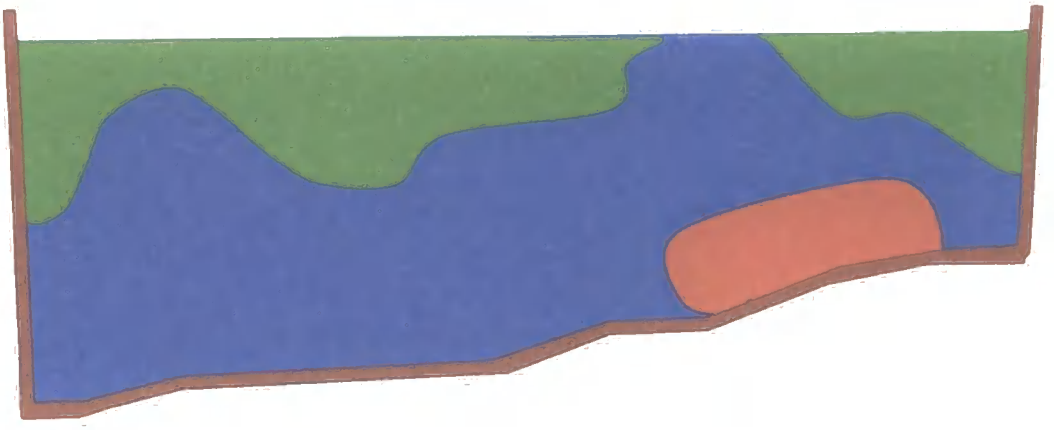
Figure 9.12 River profiles for velocity, temperature and chlorophyll at km 92.5 on 17/4/97 (10:17 to 12:37). 'X 'in upper plot signifies location of temperature probe and auto-sampler intake pipe. Drawn looking downstream. Not to scale: 32 m wide and 2.5 m deep (maximum dimensions). Each profile is based on 64 spot measurements.



Velocity (cm s^{-1}): blue = 5 - 10, green = 11 - 15, red = 16 - 20, yellow = 21- 25



Temperature (°C): blue= 12.0, green = 12.1, red = 12.2, yellow = 12.3, black = 12.4



Chlorophyll (µg L^{-1}): blue = 180 to 190, green = 191 to 200, red = 201 to 210

Figure 9.13 River profiles for velocity, temperature and chlorophyll at km 91.7 on 17/4/97 (14:50 to 15:50). 'X ' and 'Y' in upper plot respectively signify the locations of the routine sample site and sediment trap used during 1996. (Drawn looking downstream. Not to scale: 9.6 m wide and 2.8 m deep (maximum dimensions). Each profile is based on 36 spot measurements.

Table 9.4 River profile statistics. Mean and standard deviation values for velocity, temperature and chlorophyll from the river profiles shown in Figure 9.12 and 9.13. n = 36 and 64 for km 91.7 and 92.5 respectively.

	km 91.7		km 92.5	
	Mean	SD	Mean	SD
Velocity (cm s^{-1})	13.38	3.45	4.35	1.37
Temperature (°C)	12.21	0.10	11.54	0.05
Chlorophyll (μg L^{-1})	188.68	3.95	148.13	22.80

The wider downstream site (km 92.5) had considerably greater variability in chlorophyll than the upstream site, ranging from 133 to 208 μg L^{-1}, compared to a range of 185 to 202 μg L^{-1}. Temperature was more variable at the upstream site ranging from 12 to 12.4 °C compared to a difference of 11.6 to 11.7 °C at the larger downstream sites. Temperature differences need to be considered in light of general change during the survey, the temperature probe situated at km 92.5 recorded a 0.2 °C increase during the downstream survey and no change during the upstream work. Velocity was naturally faster at the narrower upstream section and this could have implications on sedimentation and mixing at this site. Chlorophyll concentrations of 149 and 157 μg L^{-1} were recorded for samples collected by the auto-sampler during the first and second survey, respectively.

Additionally to the upstream transect, spot surveys were carried out for chlorophyll and temperature in the main river and in two bays adjoining the river (the bay surveyed during 1995 and a bay on the opposite bank). The main river was found to have a significantly higher chlorophyll concentration than either of the bays (Mann-Whitney U test: $p = 0.0000$) but no difference was found between the bays or between temperature at any of the sites.

9.4 Extrinsic sources of phytoplankton

Tributaries

The three main tributaries surveyed (Figure 2.3) exhibited considerable variation in suspended chlorophyll (Figure 9.14). The chlorophyll series were decomposed using time series analysis to find the underlying trend and the resulting data compared using Mann-Whitney U Tests (Table 9.5). Tributary 2 (River Ise) has a significantly greater

chlorophyll concentration than tributary 1(Brampton Branch) and tributary 3 (Willow Brook) a significantly greater concentration than tributary 2.

The variability of chlorophyll concentration in the tributaries does not correlate with catchment area (Table 3.1), with tributary 3 having an area less than half that of the other two tributaries, but tributary chlorophyll does correlate with lake area. Tributary 3 has three lakes along its course and the contribution of these to the chlorophyll concentration in this tributary is investigated following a general review of suspended algae in the tributaries.

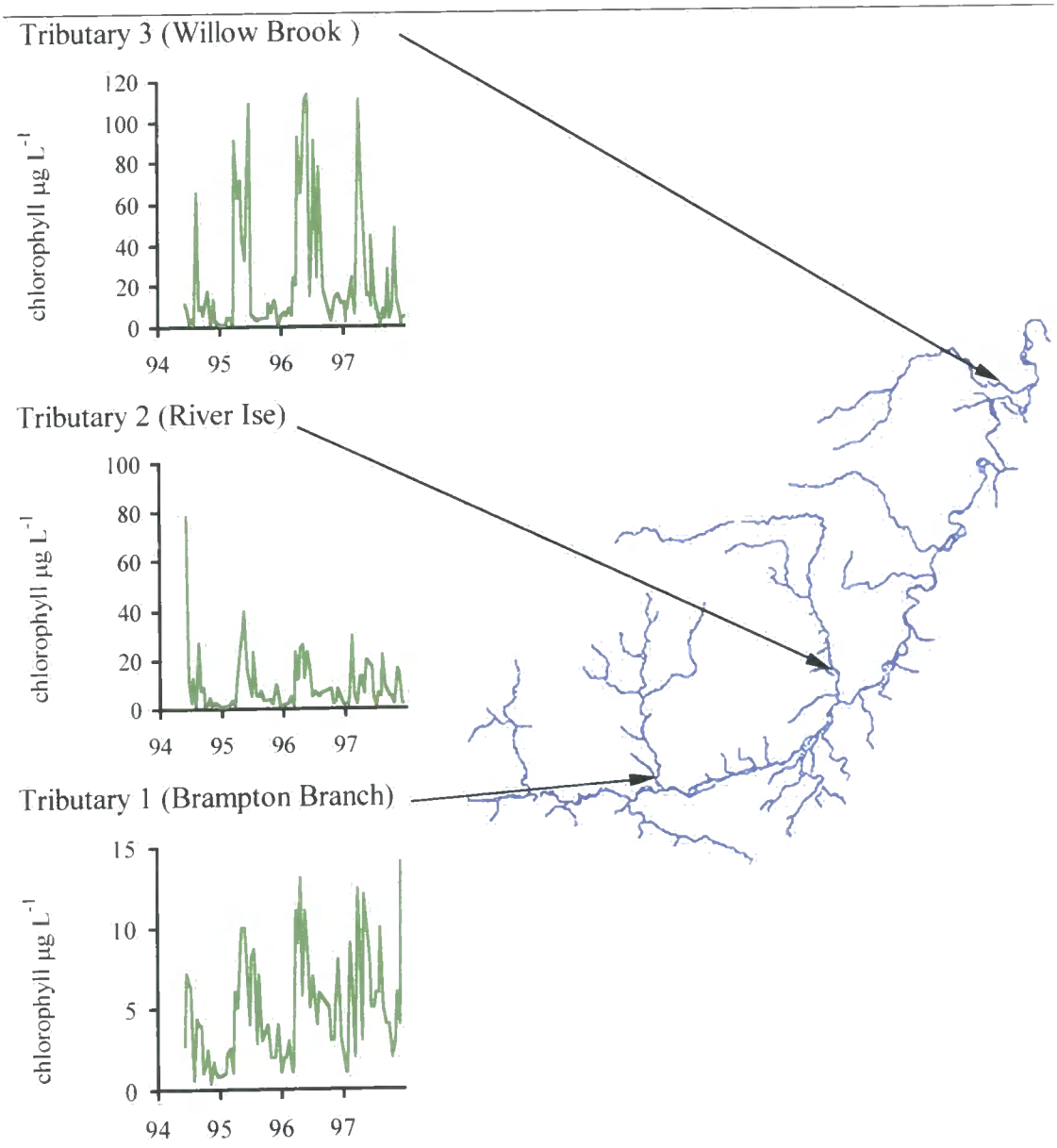


Figure 9.14 Chlorophyll concentrations at tributary sites (1994 to 1997).

Table 9.5 Chlorophyll concentrations in the tributaries. Showing overall median of data, median of fitted trend and *p* values for Mann-Whitney U tests. Tests are for paired samples working down the list (e.g. First *p* is for comparison between tributary 1 and tributary 2). Average sample frequency = 16 d and n = 84 for each series.

Site	Median chlorophyll ($\mu g\ L^{-1}$) Overall	Trend	*p*
Tributary 1 (Brampton Branch)	4.0	4.9	------
Tributary 2 (River Ise)	9.2	9.2	0.000
Tributary 3 (Willow Brook)	25.1	25.1	0.000

The principal algae of the tributaries was investigated paying particular attention to tributary 3, which had chlorophyll peaks of greatest magnitude. The low chlorophyll concentrations of tributary 1 consisted of benthic diatoms and a few green algae (as did the main river at km 22.4). The unusually high chlorophyll value recorded at the start of tributary 2 series consisted mainly of centric diatoms at a quantity not found thereafter. Tributary 3 had more prolonged periods with abundant centric diatoms early in the season followed by abundant green algae and some blue-green algae occurring later.

The origin of centric diatoms in tributary 3 was investigated on the 18/4/97 by taking chlorophyll and phytoplankton samples along its course, paying attention to the three lakes (Table 9.6). Centric diatoms were abundant throughout tributary 3 and particularly so downstream of Blatherwycke Lake (the largest of the three standing waters). The impact of tributary 3 on the main river was investigated by comparing chlorophyll concentrations in the tributary with those of the main river, up (km 85.2) and downstream (km 91.7) of the confluence (km 85.4). Time series analysis of spatial chlorophyll concentrations in the Nene identified a small but significant decrease in chlorophyll concentration between km 85.2 and km 91.7 (Table 9.1) so it seems unlikely that tributary 3 is impacting on the phytoplankton of the main river.

A comparison of contemporaneous chlorophyll concentrations in tributary 3 and km 85.2 and km 91.7 are shown in Table 9.7. Using this restricted data set no significant difference was found between any of the sites (Mann-Whitney U Tests). On a few occasions (marked 'r' in Table 9.7) tributary 3 had a chlorophyll concentration exceeding that of both km 91.7 and km 85.2, whilst km 85.2 had a lower concentration than km 91.7. It is under these conditions that a significant difference between km 85.2 and km 91.7 is most likely to occur, but none was found (Mann-Whitney U test: *p* = NS).

Table 9.6 Location (NGR), chlorophyll concentration (mg L^{-1}) and principal taxa in tributary 3. Abundance classifications (brackets) are as Table 3.5.

Site	chl.	Principal taxa
D/S Deene Lake (SP 955 928)	54	Centric diatoms > 6 to ≤ 10µm ∅ (V), *Nitzschia acicularis* (A), *Monoraphidium contortum* (A)
U/S Blatherwycke Lake (SP 971 957)	39	Centric diatoms > 6 to ≤ 10µm ∅ (C), *Nitzschia acicularis* (C)
D/S Blatherwycke Lake (TL 989 968)	123	Centric diatoms > 6 to ≤ 10µm ∅ (V), *Nitzschia acicularis* (A)
U/S Apethorpe Lake (TL 025 959)	76	Centric diatoms > 6 to ≤ 10µm ∅ (V), *Nitzschia acicularis* (A), *Monoraphidium contortum* (A)
D/S Apethorpe Lake (TL 030 945)	72	Centric diatoms > 6 to ≤ 10µm ∅ (C), *Nitzschia acicularis* (C)
Fotheringhay (routine site)	68	Centric diatoms > 6 to ≤ 10µm ∅ (A), *Nitzschia acicularis* (A), Centric diatom > 11 to ≤ 20µm ∅ (A)
Elton (TL 080 939)	65	Centric diatoms > 6 to ≤ 10µm ∅ (C), *Monoraphidium contortum* (C), *Cryptomonas* sp. (C), *Nitzschia acicularis* (C)

Table 9.7 Chlorophyll concentrations (mg L^{-1}) in tributary 3 (routine site) and the Nene at km 85.2 and km 91.7. (r) = restricted data set where chlorophyll at km 85.2 is lower than km 91.7 and tributary 3 is greater than both.

Date	km 85.2	Tributary 3	km 91.7
4 Apr 95	34	91 (r)	44
20 Apr 95	175	63	167
2 May 95	147	71	135
16 May 95	91	44	76
31 May 95	165	32	149
10 Apr 96	74	92	111
24 Apr 96	85	65	92
8 May 96	172	73	181
22 May 96	160	110	148
5 Jun 96	169	113	59
3 Jul 96	43	53 (r)	47
18 Jul 96	20	91 (r)	57
31 Jul 96	12	24 (r)	21
14 Aug 96	8	78 (r)	16
26 Mar 97	69	75	43
8 Apr 97	187	111	189
18 Apr 97	104	65	111
23 Apr 97	68	62	53

Billing marina

Following a preliminary investigation during 1994 and 1995 into the possible influence of algae from Billing Marina on the phytoplankton composition of the main river, a more extensive study was undertaken during 1996. The preliminary work identified the marina as a potential influence on the main river. High concentrations of blue-green algae (*Oscillatoria limnetica*, *O. arghadhii* and *Anabaena* sp.) were present in the marina during the summers of 1994 and 1995. The results of the 1996 survey are shown in Figure 9.15.

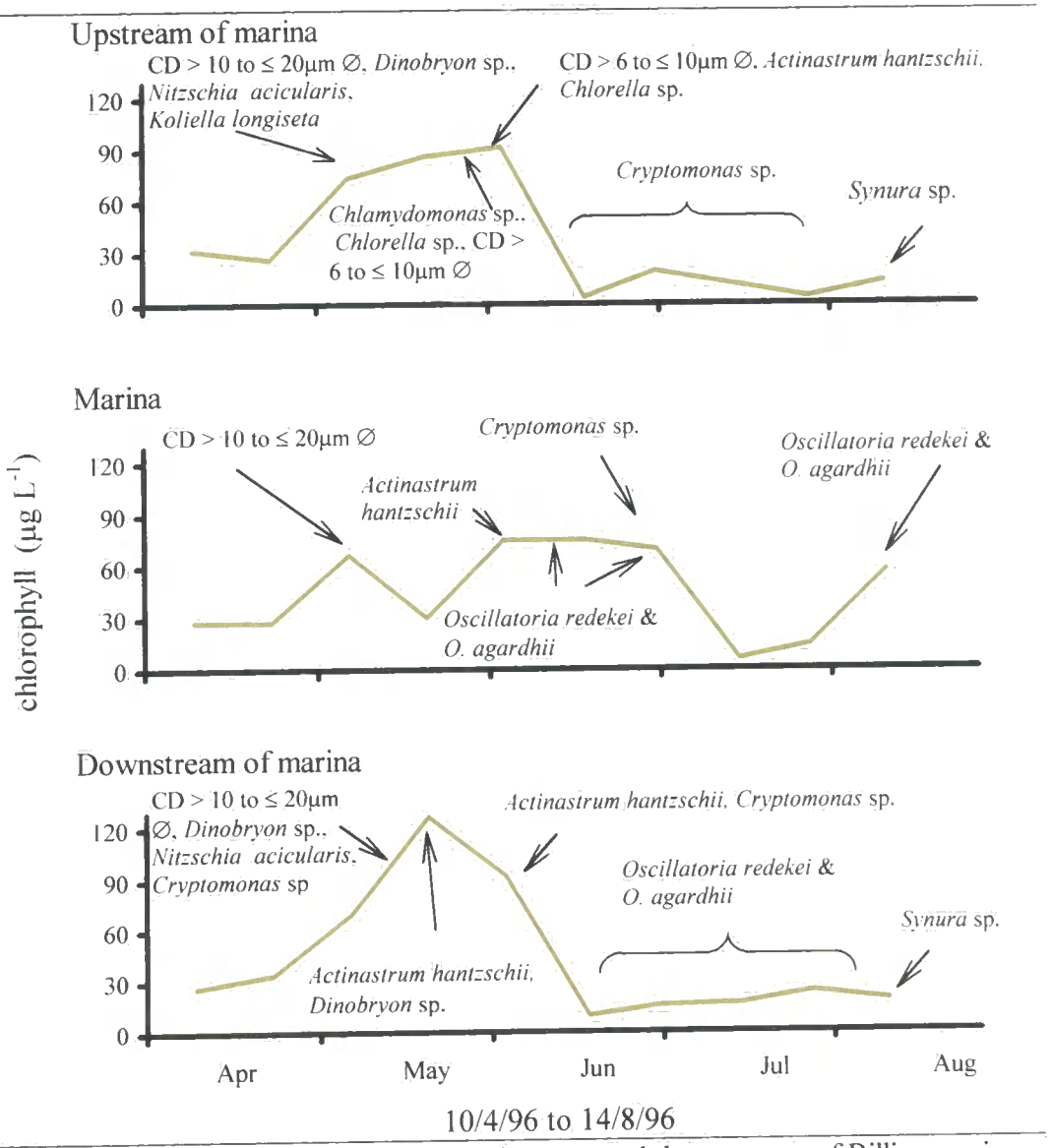


Figure 9.15 Chlorophyll and phytoplankton up and downstream of Billing marina and in the marina, during 1996. CD = Centric diatoms.

The influence of Billing Marina on the presence of blue-green algae in the main river is clear. An abundance of *Oscillatoria* spp. occurred in the marina and these

figure strongly in the plankton at the downstream site, whilst being absent upstream. *Dinobryon* sp. and *Synura* sp. were also abundant at this location but not so at any of the downstream sites. These taxa are often associated with unproductive or P-deficient environments (Sandgren, 1988) although they have been found to be abundance in P rich ponds (Reynolds, 1993). Both of these taxa are likely to be associated with the main river rather than the marina as they were also numerous at the upstream site.

Thrapston sailing lake

The outflow from Thrapston lake was investigated during 1996. This site was known to support high concentrations of blue-green algae during 1990 and 1995 (J.T. Krokowski, pers. comm.) and as this lake discharged directly into the Nene it was considered worthy of investigation. The contrast between the in and outflow of Thrapston sailing lake is illustrated in Figure 9.16. The lake receives water directly from the main river which returns via a sluice at the opposite end of the water body. On the 27/6/96 the lake had abundant *Aphanizomenon flos-aquae* and the river abundant centric diatoms. Centric diatoms entering the lake did not persist, but blue-green algae entering the river from the lake were found over a wide area, downstream of the outfall.

The chlorophyll and main phytoplankton at the Thrapston sailing lake outflow are shown in Figure 9.17. The lake experienced an early period dominated by the green alga *Chlamydomonas* sp. Following a period of low phytoplankton abundance the blue green alga *Aphanizomenon flos-aquae* became abundant in the lake, which was later succeed by *Anabaena flos-aquae*.

Thrapston lake inflow

Thrapston lake outflow

Figure 9.16 In and outflow of Thrapston sailing lake 27/6/96. Above: inflow water 'stained' brown with abundant centric diatoms flowing into *Aphanizomenon flos-aquae* 'bloom'. Below: surface 'scum' of *A. flos-aquae* adjacent to lake outflow. Arrows indicate direction of flow.

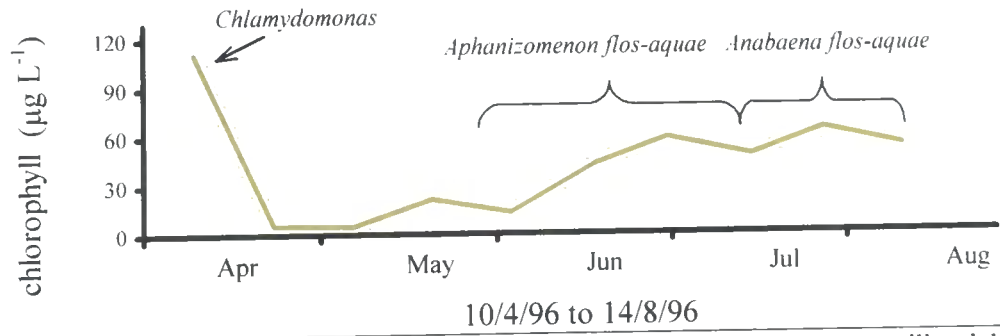


Figure 9.17 Chlorophyll and phytoplankton in outflow of Thrapston sailing lake during 1996.

Ad hoc surveys

Two additional sources of algae to the Nene were identified during 1996; Summer Leys nature reserve (SP 885 635) and Kinewell Country Park (SP 975 749). Summer Leys is of relatively recent construction (1993), whereas Kinewell lake has been established since 1983. Both of these lakes were observed to be discharging high concentrations of algae to the main river.

On the 11/4/96 Summer Leys lake was discharging *Chlamydomonas* sp. (chlorophyll = 74.1 $\mu g\ L^{-1}$) and on the 25/6/96 *Aphanizomenon flos-aquae* (chlorophyll = 337 $\mu g\ L^{-1}$). On the latter occasion the discharge was discolouring a large section of the main river.

Kinewell lake was discharging *Anabaena flos-aquae* into the main river on 25/6/96 (chlorophyll = 118 $\mu g\ L^{-1}$ - Figure 9.18). The peculiarity of the Kinewell site is it does not have a surface inflow and water is thought to enter the lake from the groundwater.

Evidence of impact on the main river during 1996

The impact of extrinsic sources of phytoplankton on the main river was not monitored in detail but several observations from the routine sample sites correspond with the various sources of phytoplankton identified during 1996.

Aphanizomenon flos-aquae were recorded in the routine sample (2078 cell mL^{-1}) from km 43.9 on the 20/6/96 and several times thereafter. This species was not seen in samples collected from km 34.0 and as km 43.9 is approximately 3 km downstream from the Summer Leys lake outfall it is likely that they originated here.

Aphanizomenon flos-aquae was not, however, recorded at km 64.6 during 1996, but was recorded along with *Anabaena flos-aquae* at km 91.7 and it is likely that these particular specimens originated from Thrapston lake (which is situated downstream of the routine site) and/or Kinewell lake. It appears that these species of blue-green algae can persist in the river over long distances if conditions are favourable.

Figure 9.18 Outflow water of Kinewell Lake containing a high concentration of *Anabaena floss-aquae* entering the main river (25/6/96). Arrows indicate direction of flow.

9.5 Discussion

Distinct and significant trends are evident in the longitudinal chlorophyll distribution. The upper catchment has relatively low chlorophyll concentrations with poorly defined seasonality, compared to the downstream locations. The dramatic increase in chlorophyll levels between km 22.4 and km 34.0 reflect a change in physical character, with the commencement of the navigation system. Suspended algae at the upstream site consist principally of benthic diatoms of the genera *Navicula* and *Nitzschia* with some ubiquitous greens like *Scenedesmus communis*, a pattern which is also evident in tributary 1.

The occurrence of phytoplankton in the navigation section is likely to reflect increased depth (Reynolds et al., 1990) rather than any other physical or chemical change in the river. Although the abundance of Chrysophytes (*Dinobryon* sp. and *Synura* sp.) at Great Billing may reflect the lower nutrient status at this site. These algae are less abundant at downstream locations which are also downstream of the Billing STW discharge and the associated increase in nutrients (Table 7.3).

Increases in chlorophyll concentration downstream from km 34.0 correlate significantly with estimated retention time to km 43.9, thereafter changes being less marked. The timing and magnitude of the chlorophyll peaks at the different sites is of considerable interest.

The data indicate a complex series of events resulting in downstream chlorophyll peaks occurring earlier and being of smaller magnitude than those at upstream sites. This pattern is apparent in the chlorophyll data but most significant in centric diatom abundance of the size class that corresponds to *Stephanodiscus hantzschii*. The disparity between chlorophyll and centric diatom abundance indicates that the absence of centric diatoms at the downstream site is being compensated for by the occurrence of another taxa, possibly *Koliella longiseta*. Although the extent of the upstream chlorophyll peaks are possibly underestimated because of the lower sample frequency there.

The early occurrence of downstream chlorophyll and phytoplankton peaks appears to be related to distance from source, which could indicate the contribution of algae from dead zones to the main river. The comparative investigation of chlorophyll and phytoplankton in the bay and main river, at km 91.7, supports this. The possible influence of a downstream temperature gradient has also been postulated (Balbi, 2000). The data indicates a downstream increase in temperature during the spring, although in the absence of contemporaneous measurements this cannot be confirmed. The influence of dead zones could also contribute to this phenomenon, although the difference between bay and main river temperatures were negligible. This finding may be misleading, however, as all measurements were taken before 10:00 and a temperature differential could occur between the two locations as the day progresses.

The occurrence of a downstream temperature gradient and contribution from dead zones offers a plausible explanation for the early occurrence of chlorophyll peaks at downstream sites. In Balbi (2000) it was suggested that the reason for upstream sites having later chlorophyll peaks of greater magnitude, than those downstream, was also related to temperature differences and facilitated by lower velocities. With suppression of abundance occurring at downstream sites, as temperature increases above the optimum for the most abundant centric diatom size class. The current work has provided an alternative explanation.

It does seem likely that temperature increases above 15°C to 18°C in the dead zones negates their importance as sources of phytoplankton to the main river and this is supported by the comparative investigation. The new insight into spatially differential chlorophyll and phytoplankton maxima is one of silica limitation. The downstream site has already been identified as being limited by available silica and if the supply to

upstream locations was greater than at downstream sites then this could explain the greater abundance of *Stephanodiscus hantzschii* there. This hypothesis is also supported by the greater abundance of *Stephanodiscus hantzschii* fo. *tenuis* at km 91.7. This species being most prevalent in silica depleted environments. This theory is supported by geographical evidence.

The first major tributary upstream from km 91.7 is tributary 3, joining the river over 6 km upstream. This tributary is likely to contain low silica concentrations as it supports considerable concentrations of diatoms. In contrast to km 91.7, both the km 64.6 and km 43.9 sites have reasonably sized tributaries within a kilometre upstream. Neither of these streams have lakes on their course so are unlikely to be silica limited for prolonged periods. This notion is tentatively supported by low frequency silicate samples at km 22.4 which exhibit high concentrations during many spring and summer samples. In the absence of spatial silica data it is difficult to postulate further.

The river profile investigations provided an insight into local variability. The downstream channel cross section was approximately three time greater than the routine site and the slower velocities resulted in greater sediment deposition there. The simultaneous measurement of velocity, temperature and chlorophyll is likely to have resulted in some disturbance of the sediment and this may have contributed to the variability in chlorophyll record. Any such occurrences are less likely to impact on measurements in the faster velocities of the upstream site. Irrespective of this possibility there was considerably greater variability in velocity and chlorophyll in the downstream channel compared to the artificial constraints at km 91.7. The greater temperature variability in the narrower channel may reflect the time of sampling rather than any other feature. Temperature was increasing during the first profile but had stabilised by the time the second was undertaken.

Both profile surveys provide confidence in sample point locations. The auto-sampler intake tube appears to have been located in a position of intermediate velocity, temperature and chlorophyll. The homogenous distributions at the upstream site vindicates the use of this sample point also.

Lentic systems within the Nene catchment can have a significant impact on the phytoplankton composition of the main river. This is particularly apparent during drier years and when intrinsic phytoplankton abundance is low. However, tributary 3 did not

have a detectable impact on the chlorophyll concentration of the main river, although this tributary appears to be a source of *Oscillatoria redekei.*

The results presented here highlight the difficulties of selecting representative sample sites on the Nene. The apparent influence of silica limitation and contribution of algae from lentic environments can be considerable.

9.6 Summary

1 The Nene supports a distinct phytoplankton with pronounced seasonality, at sites downstream from the commencement of the navigation.

2 Phytoplankton abundance appears to be restricted in the upper sections of the navigation by residence time, chlorophyll concentrations not achieving their maximum for approximately 20 km.

3 Chlorophyll and centric diatom peaks occurred earlier and were of lesser magnitude at downstream sites compared to upstream locations. This phenomenon was attributed to a downstream temperature gradient, contribution of dead zones and silica limitation at the downstream site.

4 Chlorophyll concentration and centric diatom abundance respond similarly to temperature throughout the river.

5 Lentic systems have a significant impact on the phytoplankton composition of the main river, particularly as sources of blue-green algae during dry years and when phytoplankton abundance is low in the river.

6 Some phytoplankton taxa in the upper catchment are normally associated with a lower nutrient status than those further downstream, which are downstream of the major STW discharges.

7 The most chlorophyll rich tributary, tributary 3, did not have a significant impact on the main river but this tributary appears to be a source of *Oscillatoria redekei.*

8 The results highlight the difficulties of selecting representative sample sites for evaluation river phytoplankton in the Nene. Spatial distributions can vary considerably and the apparent influence of silica concentrations and lentic sources of algae can be significant.

10 BIOLOGICAL INTERACTIONS AND LOSS FACTORS

10.1 Introduction

This chapter explores interactions between phytoplankton and other biota with particular reference to the loss of algae from suspension. Loss factors that will be considered are zooplankton, macrophytes and loss through sedimentation, which may be partly the result of biological factors. The aim of this investigation is to identify and qualify the conditions under which loss occurs. All examples and data presented are from km 91.7 (except rotifers in 1994 which were from km 94.3).

10.2 Zooplankton

The impact of planktonic rotifers will be considered for 1994 and 1996, years that had great contrast in the abundance of suspended chlorophyll. In both years the most abundant zooplankton taxa were *Keratella* sp., *Synchaeta* sp. and *Brachionus* sp., constituting 75% and 94% of total rotifers for 1994 and 1996, respectively. The abundances of all rotifers and the main rotifer taxa are shown in Figure 10.1.

The magnitude and extent of the chlorophyll peak was considerably greater during 1996 and this is reflected in both the magnitude and taxa composition of the rotifers. *Synchaeta* sp. were the most numerous taxa during 1994, having their peak in early May. This coincided with the *Brachionus* peak and was followed by a small number of *Keratella*. During 1996 *Synchaeta* sp. also had their peak abundance during early May but this was preceded and followed by several smaller *Synchaeta* peaks. Again in 1996 the *Synchaeta* peak was followed by a *Keratella* peak, but 1996 differed in the considerable abundance of this taxon.

A positive relationship exists between total rotifer abundance and chlorophyll concentration (Figure 10.2). The combined 1994 and 1996 data sets were significant ($r^2 = 0.67, p < 0.05$, n = 55), although there was far more variability in the 1994 data (1994; $r^2 = 0.44, p < 0.05$, n = 24; 1996: $r^2 = 0.82, p < 0.05$, n = 31).

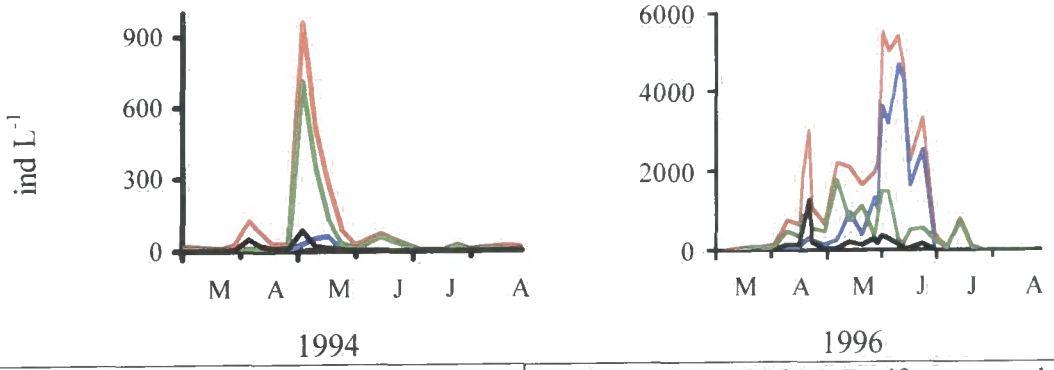


Figure 10.1 Rotifers abundance (ind L^{-1}) during 1994 and 1996. Rotifer taxa: red = all rotifers; blue = *Keratella* sp.; green = *Synchaeta* sp.; black = *Brachionus* sp.

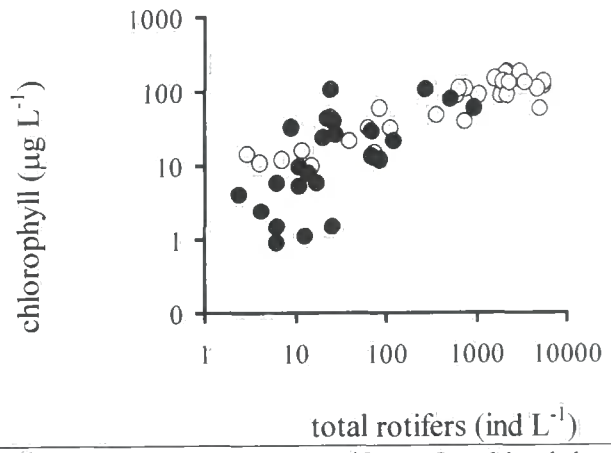


Figure 10.2 Chlorophyll plotted against total rotifers. Combined data for 1994 (solid circles) and 1996 (open circles).

The influence of discharge on total rotifer abundance was significant, with most variability in the latter half of the year (Figure 10.3), as seen in the phytoplankton data. Data from the first half of the year correlated most significantly ($r^2 = 0.80$, $p < 0.05$, n = 37) and the combined influence of chlorophyll and discharge on total rotifer abundance increased the significance of the relationship ($r^2 = 0.84$, $p < 0.05$, n = 37; total rotifers (ind L^{-1}) = 10^[3.48 - 2.74($\log_{10}$ discharge (m^3 s^{-1})) + 0.713($\log_{10}$ chlorophyll (µg L^{-1}))]; discharge and chlorophyll from 1 January and 30 June). Rotifer abundance correlated most significantly with discharge expressed as a 14 day average.

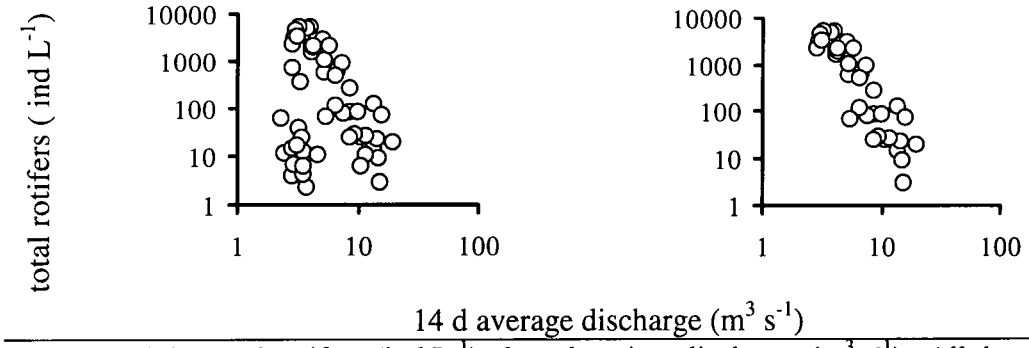


Figure 10.3 Total rotifers (ind L^{-1}) plotted against discharge (m^3 s^{-1}). All data (left) and data from 1 January to 30 June (right).

The food preference of *Keratella* sp. and *Synchaeta* sp. was investigated by multiple regression analysis, with the rotifer data expressed as individual and combined years. *Synchaeta* sp. were associated most significantly with green algae ($r^2 = 0.67$, $p < 0.05$, n = 42) and *Keratella* sp. with centric diatoms and green algae ($r^2 = 0.80$, $p < 0.05$, n = 42). Both taxa were associated more strongly with greens during 1996 and diatoms during 1994.

As rotifer periodicity does not appear to relate to food availability the influence of temperature was evaluated as a causal factor for the timing of rotifer abundance (Figure 10.4). *Synchaeta* sp. are at their most abundant at a temperature of 11.5°C and *Keratella* sp. at 17.5°C, with *Brachionus* sp. having a temperature optimum similar to *Synchaeta* sp.

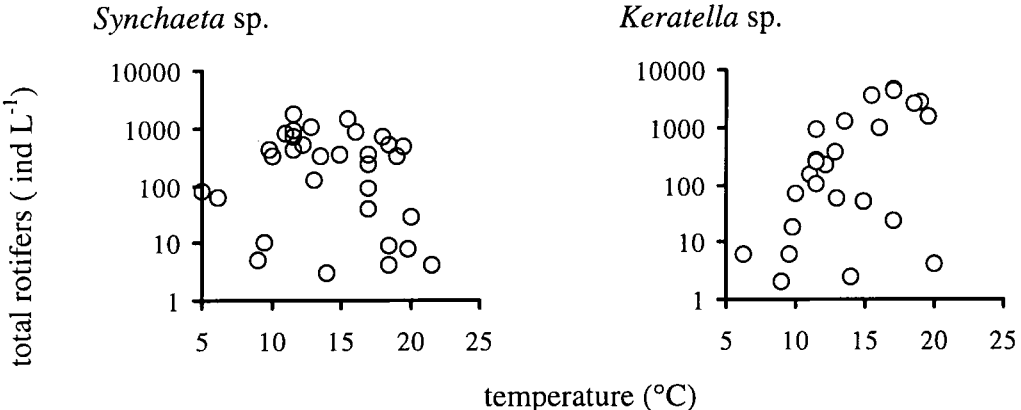


Figure 10.4 *Synchaeta* sp. and *Keratella* sp. (ind L^{-1}) plotted against temperature (°C).

A comparison between total rotifer abundance and discharge, temperature and chlorophyll is shown in Figure 10.5. Discharge up to June 1994 was mostly greater

than 10 $m^3 s^{-1}$, with the exception of a short period at the beginning of May when it declined to less than 5 $m^3 s^{-1}$. This period of reduced discharge coincided with the peak of rotifer abundance. In 1996 flow declined below 5 $m^3 s^{-1}$ during April and remained below this level from May onwards.

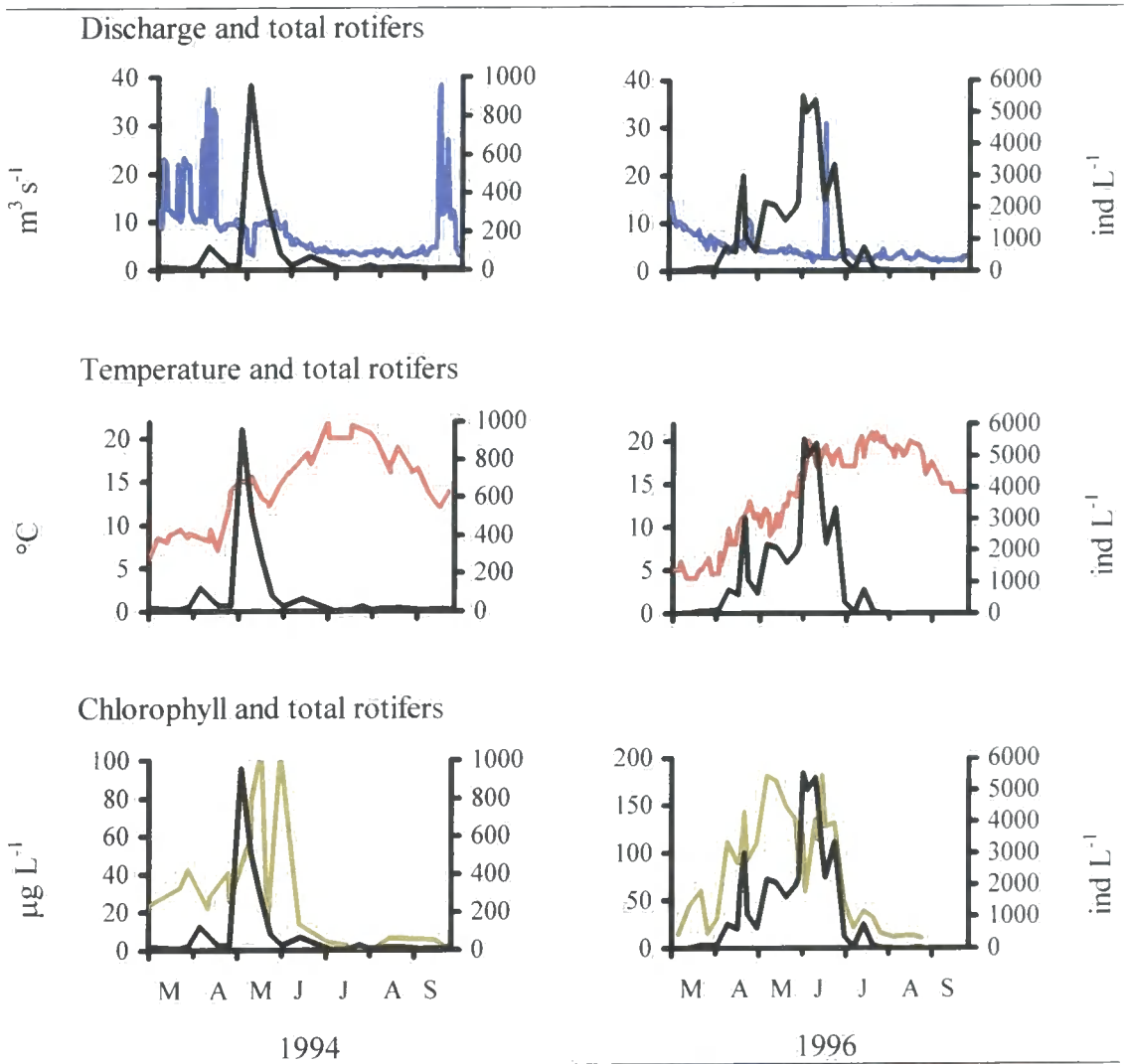


Figure 10.5 Total rotifers (ind L^{-1}) plotted against discharge ($m^3 s^{-1}$), temperature (°C) and chlorophyll (µg L^{-1}). Black = total rotifers.

Temperature was favourable during both years from May onwards although this was not reflected in the abundance of rotifers, therefore rotifer maxima were probably more influenced by discharge than temperature, especially during 1994.

The chlorophyll concentration had a short lived peaks of low magnitude during May and June 1994. The magnitude of these peaks is likely to result from high discharge and the reduced chlorophyll that occurred between the two peaks could partly relate to a concurrent reduction in temperature and slightly elevated discharge. It

appears that during 1994 the decline in rotifers was the result of increased discharge, rather than food availability.

In 1996 the peak rotifer abundance occurred in the early part of June, during a period of stable discharge and temperature. It is tempting to attribute the concurrent reduction in chlorophyll concentration that occurred at the time of high rotifer abundance to grazing, but chlorophyll was in decline from the middle of May when rotifers were in low abundance.

Ciliates were not monitored during 1994 but during 1996 they achieved abundances of 3120 and 2168 cell mL^{-1} in April and May respectively. Ciliate abundance was positively associated with chlorophyll concentration ($r^2 = 0.74$, $p < 0.05$, n = 25) and was most significantly associated with centric diatoms and green algae ($r^2 = 0.71$, $p < 0.05$, n = 19). Ciliate peaks occurred throughout the period of phytoplankton abundance and therefore had a wide temperature range.

Copepods (particularly nauplii) and occasionally cladocera were present in the zooplankton samples during 1994 and 1996. Nauplii achieved maximum abundances of 22 and 38 ind L^{-1} during 1994 and 1996, respectively. Adult copepods and cladocera rarely occurred.

10.3 Macrophytes

The influence of suspended chlorophyll concentration on submerged macrophyte abundance was assessed by investigating their relationship. Data from three sites were combined using standardised variables for percentage cover of submerged macrophytes and average annual chlorophyll preceding each macrophyte survey (Figure 10.6).

The survey sites were Cogenhoe Back Channel (a few km downstream from km 34.0), White Mills (a few km upstream from km 39.8) and km 52.2 (routine site) and the surveys took place between 1993 and 1996. A significant negative relationship was found between the abundance of submerged macrophytes and preceding annual average chlorophyll concentration ($r^2 = 0.82$, $p < 0.05$, n = 15). The greatest abundance of macrophyte and lowest chlorophyll concentrations occurred during 1994 whilst the opposite trend was seen in 1995 and 1996. A fourth site (Ditchford Lock - few km upstream from km 52.2) was rejected as four out of the five samples from this impounded stretch behaved differently from those of other locations.

The influence of average temperature and discharge on macrophyte abundance was examined and both were non-significant (temperature: $r^2 = 0.04$, p = NS, n = 15; discharge: $r^2 = 0.19$, p = NS, n = 15).

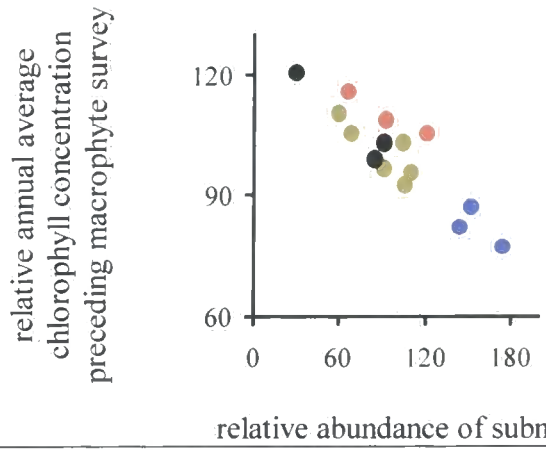


Figure 10.6 Submerged macrophyte abundance plotted against chlorophyll. Data is from three sites and standardised for percentage cover of submerged macrophytes and average annual chlorophyll preceding each macrophyte survey. 1993, red; 1994, blue; 1995, green; 1996, black.

10.4 Sedimentation

The results from the sediment trap used at km 91.7 during 1996 indicate the presence of physical and biological loss factors. Estimated daily sediment deposition, physical and chemical variables are shown in Figure 10.7. Inorganic sedimentation was variable, being lowest in March and September and greatest in early May. Organic sediment was greatest in late July, but approximately proportional to inorganic sediment throughout the survey ($r^2 = 0.88$, $p < 0.05$, n = 14).

High inorganic sedimentation in May is likely to result from spate flows in late April. The organic peak in late July could result from sedimentation of phytoplankton following their decline.

The z_{eu} readings indicate non phytoplankton turbidity both preceding and following the chlorophyll peak. High turbidity in March is likely to result from a sediment load caused by high rainfall and maintained by high discharge. Turbidity following the chlorophyll peak could result from dead algae and detritus resulting from the period of phytoplankton abundance.

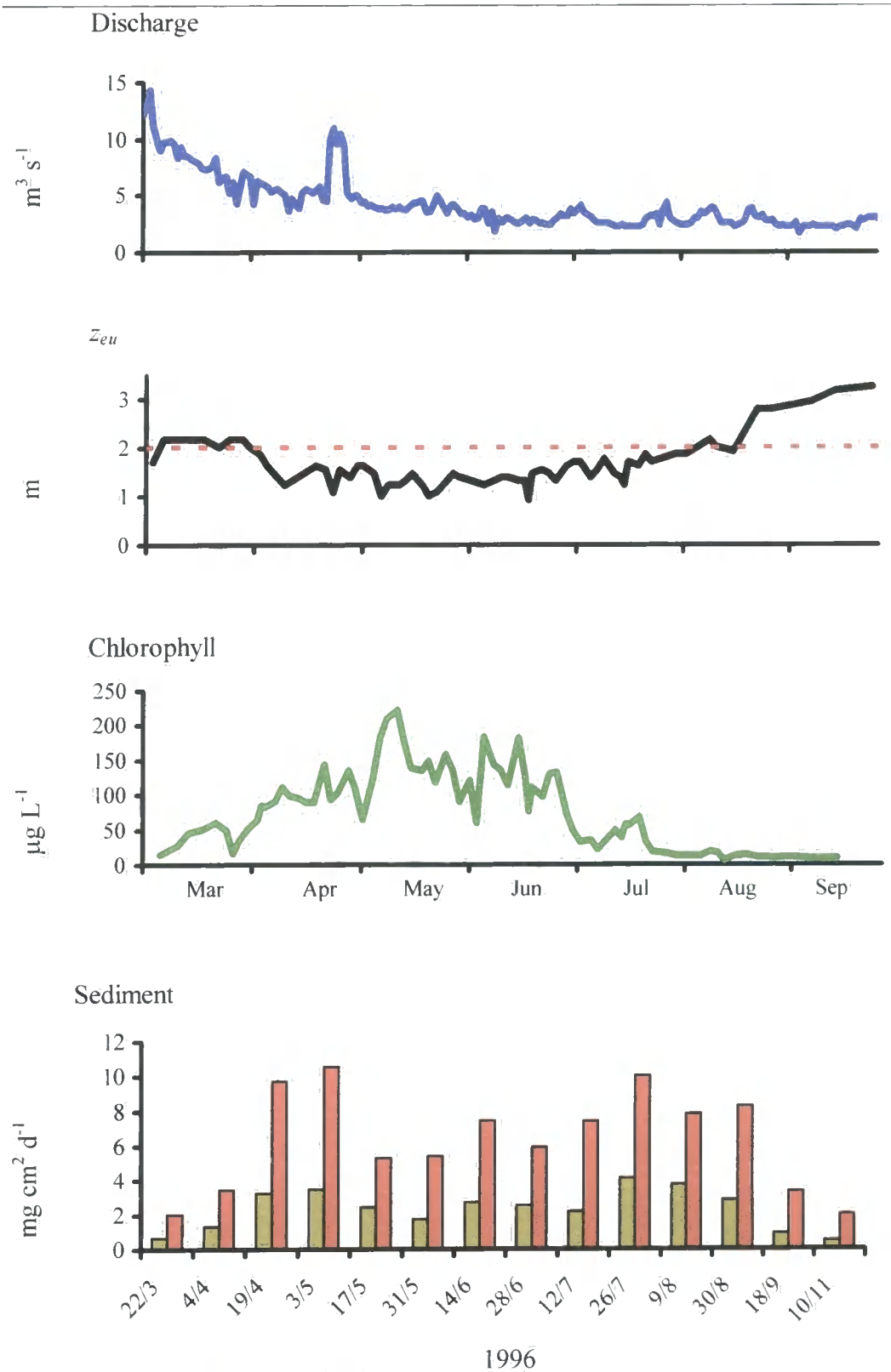


Figure 10.7 Discharge , z_{eu}, chlorophyll and sedimentation during 1996. Sedimentation: inorganic, organic. Dashed line on z_{eu} plot indicates average river depth.

The numerically most important taxa from the sediment trap are shown in Table 10.1, and constitute 88% of live and 77% of dead cells found. The greens consisted of a wide range of taxa represented in the plankton.

Table 10.1 Percentage occurrence of most abundant live and dead cells in sediment trap during 1996. Total abundance of cells = 21.3×10^6 and 23.6×10^6 for live and dead cells respectively.

Taxa	Condition	%
Centric diatoms ≤ 5 μm ∅	live	4
	dead	10
Centric diatoms > 5 to ≤ 10 μm ∅	live	22
	dead	54
Centric diatoms > 10 to ≤ 20 μm ∅	live	6
	dead	8
Cocconeis spp.	live	0
	dead	5
Greens	live	56
	dead	0

Dead centric diatoms were represented by clean intact frustules which were devoid of cell contents and their occurrence was possibly the result of parasitism. This phenomenon was manifest in all centric size classes. A comparison of live and dead cell abundances in the sediment trap and plankton is shown in Figure 10.8. In this Figure the plankton record is an average of samples taken during each deployment of the trap (ca. 14 d).

The centric diatoms start with equal proportions of dead and live specimens captured in the sediment trap, but following mid-April the proportion of dead cells increases exponentially. The difference between the proportion of live and dead diatoms was most pronounced in early May. This situation was similar for centric diatom ≤ 5 μm ∅ but the largest diatoms had their biggest differential in the middle of June.

Very few dead greens algal cells were found and the proportion of live cells found in the trap mirrored those of the plankton ($r^2 = 0.85$, $p < 0.05$, n = 14). One of the largest outliers in this data set is associated with late July and possibly represents the sedimentation of green algae as the period of plankton abundance ends. The high degree of consistency between live green algae in the plankton and those in the sediment trap suggests a continuous sedimentation rate in proportion to abundance. The relationship between live centric diatoms in the trap and the plankton was not so strong

as for green algae but was also significant ($r^2 = 0.66$, $p < 0.05$, n = 9), the greater variation presumably arising from large numbers of dead cells.

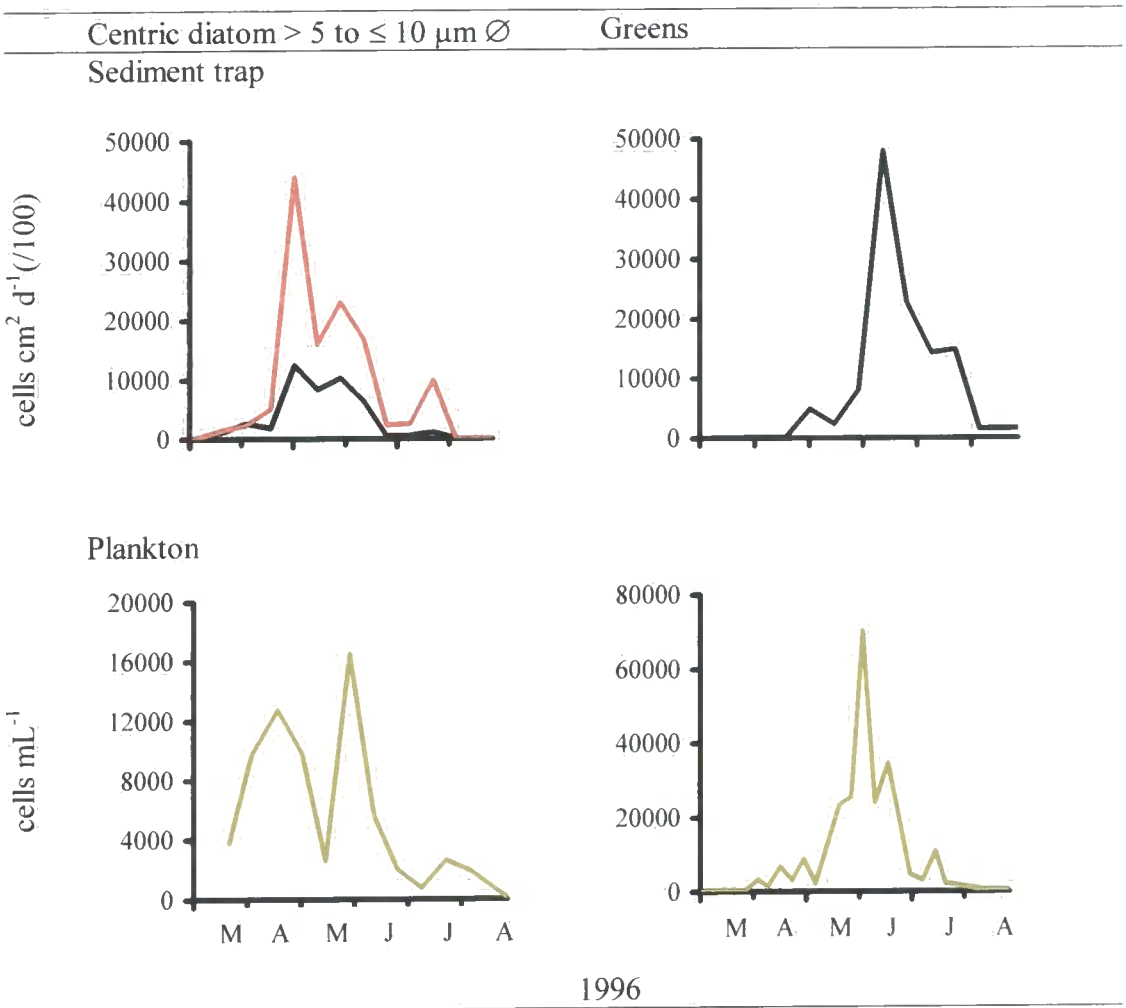


Figure 10.8 Centric diatoms and green algal cells in the sediment trap (above) and in the plankton (below). Sediment trap: black = live cells; red = dead cells. Plankton data are averaged over each period of the sediment traps deployment.

The possibility that the exponential increase in abundance of dead centric diatoms in the traps was related to parasitism was tentatively explored, assuming that small flagellates ≤ 5 µm GALD were involved in the loss process. Contemporaneous values for discharge, temperature dead diatoms in the traps, diatoms in the plankton and small flagellates are shown in Figure 10.9.

The loss of planktonic centric diatoms and increase of dead cells in the sediment trap do not relate to variations in either discharge or temperature. With both these physical variables being favourable to phytoplankton development. Increasing flagellates correspond with a decrease in planktonic centric diatoms and an increase in dead cells in the sediment trap. Planktonic flagellates and centric diatoms exhibit a

phased response that is often evident in 'predator-prey' relationships (Begon et al., 1987).

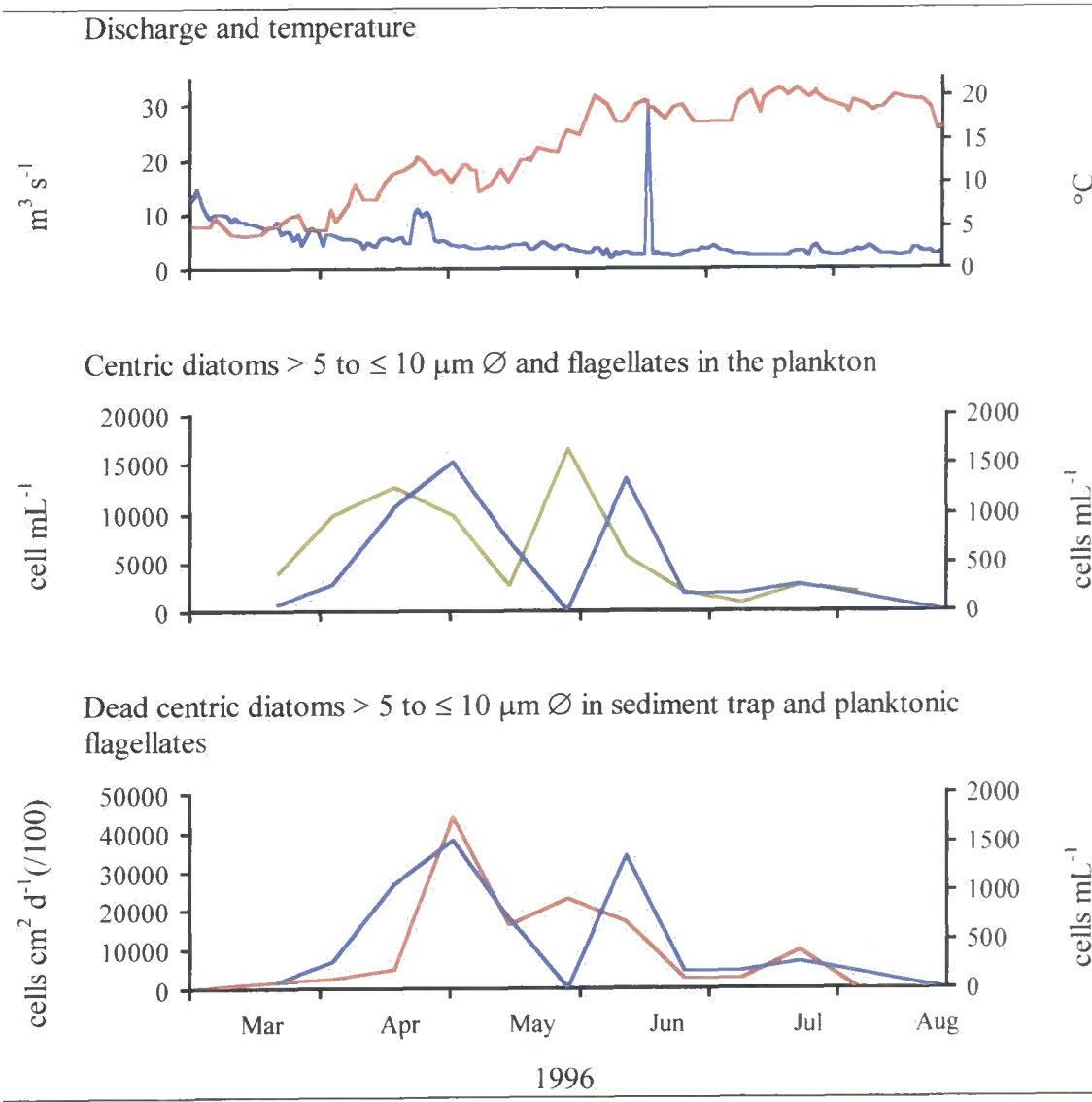


Figure 10.9 Discharge and temperature (top), Centric diatoms > 5 to ≤ 10 µm ∅ in the plankton and unidentified flagellates ≤ 5 µm GALD in the plankton (middle) and dead Centric diatoms > 5 to ≤ 10 µm ∅ in the sediment trap and unidentified flagellates ≤ 5 µm GALD in the plankton (bottom trap). Plankton data averaged over period of each sediment trap deployment. Diatoms left axes, flagellates right axes.

The possibility that small flagellates could be related to loss of phytoplankton from suspension was further explored using 1994 data (Figure 10.10). As with the 1996 data the flagellates and centric diatoms > 5 to ≤ 10 µm ∅ exhibit a classic 'predator-prey' relationship. However, the occurrence of parasitism does not explain the ultimate decline in suspended algae that occurred in 1994 and 1996.

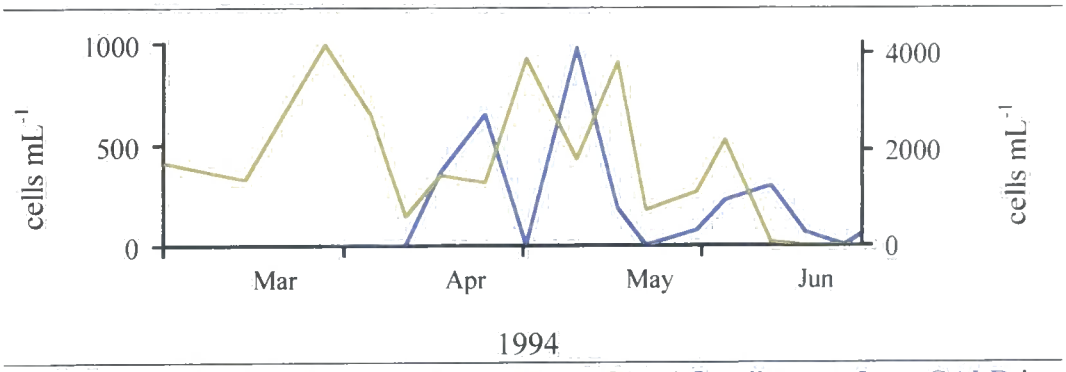


Figure 10.10 Centric diatoms > 5 to ≤ 10 µm ∅ and flagellates ≤ 5 µm GALD in the plankton during 1994.

Other notable patterns in the taxa found in the sediment trap were, the occurrence of dead *Cocconeis* spp. which only occurred from July onwards and live *Glenodinium* sp. during June and July.

10.5 Discussion

It is difficult to quantify loss factors, in view of the range of complex interactions and processes occurring simultaneously. However, with the aid of published or theoretical values the importance of each component can be estimated.

Rotifer abundance and periodicity was controlled by temperature, discharge and food availability. Rotifer abundance correlated most significantly with daily discharge averaged over longer periods than those used for the phytoplankton, and this probably reflects the slower growth rate of these organisms.

Rotifers grazing has the potential to significantly reduce phytoplankton populations. The filtration rate of these animals will vary according to temperature, size and density of prey. Filtration rates of 0.02 to 0.11 mL d^{-1} have been cited (Reynolds, 1993). These values would result in filtration volumes ranging from 2 to 105 mL d^{-1} and 11 to 646 mL d^{-1} respectively for the maximum rotifer abundances that occurred during 1994 and 1996. From these values it appears unlikely that grazing was having an appreciable impact on phytoplankton abundance during 1994, although during 1996 some 'top down' control of the phytoplankton could have been occurring.

Rotifer abundance will also be limited by food particle size. The principal rotifers found in the Nene would be able to handle particles up to about 12 and 18µm for *Keratella* sp. and taxa of a similar size to *Synchaeta* sp., respectively (Reynolds, 1993). Using these values it is unlikely that the early decline of rotifers during 1994 was due to

food limitation. Although this may be so during 1996 where rotifer decline closely followed the suspended algae.

It appears that periods of high rotifers abundance did not result in a large loss of suspended algae, either during 1994 or 1996. Rotifers seem to have been limited by discharge during 1994 and although there was a great abundance of *Keratella* sp. during 1996 they did not result in an appreciable decline in phytoplankton abundance. In the Nene rotifers are likely to grow more slowly than phytoplankton (rotifers: Sanderson, 1998; phytoplankton: Reynolds, 1993) and as optimal conditions for phytoplankton species will correspond with optimal conditions for rotifers it seems likely that rotifers are controlled by phytoplankton abundance, rather than phytoplankton being controlled by rotifers. The negligible impact of rotifer grazing on phytoplankton abundance is in contrast to the dramatic population crashes in centric diatoms, that appear to be the result of parasitism.

There is good evidence of significant parasitism of the centric diatom populations. This is supported by the high quantity of dead diatoms in the sediment trap, their condition and the concurrent disappearance of centric diatoms from the plankton. The apparent relationship between the increased abundance of small flagellates and reductions in planktonic diatoms is of considerable interest, but not a key piece of evidence for the occurrence of parasitism.

As mentioned above, the dead centric diatoms were predominantly intact frustules completely devoid of cell contents. The only plausible explanation for this phenomenon is parasitism (Canter, 1979). Chytrids were frequently seen attached to centric diatoms, but no obvious pattern or frequency of occurrence was noted. Likewise, dead diatoms in the aforementioned condition were occasionally seen in the plankton, but these only constituted a small percentage of total cells and it appears that dead cells are quickly lost from suspension. The low abundance of dead green algae in the sediment trap either indicates low parasitism of this group or that dead greens remain in suspension. As the latter situation was not observed it seems likely that parasitism of greens was minimal, compared to the centric diatoms.

Ciliates were present throughout the period of phytoplankton abundance and probably represent a range of species. Some planktonic ciliates feed preferentially on phytoplankton (Foissner and Berger, 1996) and these are likely to exert loss pressure on the phytoplankton community.

The sediment trap produced invaluable data and functioned satisfactorily over a range of conditions. The trap had a $l{:}\varnothing$ ratio of 6.5, which is somewhat less than that recommended for use in turbulent conditions (Bloesch and Burns, 1980). Nevertheless, the trap retained fixative throughout each period of deployment indicating an absence of currents within the chamber. Results from sediment traps can be inherently misleading, with some devices over-trapping sediment (Kozerski, 1994). Notwithstanding this, the sediment trap provided valuable data that would have otherwise been unattainable or overlooked.

Sedimentation rates are extremely heterogeneous in rivers (Tipping et al., 1993). It is therefore dangerous to draw river-wide conclusions about sedimentation from a single point. The location of the trap, at km 91.7 (Figure 9.13), was chosen for ease of access, security and river depth. The river section at this point is uncharacteristically narrow and consequently has an atypically high velocity (Chapter 9). The consequences of this on the collection of sediment is unclear, but the location should have ensured a continuous and unidirectional passage of water and eliminated the possibility of disproportional deposition that can occur in eddies.

Throughout the study of sedimentation the deposition of live cells was approximately proportional to their concentration in suspension, and sedimentation rates appear to be independent of variations in discharge. This factor indicates the presence of turbulent flow and entrainment of cells throughout the period of phytoplankton abundance.

The relationship between submerged macrophyte abundance and preceding chlorophyll concentration could be of great significance. The data indicate that phytoplankton can suppress submerged macrophyte growth, presumably through shading. Following the 1976 drought macrophyte growth was so restricted in the Nene, compared to the pre-drought conditions, that 'weed' cutting was abandoned. Brierley et al. (1989) concluded that this situation was mainly caused by excessive winter scouring and dredging practices. However, the data indicates that a series of cool, wet summers could have promoted prolonged periods of phytoplankton abundance, which in turn could have suppressed macrophyte growth (Balbi, 2000).

The theory that phytoplankton suppress submerged macrophyte growth was supported by the sediment trap data. *Cocconeis* sp. are associated with submerged aquatic plants (Pentecost, 1984; Cox, 1996) and the absence of this taxa throughout

periods of phytoplankton abundance indicates the absence of submerged macrophytes. Likewise the sudden occurrence of these diatoms following the decline in phytoplankton is thought to indicate the occurrence of submerged plants.

The impact of submerged macrophytes on phytoplankton is less easy to establish from the data, although the literature suggests that macrophytes can inhibit phytoplankton development in a number of ways (Scheffer, 1999). In the Nene it is likely that the most important impacts of macrophytes are their interference with turbulent flow and inhibition of algal resuspension.

10.6 Summary

1 Rotifers can be abundant in the Nene and their abundance and periodicity is limited by temperature, discharge and food availability. The slower growth rate of rotifers, compared to phytoplankton, results in them requiring periods of greater stability to proliferate than are required by phytoplankton.

2 The evidence from 1994 and 1996 suggests that rotifer abundance tends to mirror periods of phytoplankton abundance rather than control them.

3 The sediment trap data suggests that centric diatoms suffer from severe parasitism and this appears to result in significant reductions in the abundance of centric diatoms. There was also some evidence that unidentified flagellates ≤ 5 µm GALD are implicated in parasitism.

4 Submerged macrophyte abundance correlates negatively with the annual average chlorophyll concentration preceding the plant surveys. Therefore phytoplankton appear to be restricting submerged macrophyte growth through shading.

5 The sudden occurrence of *Cocconeis* sp. in the sediment traps following the decline of phytoplankton is thought to indicate a rapid increase in submerged macrophyte abundance, which in turn inhibits the return of abundant phytoplankton.

11 DISCUSSION

11.1 Synopsis and introduction

The aims of this research have been achieved, although the hypothesis that 'the key factors controlling phytoplankton populations in a small river are not dissimilar to those acting in larger systems' can only be partially accepted (Chapter 1). The Nene supports high concentrations of phytoplankton which are controlled by similar processes to those of larger systems, but the small size of the Nene combined with geological and geographical factors result in it sometimes behaving very differently to larger river systems.

The apparent 'switching' of the Nene from phytoplankton to macrophyte dominance is not reported from larger systems. This feature of the Nene results from its relatively shallow depth which allows submerged macrophytes to flourish under certain conditions. The geological and geographical features of the Nene catchment result in a wide flow regime (impermeable geology and low rainfall), which may have exerted selection pressures on the phytoplankton which are absent in larger systems. For example, the Nene's unusually wide flow regime may have produced selection pressures, through silica limitation, for a low temperature optimum in some of the centric diatom populations.

These features of the Nene will be explored more fully in this chapter, which is intended to consolidate the results of Chapters 4 to 10 into a unified consensus. This will be undertaken with an overview of phytoplankton in the Nene followed by a detailed appraisal of controlling factors, consideration of modelling and concluding with a summary of methodological approaches to the Nene and river phytoplankton in general.

11.2 Overview of phytoplankton in the Nene

The chlorophyll concentration at km 91.7 over the 22-year period exhibits considerable variation. There appears to have been a higher chlorophyll concentration in the early part of the period and higher light and lower discharge latterly (Table 7.1), although significant inter-year variation was seen in all variables examined. Despite this variation there is considerable inter-year constancy in chlorophyll concentration in

relation to physical variables. Fortuitously, during the period of phytoplankton analysis considerable inter-year contrast occurred. 1994 had the lowest chlorophyll maxima for the 24-year period and 1996 had a high and prolonged chlorophyll concentration.

Chlorophyll concentrations and similarities and differences in taxa composition between 1994 and 1996 are illustrated in Figure 11.1. The years were clearly different in the magnitude and extent of chlorophyll, with 1996 having a maximum over twice that of 1994. However, irrespective of this difference the form of the phytoplankton was very similar in the early part of both years.

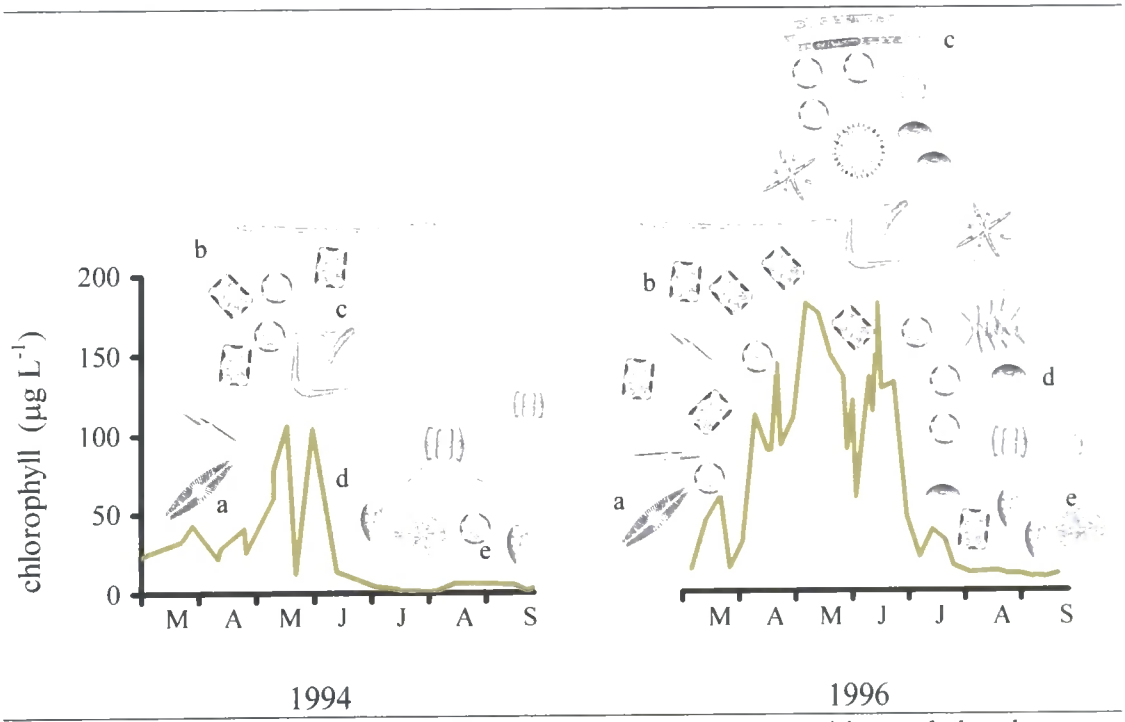


Figure 11.1 Schematic representation of phytoplankton composition and abundance during 1994 and 1996 at km 91.7. Letters refer to the description in the text. Chlorophyll = green line. Illustrations of phytoplankton modified from Belcher and Swale (1978 and 1979). Influence of spate flows during September 1994 not represented. Illustrations not to scale.

Both 1994 and 1996 commenced with the occurrence of *Navicula lanceolata*, *Nitzschia acicularis* and centric diatoms (a: Figure 11.1). As time progressed centric diatoms became more abundant and both years also had the narrow filamentous blue-green algae *Oscillatoria redekei* and *O. limnetica* (b). From mid-May onwards, green algae became more abundant (c) but the abundance and diversity of greens was far greater in 1996, with *Actinastrum hantzschii*, *Monoraphidium contortum* and *Chlorella*. spp. becoming particularly numerous. *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* were also present in 1996, but absent from 1994.

From mid-June 1994 the abundance of phytoplankton declined to a very low level (d) and remained so until the September spate (Chapter 9). This period was characterised by *Rhodomonas lacustris* var. *nannoplanktica*, *Scenedesmus communis* and benthic pennate diatoms like *Cocconeis* spp. (e). There were also abundant picoplankton during the summer of 1994, when other algae were scarce (not represented in Figure 11.1). A similar period of low phytoplankton abundance with suspended benthic diatoms also occurred in 1996, but two months later than in 1994 (e). The summer of 1996 (d) was characterised by a continuation of centric diatoms and mixed green algae.

The similarities and differences seen between 1994 and 1996 characterise the phytoplankton of the 22-year period and provide a starting point from which generalisations can be made. The first half of the year appears to contain far greater consistency in phytoplankton abundance and periodicity than occurs between July and December. This temporal trend was seen in the long term chlorophyll data and the more recent species analyses. The processes involved in phytoplankton trends in the Nene lie in an understanding of the key controlling variable.

11.3 Controlling variables

Those factors identified as important controls of phytoplankton are listed in Table 11.1. Other factors, like N and P, are not thought to be important at this time. Some of the factors listed in Table 11.1 have multiple effects on the timing, magnitude and species composition of phytoplankton populations. Each of these factors will now be considered.

Table 11.1 Principal factors controlling phytoplankton abundance in the Nene

Physical	Depth
	Discharge
	Extrinsic sources
	Dead zones
	Light
	Retention time
	Temperature
Chemical	Silica
Biological	Grazing
	Macrophytes
	Parasitism

Depth and retention time

The influence of water depth on phytoplankton development is clear in the Nene. A characteristic river phytoplankton does not occur in the Nene until the commencement of the navigation, at Northampton. Depth influences phytoplankton development by facilitating turbulent flow, which cannot occur so readily in shallow rivers (Smith, 1975).

Retention time in the Nene was identified as a factor influencing phytoplankton development. Chlorophyll maxima at km 34.0 were significantly lower than the next downstream sample site (km 39.8) and km 39.8 had a significantly lower concentration than km 43.9. Significant differences did occur downstream of km 43.9 but these were small in comparison to the upstream sites. Retention time is, of course, an approximation as it assumes non-laminar flow and ignores the influence of eddies and dead zones. Regardless of these shortfalls the Nene appears to require a distance of about 20 km before phytoplankton can achieve their maximum abundance. The influence of retention time cannot be evaluated further in the absence of silicate data for these sites.

The depth of the navigable Nene is reasonably consistent, at about 2 m. This depth is likely to create a favourable light climate for suspended algae in a turbulent river system. The minimum euphotic depth in the Nene is about 1 m and under these conditions algae spend approximately half their time below the photic-zone. Consequently, as river depth increases suspended algae will spend increasingly longer in darkness. This is illustrated in the River Meuse where a downstream decrease in phytoplankton biomass was attributed to increasing depth (Descy and Gosselain, 1994).

Discharge

The multiple-influences of discharge are illustrated in Figure 11.2. High discharge can result in a high suspended sediment load, which will reduced the euphotic depth and restrict phototroph abundance. In the Nene this situation is often apparent in the early spring when the high winter flows are carrying suspended matter washed in from agricultural land. Suspended chlorophyll at this time is low and the first peak normally coincides with increased light attenuation. The early occurrence of *Navivula lanceolata* in the plankton may also be related to this factor with recruitment probably originating from the shallower margins. The occurrence of this large diatom in suspension illustrates another important feature of discharge, the creation of turbulence.

The influence of turbulent flow is evident in the annual succession of taxa: Pennate diatoms to centric diatoms to colonial and flagellate forms. The data from the sediment trap indicated loss from suspension in proportion to abundance in the plankton and this was cited as evidence of turbulent flow (Chapter 10). However, turbulence flow is likely to have been decreasing with decreasing discharge, hence the early occurrence of the large pennate diatom *Navivula lanceolata*.

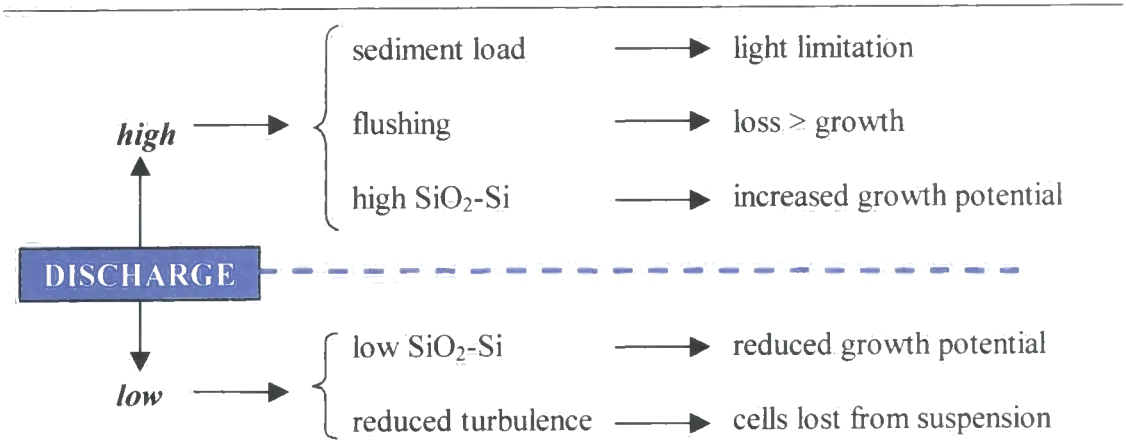


Figure 11.2 Schematic representation of the influences of discharge on phytoplankton in the Nene.

Flushing, or the influence of increased discharge, will ultimately result in loss of phytoplankton, but the short-term effect of flushing is not straightforward. An increase in water entering the river will result in increased velocity and loss of phytoplankton. The influence on phytoplankton abundance will be through dilution and enhanced removal rate. Increased discharge will normally be the result of recent precipitation and if occurring over a prolonged period will ultimately result in loss of phytoplankton. Localised heavy rain will quickly lead to loss through dilution although heavy rainfall in the headwaters will result in a slower loss process. This will occur not only because of a delayed response due to distance but because the whole upstream system has to be flushed before the impact is apparent. The spate flows of September 1994 illustrate this point (Chapter 8).

On the day of the spate flows that occurred during September 1994, heavy rain fell across the length of the Nene catchment and resulted in a three-fold increase in discharge that day and a further three-fold increase, which lasted for the subsequent two days. The very rapid increase in blue-green algae following the inundation indicates local flushing from lakes (probably located along tributary 3). However, the sustained

high occurrence of other algae normally present in the river (e.g. *Scenedesmus communis*), over at least two weeks indicates a flushing of algae from the length of the river. The protracted period with high phytoplankton abundance following the spate indicates that despite the elevated discharge much of the pre-spate river water was only partially mixing with the rain water, with a large proportion of the original river water being retained and gradually flushed downstream.

The negative influence of flushing is balanced against the positive effect of increased silica concentrations. High discharge increases the concentration of silica but also increases loss through flushing. Whereas low discharge provides a more favourable physical environment but a less favourable chemical media for sustained diatom growth. These conflicting trends were well illustrated during this study. April and May of 1994 and 1996 had average silica concentrations of 2.9 and 0.5 mg L^{-1} respectively. The average discharge during these periods was 10.9 and 5.1 $m^3 s^{-1}$ respectively for 1994 and 1996. Therefore spring phytoplankton abundance was limited by discharge during 1994 and probably by silica at times during 1996.

Summer periods of elevated rainfall and discharge are often associated with lower temperature, which in turn may promote phytoplankton abundance.

Temperature

A simplified representation of a phytoplankton temperature gradient for the Nene is shown in Figure 11.3. Here taxa are listed and their optimum temperature identified (Chapter 8), although some species have a wider temperature range than others. These values were derived from nature and therefore in the presence of other controlling variables. The data suggest that the temperature range of the Nene extends beyond the optimum for some species whereas others exhibit increased growth throughout the range.

Hypothetical growth curves are illustrated in Figure 11.4 for *Stephanodiscus hantzschii* and *Actinastrum hantzschii*. At low temperature the centric diatom grows more quickly then the green alga, but when temperature increases above 15°C to 18°C growth rate declines and eventually becomes negative. *Actinastrum* grows more slowly at low temperature compared to the diatom but continues to exhibit increased growth with increasing temperature.

The response curves are typical for the algal groups they represent (Garnier et al., 1995) but the interesting feature of the *Stephanodiscus hantzschii* curve is that the

optimum is several degrees lower than seen for this species in other British rivers (Swale, 1969). As postulated earlier, a plausible explanation for a lower temperature optimum in *S. hantzschii* is selection pressure created by silica limitation. As described above, a balance exists between the negative and positive affects of high and low discharge on centric diatom development in the Nene. To achieve maximum abundance *S. hantzschii* has to time its maximum growth at a time when silica concentrations are adequate. This period will correspond with high discharge, and will tend to occur early or late in the year when temperature is low. This theory is supported by the larger centric diatoms, which consist mainly of *S. hantzschii* fo. *tenuis*, having a higher temperature optimum, this species being a poorly silicafied form of *S. hantzschii* (E.Y. Haworth, pers. comm.).

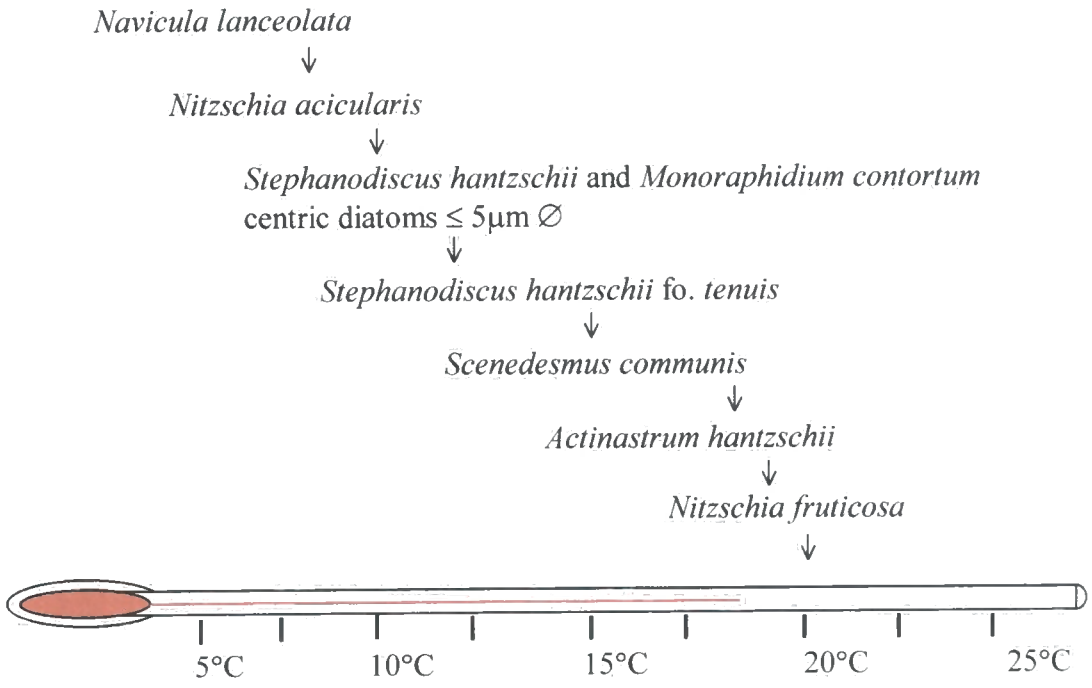


Figure 11.3 Schematic representation of phytoplankton temperature gradient. Arrows indicate approximate temperature optima.

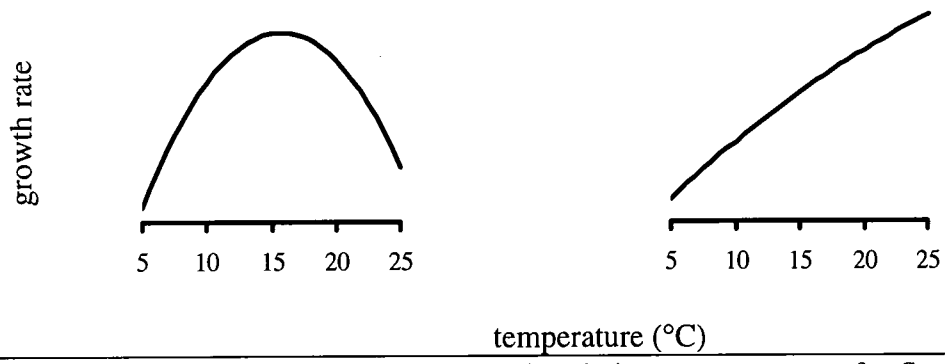


Figure 11.4 Hypothetical growth curves in relation to temperature for *Stephanodiscus hantzschii* (left) and *Actinastrum hantzschii* (right) in the Nene.

Water temperature in the Nene is low relative to other Northern European rivers and this may relate to differences in discharge and length. The Nene has a maximum temperature of about 21°C whereas other rivers exceed this value by several degrees (Descy and Gosselain, 1994; Gosselain et al., 1994).

Light

Although light is included in the list of key factors controlling phytoplankton abundance in the Nene (Table 11.1), it is difficult to disentangle the influence of light from temperature, although this can be done experimentally. Algal cell temperature can be influenced by solar radiation, particularly when cells are near the surface and infrared wavelengths are transmitted (DeNicola, 1996). However, light is more likely to influence growth by convection transfer of heat from surrounding waters.

Ultraviolet radiation can be damaging to algal cells (Soeder and Stengel, 1974) although the negative impact of light in a turbulent environment is difficult to determine.

Dead zones

The contribution of algae from dead zones has the potential of significantly influencing the algae of the main river. Although in the Nene this period of influence appears to be restricted to a times when the temperature is below 15°C. Under these conditions the bay at km 91.7 did contain a greater concentration of phytoplankton than

the main river (Chapter 9) and these were likely to be augmenting the algae of the main river, through diffusion. Bays and other dead zones are also likely to be more effective at retaining algae during spate flows than the main river and thus facilitate rapid recolonisation.

Dead zones are likely to heat and cool more rapidly than the main river (Reynolds and Glaister, 1992) and in the case of the Nene this factor could contribute to the downstream temperature gradient, which is apparent in the spring. The differentially higher temperatures in dead zones would promote the growth of *Stephanodiscus hantzschii* below 15°C and inhibits growth at higher values. The bay at km 91.7 did not contain more green algae than the main river during the summer and the reason for this is unclear. One possible explanation for this disparity is that the bay contained a stand of *Glyceria maxima* and these could have been creating shade.

Extrinsic sources of phytoplankton

The significance of extrinsic sources of phytoplankton to the Nene is one of species composition and frequency of occurrence. The data indicate that centric diatoms entering the Nene from tributary 3 have an insignificant impact on the main river. However, sources of blue-green algae from standing waters adjoining the river are of importance.

Oscillatoria limnetica, *O. redekei* and *O. agardhii* occur in the Nene and appear to tolerate turbulent riverine conditions, although there is no evidence of continued growth in the river. Other blue-green algae, like *Aphanizomenon flos-aquae and Anabaena flos-aquae,* are less frequent in the Nene phytoplankton. However, with the increased abundance of standing waters adjoining the river and the possibility of warmer summers these taxa could become a more frequent occurrence in the Nene. Notwithstanding this possibility, most the blue-green algae originating from lentic sources could probably be prevented from entering the river. For example, the diversion of channels around standing water rather than through them and the use of reed beds to 'filter' algae from lake outflow waters would contribute significantly to the prevention of algae entering the river.

One of the unresolved questions regarding river phytoplankton is sources of inocula (Wehr and Descy, 1998). The studies of extrinsic sources of phytoplankton demonstrates the potential sources of phytoplankton inocula to the main river. The Nene contains numerous marinas, adjoining flooded gravel pits, backwaters and side

channels. All these locations and much of the main river can retain sources of algal inocula, as algal cells or resting stages. The study of tributary 3 showed how centric diatoms can be carried over long distances, therefore any standing water within the catchment is a potential source of algae to the river.

Grazing

Grazing by Cladocera is an important controlling factor for phytoplankton in standing waters, and considered a key component in 'top down' control in many biomanipulation projects. However, in the Nene grazing appears to be of lesser importance than in lakes. Planktonic grazers in the Nene are mostly restricted to rotifers and ciliates and although large populations can develop they do not appear to significantly impact on phytoplankton abundance. This is despite rotifer populations in the Nene achieving abundances that are thought to limit phytoplankton numbers in larger systems (Gosselain et al., 1998).

Grazing by bivalves molluscs has not yet been considered in the Nene. Large bivalve of the genera *Anadonta* and *Unio* are ubiquitous throughout the deeper freshwater sections of the Nene (EA, unpublished data). These organisms are relatively slow growing, taking several years to mature (Ellis, 1978). Bivalves will almost certainly be filtering algae during periods of phytoplankton abundance, but the degree of impact they have on phytoplankton populations is unknown. However, filtering by bivalves will be relatively continuous and is unlikely to be responsible for sudden reductions in phytoplankton abundance, such as those that appear to occur from parasitism.

Parasitism

The data suggest that in the Nene parasitism has a major impact on phytoplankton abundance, being responsible for significant population reductions. Parasitism by chytrids cause population crashes in rivers (Clarke, 1989) and lakes (Canter, 1979) and they appears the most likely explanation for sudden declines in phytoplankton abundance in the Nene. Significant reductions in centric diatoms population, which cannot be explained by physical or chemical variables, are likely to result from parasitism. This was confirmed during 1996, with the concurrent disappearance of centric diatoms from suspension and their condition and occurrence in the sediment

trap. Numerous intact frustules devoid of cell contents are more likely to be the product of parasitism than any other process (Chapter 10).

Macrophytes

Abundant submerged macrophytes are often cited as being responsible for improved water clarity, although the mechanisms for this phenomenon are numerous (Scheffer, 1999). In the Nene high chlorophyll concentrations do appear to restrict the abundance of submerged plants. However, it does not necessarily follow that abundant macrophytes will limit chlorophyll levels.

One of the main sources of unexplained variation in the phytoplankton data is summer periods with protracted low chlorophyll levels. The occurrence of these periods was established in the long-term chlorophyll data set with 25% of the data ('low chlorophyll summer periods') having a significant influence on the whole data set.

Macrophytes could be implicated in the perpetuation of low chlorophyll summers, but probably not in their initiation. That is, some factor other than macrophytes is responsible for causing the decline of phytoplankton long enough to permit the growth of submerged macrophytes, which will then prevent the return of phytoplankton abundance. The decline of the summer chlorophyll and events that could result in submerged macrophyte growth will now be considered.

11.4 Summer decline of phytoplankton

The exploration of factors that could lead to the onset of low chlorophyll summers will commence by looking at a year that didn't experience such a phenomenon, 1980. Discharge, temperature, silicate and chlorophyll at km 91.7 during 1980 are shown in Figure 11.5.

During 1980 an early chlorophyll peak (a, Figure 11.5) occurred at low temperature (7°C) and high discharge (11 $m^3 s^{-1}$), but high amplitude peaks did not occur until temperature increased above 10°C and discharge declined below 10 $m^3 s^{-1}$ (b). This and the subsequent chlorophyll peak resulted in a sharp decline in silicate and are therefore likely to constitute abundant centric diatoms, with possible silica limitation and parasitism. The latter possibly resulting in a significant decline in chlorophyll (c), although declining temperature may have also contributed.

The second major decline in chlorophyll during June 1980 (d) may have again resulted from parasitism, but also corresponded with temperature increases above 18°C. which is likely to have reduced centric diatom abundance.

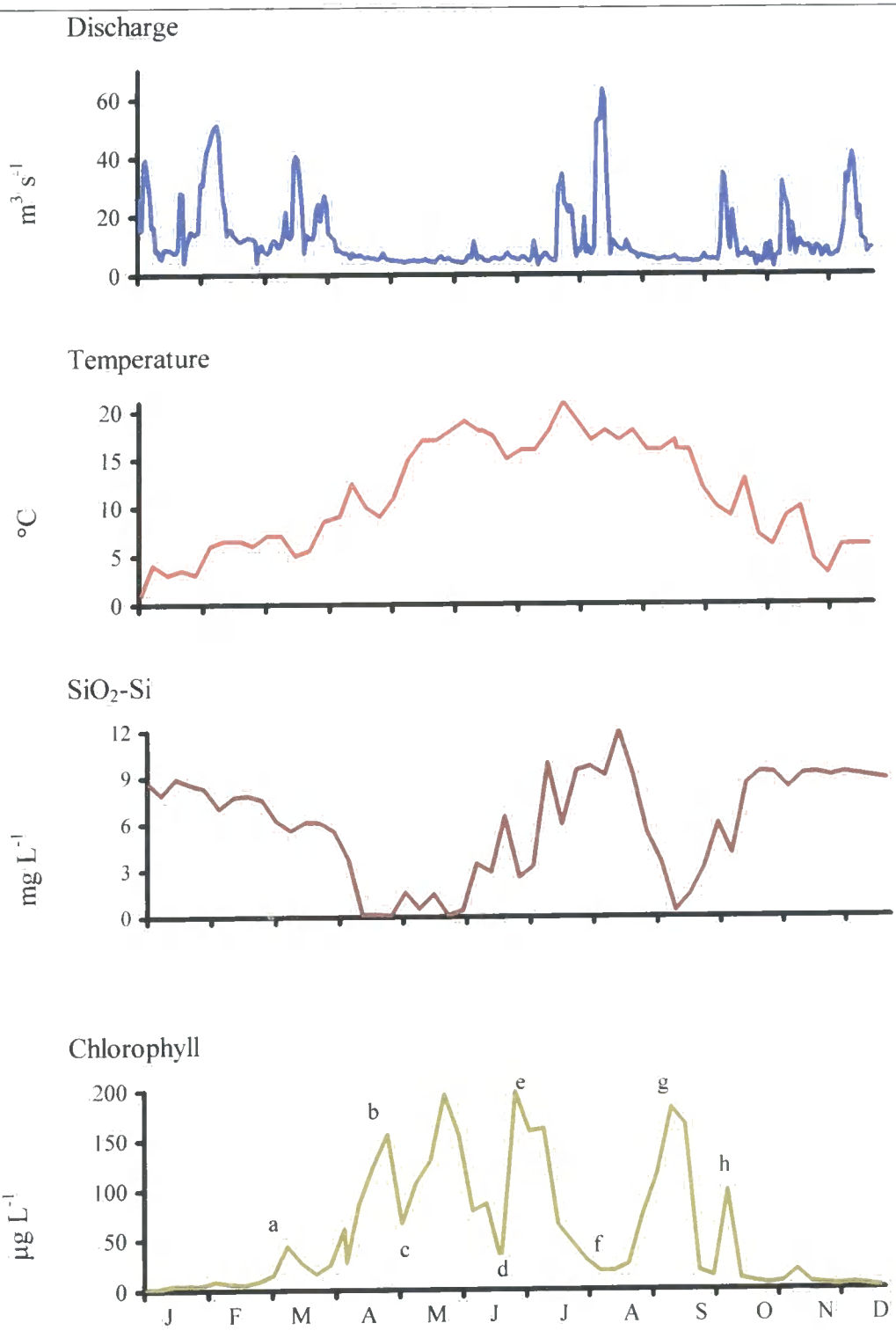


Figure 11.5 Discharge, temperature, silicate and chlorophyll at km 91.7 during 1980.

Likewise, the large chlorophyll peak that occurred in late June (e) corresponded to a reduction in temperature and the less pronounced reduction in silicate possibly suggests an increasing abundance of suspended green algae. Although this peak was probably dominated by diatoms because its decline again corresponded with an increase in temperature to 21°C. The duration of this trough (f) was extended by high discharge during August/September. The return of discharge to values below 10 $m^3 s^{-1}$ was soon followed by an increase in chlorophyll which coincided with a temperature favourable to diatoms and resulted in the depression of silicate.

The decline of the September chlorophyll peak may have been caused by parasitism, but declining temperature probably resulted in the depressed chlorophyll peak that followed (h), which was annulled by increasing discharge.

This description of the probable factors controlling chlorophyll during 1980 propose a series of events that would inhibit the occurrence of macrophyte growth, through shading caused by high suspended chlorophyll. It is also worth noting that 'weed' growth was negligible during 1980 (Brierley et al., 1989).

The significance of temperature as a controlling mechanism in the Nene should not be underestimated. Sustained temperature above 18°C will result in a significant reduction in centric diatoms and promote macrophyte growth. Sustained high discharge will also reduce phytoplankton abundance and provide favourable conditions for submerged macrophyte growth, as occurred during 1994.

In 1996 the temperature increased above 18°C in early July and remained so until the end of August, peaking at 21°C. The occurrence of this prolonged period with high temperature is likely to have produced favourable conditions for macrophyte growth.

The simplified pattern of temporal phytoplankton dynamics introduced in Figure 11.1 can be further expanded by subdividing the annual occurrence of phytoplankton into four phases, based on variations in temperature and discharge.

11.5 Phytoplankton phases

Four possible phases of annual phytoplankton occurrence are shown in Figure 11.6. These phases are not necessarily sequential and not all phases will occur each year.

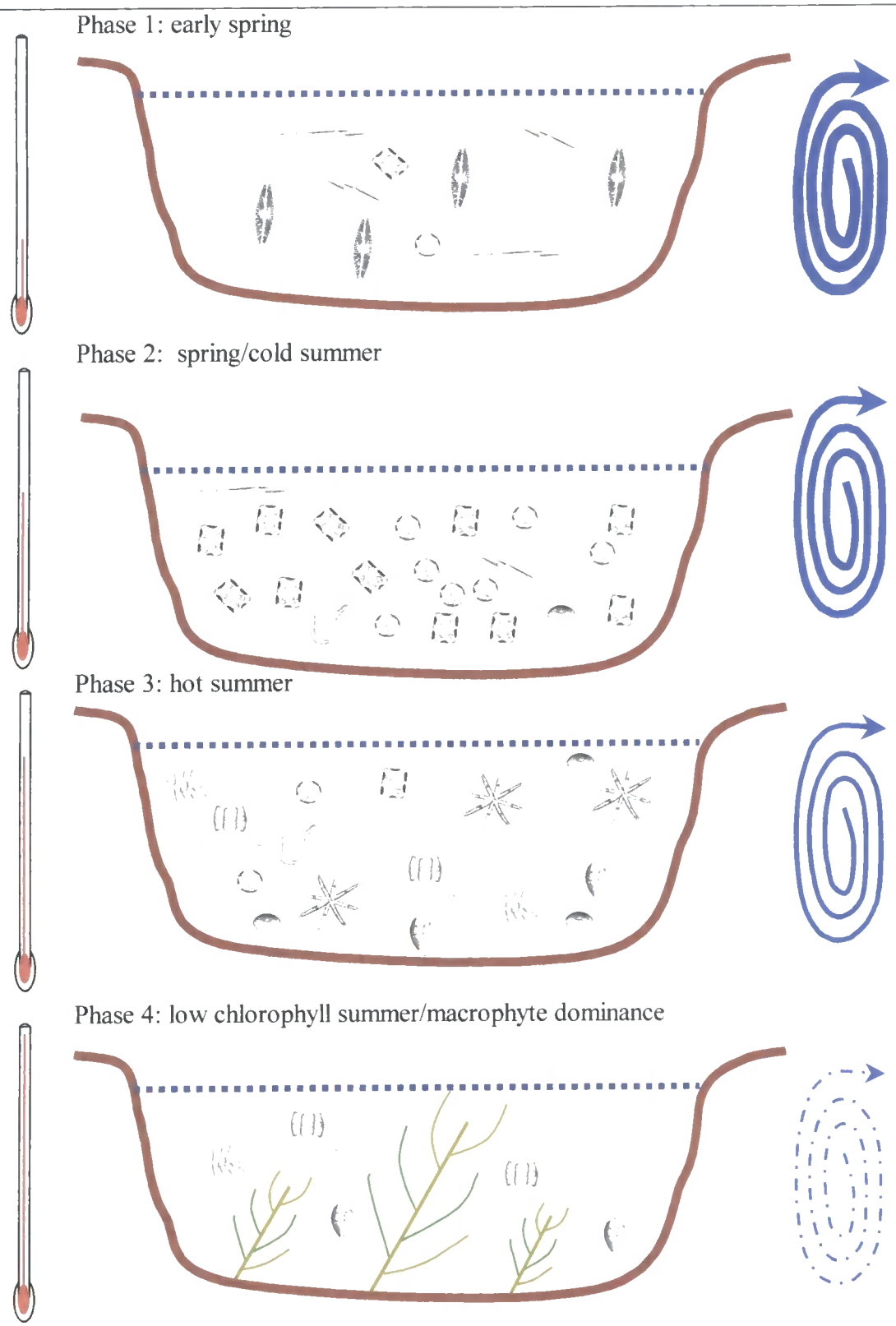


Figure 11.6 Phases of phytoplankton abundance in the Nene and the influence of temperature and discharge/turbulence. Illustrations of phytoplankton modified from Belcher and Swale (1979 and 1979). Temperature represented by length of red line (left) and discharge/turbulence by thickness of blue swirl (right).

Phase I probably occurs in some form most years at low temperature and high discharge, and is characterised by *Navicula lanceolata* and *Nitzschia acicularis*. Phase II is the key period of high phytoplankton abundance and dominance by centric diatoms. Increasing temperature and discharge can result in Phase III, green algae dominance. Prolonged high temperature can lead to Phase IV where submerged macrophytes become abundant and phytoplankton scarce.

These four phases of phytoplankton abundance, albeit great generalisations of nature, explain the major temporal trends seen in the Nene. Exceptions to these phases do occur, such as the severe drought of 1976, but the influence of temperature and discharge do generally hold true. Phase II is the key period of phytoplankton abundance and occurs every spring to some extent. Phase I precedes Phase II most years, but Phases III and IV are not necessarily represented every year (e.g. 1980).

These simplifications and the gradual introduction of levels of greater complexity are a sound basis for modelling phytoplankton abundance in the Nene.

11.6 Modelling

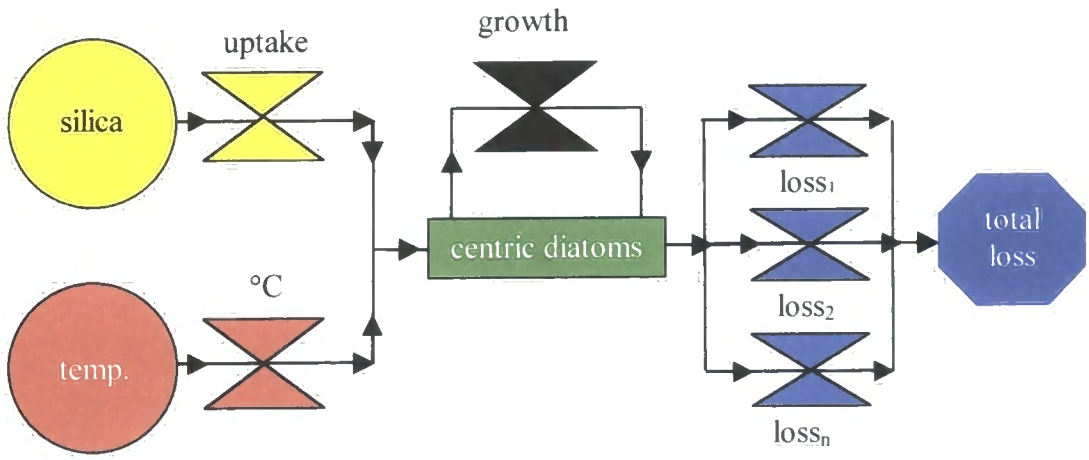
Simple linear models describing chlorophyll concentration in the Nene at km 91.7 were constructed using temperature and discharge as predictors (Chapters 7 and 8). These models were produced by transforming temperature and discharge to approximate linear functions. Unfortunately, these technique inadequately describe the non-linear nature of the relationships and under these circumstances phytoplankton dynamics are most satisfactorily modelled by numerical methods (Bowker, 1996).

The Nene data lend themselves well to modelling. The simplest form of dynamic models are deterministic and assume that future trends can be determined from the current state (Gurney and Nisbet, 1998). The production of a dynamic model to describe phytoplankton in the Nene at km 91.7 is the next logical step to this research. Although modelling is beyond the scope of this work the conceptualisation of a model is a useful summation of key processes.

Some models of river phytoplankton focus on chlorophyll (Whitehead et al., 1997) although an approach using key taxa (Garnier et al. 1995) is preferable for the Nene. As the centric diatom *Stephanodiscus hantzschii* is the most abundant taxon in the Nene it is suggested that this species should be used for the initial model. Once the model of a single taxa has been produced and verified then other taxa could be introduced. The results of combined taxa models could be converted to chlorophyll (based on cell

volume) for comparison with the long-term data. A flow chart for the initial model is shown in Figure 11.7.

The model would be based on a daily timeframe and require daily values for discharge, water temperature and silicate concentration, standardised to a predetermined time (e.g. 12:00). Daily discharge data would be readily available for km 91.7 (following scaling) but temperature and silicate concentration would have to be modelled from other variables. Initial attempts to model water temperature have been made using six day average solar radiation and two day average discharge ($r^2 = 0.71$, $p < 0.05$, n = 304). Silicate has not yet been modelled but would have a minimum data requirement of centric diatom abundance and discharge. Once the wherewithal for obtaining these daily variables is established the modelling proper could proceed.



Loss factors	Key		
discharge	rectangle	=	state variable
parasitism	driving variable	=	circle
sedimentation	flow	=	lines and arrows
grazing	processes	=	valves
macrophytes	sink	=	polygon

Figure 11.7 Flow diagram of phytoplankton dynamics. The state variable 'centric diatoms' is governed by the controlling variables temperature and silica concentration (above). Table of loss factors and a key to symbols used in the diagram (below).

The first step would be to establish equations to describe growth, in respect to temperature and the availability of silicate. Growth rate at different temperatures can be estimated from data collected during this work, when silica was not limiting. The impact of silica limitation can be estimated from Swale (1963). Once these two relationships have been established then gross phytoplankton abundance can be

estimated and these values minus total loss would provide estimates of net phytoplankton abundance.

The impact of loss factors has to be calculated on a daily basis and each factor listed in Figure 11.7 will be considered in turn.

Discharge

There are two main facets of the loss impacts of discharge on centric diatoms, namely dilution and turbulence. Dilution impacts could be quantified from the linear relationship between chlorophyll/centric diatoms and discharge. The minimum discharge to produce sufficient turbulence for centric diatoms could also be extrapolated from the data.

During periods of high phytoplankton abundance the impact of short-lived small (below 10 $m^3 s^{-1}$) fluctuations in discharge would be negligible. Large and sustained increases in discharge would initially lead to increased phytoplankton abundance, due to flushing of algae from the system, followed by general decline in phytoplankton abundance.

Parasitism

The impact of parasitism on centric diatom abundance would best be achieved by modelling chytrid abundance from the abundance and duration of centric diatom populations, assuming an exponential rate of increase in infection. The frequency of parasitism would have to be estimated from trends observed during this work.

Sedimentation

Sedimentation losses of centric diatoms could be estimated as a proportion to their concentration in suspension.

Grazing

The abundance of rotifers (and possibly ciliates and bivalves) could be estimated from temperature, discharge and abundance of suspended algae. Standard filtering rates for rotifers can be applied to abundance values.

Macrophytes

To assess the impact of macrophytes it would be preferable to model general macrophyte abundance, based on temperature and underwater light. Light transparency

being estimated from phytoplankton abundance/chlorophyll concentration. Loss of macrophytes would be assumed to occur at high discharge (> 20 $m^3 s^{-1}$) and consideration has to be given to the EA annual 'weed' cutting programme.

The uncertainty of the impact of macrophytes on phytoplankton abundance could be further investigated using the model. Once the first half of the year has been adequately represented by the model, then the range of conditions that result in macrophyte dominance can be evaluated by adjusting the controlling variables or by the introduction of new variables.

Spatial modelling

Modelling of spatial trends in phytoplankton abundance in the Nene could follow temporal modelling at km 91.7. Initial spatial modelling would be preferably limited to km 64.6 and km 43.9 and the model could be used to describe the additional silicate resource that appears to be available at these sites (Chapter 9). Modelling at km 34.0 would require some adjustment to growth/loss factors to account for reduced retention time that appears to impact here (Chapter 9).

Extrinsic sources of algae

The modelling of extrinsic influences would be more problematic and require an element of local knowledge. The abundance of blue-green algae would need to be estimated/modelled in lakes. However, some species of blue-green algae like *Oscillatoria redekei* can occur in standing water throughout much of the year (Whitton and Peat, 1969) so the influence of these taxa would involve modelling their abundance in standing waters and consideration of proximity and discharge. The latter would represent flushing rate of algae into the river.

Extrapolation of unknown conditions

The modelling of phytoplankton abundance under physical conditions not encountered during this study would also be problematic. For example, periods of sustained low discharge, as seen in 1976, can have a major influence on the pattern and magnitude of phytoplankton abundance. Under these conditions consideration would have to be given to other physical variables, like wind speed and direction as found in a study of phytoplankton in an impounded stretch of the River Welland during 1996 (Balbi, 1997).

Validation and use of model

The modal would need to be validated before it can be used with confidence and this would be undertaken using actual data from the Nene (probably chlorophyll concentration). If the model cannot be made to function adequately then additional data on macrophyte/phytoplankton relationships, parasitism and grazing may be required.

Modelling of the Nene could also have important implications for river management, particularly with the advent of the Water Framework Directive, which has a requirement for phytoplankton monitoring and is due to be adopted in the UK shortly. Additionally, if river phytoplankton monitoring does become a requirement of this Directive then many of the methods developed here would have a wider application.

11.7 Methodology and sampling strategy

Phytoplankton enumeration

The methodology development undertaken during this research provides considerable scope for further use, particularly the phytoplankton enumeration techniques. Sedimentation techniques of phytoplankton enumeration are widely referenced in the literature. Utermöhl (1958) has been cited over 1000 times in the literature since 1981 and Lund et al. (1958) more than 590 times (ISI, Science Citation Index). However, these citations are often inappropriate or incomplete.

The Lund et al. (1958) methodology was based on a complete counting of settled algae in a sedimentation chamber and the statistical treatment described is often inappropriately transferred to other techniques without consideration of additional sources of error that could be introduced. Edgar and Laird (1993) reviewed the Poisson-based methods of assigning confidence to count estimates and concluded that the techniques underestimated confidence intervals by between 5% and 15%.

The method proposed by Lund et al. (1958) has also been criticized for the way confidence intervals from a single count are used to embrace the population in a lake, or part thereof. Other studies have concentrated on levels of error, from sample to final determination, and quantified the errors at each stage (Venrick, 1981; Irish and Clarke, 1984), with sample error often cited as being the largest source or variance.

Utermöhl (1958) cited many possible alternative techniques of counting phytoplankton in sedimentation chambers and offered little in the way of statistical

validation. Irrespective of this the 'Utermöhl Method' is often cited without any indication of which techniques have been used or statistical confidence attained.

The significance of the discrepancies in counting techniques listed above will depend greatly on the nature of the study. Although when inferences are being drawn about population trends it is imperative that a knowledge of the confidence of each estimate is gained.

The 'spaced fields' method developed and used during this research provides a robust modification to the traditional approaches and although the confidence intervals achieved per unit effort are wider than those quoted by Lund et al. (1958) they are more realistic and reliable.

The spaced fields technique is likely to be criticized by statisticians because of the non-random nature of field placement. Cassie (1962) suggests that systematic sampling will always produce more accurate estimates than random sampling, and that statement was verified here. The disadvantage of non-random sampling is that probability theory cannot be used to assign confidence. The necessity to apply confidence intervals in this way is not a issue with the spaced fields method, as non-parametric techniques or the relationships resulting from the simulations can be used.

The spaced fields method is not necessarily a replacement for other methods but an alternative which is ideal for use when the identification of small changes in population density need to be confidently attained. This method would be useful for monitoring phytoplankton cultures and in monitoring toxic or nuisance phytoplankton which requires high levels of accuracy. Accurate enumeration of potentially toxic planktonic blue-green algae is becoming increasingly important in the water industry as litigation or cessation of sporting activities may result from cell counts exceeding a predetermined level (although the validity of cell count 'warning thresholds' is dubious).

Sediment traps

The use of a sediment trap in the Nene produced invaluable results. On reflection it would have been preferable to empty the trap more frequently (at least weekly) to identify the impact of parasitism more precisely. Irrespective of this, the method has potential for application in other systems although traps must be located with care, preferably in sections where a unidirectional flow is maintained.

Sampling and analysis strategy

The assessment of phytoplankton abundance was approached at several levels during this research. Increasing effort normally produces additional information, but the increase in information is not always proportional to effort. As time is always a limited resource careful targeting of effort is vital. In terms of pure research, time will always be wasted in the pursuit of knowledge. However, in terms of routine river monitoring all work has to be justified and carefully thought out strategies are required.

The key to a optimal sampling and analysis strategy lies in flexibility and ensuring that effort is targeted where it is most effective. Unfortunately, routine monitoring programmes rarely allow for flexibility and sample collection has to be scheduled and budgeted for.

Chlorophyll samples have been collected in the Nene over many years, although notwithstanding this work, their intended use is unclear. The UWWT Directive classifies rivers as eutrophic if they have an annual maximum chlorophyll > 100 $\mu g\ L^{-1}$ or annual average > 25 $\mu g\ L^{-1}$. Such a simplification of complex systems has obvious shortfalls in respect to the timing and frequency of sampling. Sample effort would be most effectively targeted at times of greatest phytoplankton abundance, but this would bias the average chlorophyll concentration. If targeted sampling is not undertaken then the peaks chlorophyll concentration could be overlooked. Additionally, samples taken in the morning often have lower levels of chlorophyll that those collected in the middle of the afternoon.

Nevertheless, chlorophyll is a readily obtained determinand, and despite numerous shortfalls, forms a sound basis for a routine sampling programme. The contribution of degradation product must be considered and can be a significant proportion of total chlorophyll. However, chlorophyll determination alone is inadequate to gain a thorough understanding of regulatory processes in rivers, and phytoplankton species-composition must be considered.

Flexibility of phytoplankton analysis is as important as flexibility of sampling, although analysis flexibility is compatible with a scheduled sampling programme. It is suggested that samples (concentrated if necessary) should be examined live, species identified and abundance assessed using a semi-quantitative method (Chapter 3). The results of the semi-quantitative method could then used in conjunction with chlorophyll data to draw qualitative inferences about temporal or spatial trends (Chapter 9).

Quantitative phytoplankton analysis is essential for assessing trends and the identification of small changes. For example, in the assessment of eutrophication, temporal trends in chlorophyll could be less obvious than species trends. Species will always be better ecological indicators than general measurements like chlorophyll.

Recent developments in HPLC techniques provide methods of quantifying taxonomic groups without counting. Pigments identified using HPLC techniques can be used as quantitative markers (Descy and Metens, 1996) and software has been developed to aid this process (Descy et al., 2000). The HPLC analysis provides a potential intermediate method of analysis between chlorophyll and counting, and should be considered for use in routine monitoring.

Spatial distribution of sample sites for effective river monitoring also requires consideration. During this work effort was targeted at km 91.7, the most downstream site, and selective analysis was undertaken at other sites. This has proved to be an effective approach on this river and km 91.7 could be used as a key site in routine sample programmes, although the differences in spatial distributions of phytoplankton/chlorophyll would need to be considered.

A suggested monitoring programme for km 91.7 is listed in Table 11.2, and this could be repeated at km 64.6 and km 43.9 if resources allowed. This programme would produce a sound data set and could be used in conjunction with modeling to help further elucidate the processes involved in phytoplankton dynamics in the Nene.

Table 11.2 Suggested routine monitoring programme for km 91.7.

Samples and measurements	Treatment/notes
Temperature	Field record
Secchi depth	Field record
Chlorophyll/HPLC	Stored in dark below 4°C and analysed within 24 h
Live	Stored in dark below 4°C and analysed semi-quantitatively on day of collection
Lugol's fixed	Store out of direct sunlight and analysed quantitatively within 12 months, adding more preservative during storage if necessary. Samples can be selected for analysis according to chlorophyll/HPLC/semi-quantitative results and should be counted to produce population estimates of dominant taxa within confidence intervals of ± 25%
SiO_2-Si	
(*Routine chemical programme would provide sufficient nutrient data*)	
Frequency (*Period 1 should to be extended during mild autumns/winters*)	
Period 1: weekly between 1 March and 31 October	
Period 2: 2-weekly between 1 November and 28/29 February	

12 SUMMARY

1 Phytoplankton dynamics were investigated in the 96-km length of the River Nene. Trends in chlorophyll data for a downstream site (km 91.7), spanning 24-years, were explored using physical and chemical variables as predictors. The interpretation of these data was aided by additional sampling at eight main river and three tributary sites between 25/6/1993 to 31/12/1997 (km 22.4 to km 91.7).

2 The additional sampling programme included investigations into light attenuation, sedimentation, filterable reactive and total phosphorus, phaeopigments, species composition, picoplankton, zooplankton, small-scale and short term trends in chlorophyll and phytoplankton. Phytoplankton analysis was targeted at four main river and the tributary sample sites.

3 Phytoplankton were abundant in the deeper navigation sections of the river and were characterised by spring peaks which were followed by either continued high chlorophyll or very low concentrations. Chlorophyll concentrations in the upper part of the navigation appeared to be restricted by retention time.

4 Discharge and temperature were significant predictors of chlorophyll concentration and phytoplankton abundance, and correlated most significantly with data from the first-half of the year. The greater variability later in the year was due to increased species diversity and some summers having extended periods of very low chlorophyll concentration.

5 188 taxa were recorded for km 91.7 and the 20 most abundant taxa represented 88% of all taxa. Centric diatoms represented 44% of taxa and over half of these were of the size class $\geq 5 < 10$ µm diameter and consisted mostly of *Stephanodiscus hantzschii*.

6 32 taxa groups were formed to investigate phytoplankton dynamics further and these based on abundance/biovolume, periodicity and ecological considerations. These groups were assessed by scrutiny of time-plots, correlating with discharge, temperature and light and by multivariate analysis. These three approaches were

complementary and identified taxa gradients in relation to physical and chemical variables.

7 Spring phytoplankton peaks were characterised by abundant centric diatoms, which followed increasing temperature and decreasing discharge. Further increases in temperature and decreases in discharge were characterised by increasing numbers for green algae. Picoplankton were often abundant at times when larger phytoplankton were scarce and blue-green picoplankton were more abundant than greens.

8 High frequency sampling at Wansford identified short-term trends in chlorophyll and phytoplankton abundance that related to discharge, temperature, light and silica.

9 There is considerable evidence that centric diatom abundance was limited by the availability of silica and limitation appears to be more pronounced at some locations than others.

10 Rotifers were abundant in the Nene and were limited by temperature, discharge and food availability. Rotifers required periods of greater stability than phytoplankton to proliferate. Grazing did not appear to significantly impact on phytoplankton abundance.

11 The data suggest that centric diatom populations are severely impacted by parasitism. This premise is supported by the loss of centric diatoms from the plankton, in the absence of any physical or chemical explanation, and their concurrent appearance and condition in a sediment trap.

12 Chlorophyll and phytoplankton peaks at km 91.7 occur earlier and are of lower magnitude than at upstream sites. Although the reason for this pattern is not entirely clear it appears to relate to a downstream temperature gradient, the contribution of dead zones and differential silica limitation.

13 Lentic sources of centric diatoms do not appear to have any influence on the main river but several locations were discharging blue-green algae into the river, which persisted over long-distances.

14 Neither N nor P appear to be limiting phytoplankton abundance in the Nene and from the prevailing concentrations of these nutrients the river can be classified as eutrophic to hypertrophic. However, there is an abundance of some phytoplankton taxa at km 22.4 that are normally associated with unproductive or P limited environments.

15 Summer periods with low chlorophyll concentrations are thought to result from an abundance of submerged macrophytes. The data suggests that abundant phytoplankton inhibit submerged macrophyte growth, through shading. However, once macrophytes become established they prevent the development of large phytoplankton populations. Protracted periods of high temperature (> 18°C) are thought to be a causal factor for the decline of phytoplankton numbers and increased submerged macrophyte growth.

16 Proposals for modelling phytoplankton dynamics are presented.

17 Phytoplankton enumeration techniques in sedimentation chambers were evaluated by empirical methods and computer simulation. Traditional methods produced errors which were greater than predicted by parametric techniques. A new approach called spaced fields was developed, which performed more satisfactorily than traditional methods and permitted the accurate identification of small changes in phytoplankton population.

18 The spaced fields counting method was used to evaluate sample, primary and secondary-subsample error. All three stages of sampling were found to contribute an approximately equal variance to the overall process.

19 A sampling programme for future monitoring of the Nene is suggested.

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