Nutrient cycling at Bakethin reservoir, Northumberland

Mattin, Vanessa Judith

Nutrient cycling at Bakethin Reservoir, Northumberland

by

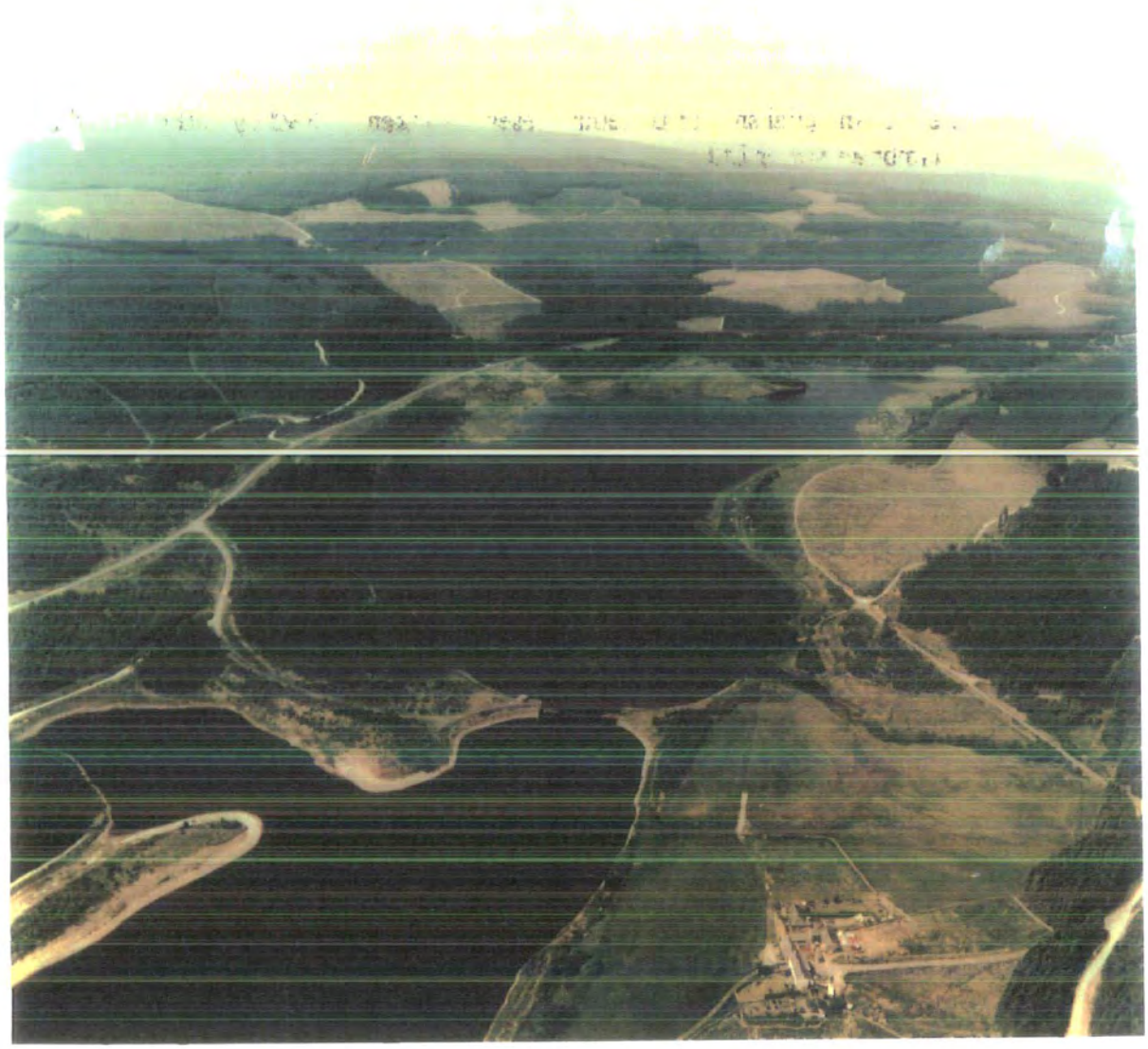
Vanessa Judith Mattin B.Sc.

A thesis submitted for the degree of Master of Science in the University of Durham, England

Department of Biological Sciences

December 1994

Figure 1.1
Arial shot of Bakethin Reservoir taken 13/06/1992 by AirFotos Ltd for Northumbrian water Ltd



Declaration

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

Vanessa Mattin

Abstract

The aims of this research were to investigate the nutrient regime of a small upland water body, Bakethin Reservoir, situated in the middle of Kielder forest Northumberland, England. The research consisted of three main sections: to identify possible inputs of nutrients to the Reservoir, to investigate the seasonal changes in chemical variables and photosynthetic organisms, and to investigate whether certain organisms are P-limited at the Reservoir.

Possible sources and sinks of nutrients were identified and it is likely that the major source of nutrients is the River North Tyne, which receives Butteryhaugh sewage treatment works and the Kielder Salmon hatchery. A small stream entering the Reservoir on the northern shore, termed here the Calcareous Flush, is very different in terms of water chemistry and flora to the Reservoir and other freshwater sites in the area. This inflow has a high merit in terms of conservation and general ecology and was therefore included in the study.

Water samples were taken monthly from March 1993 until July 1994 at 6 sites around the Reservoir and from April 1993 until July 1994 at a site upstream of the STW. 18 physical and chemical variables were measured and these were all found to vary over the seasonal cycle and between sites. However, certain characteristics of the water can be highlighted and these are the low temperature (annual mean of 6.9^{0}C), the brown colouration (mean absorbance 0.05 at 420 nm), and the low transparency (mean annual Secchi depth of 0.94 m). The annual range of inorganic combined N at Bakethin Reservoir is 46.2 to 166.3 $\mu g\ l^{-1}$. Total filtrable phosphate ranged from 11.4 to 49.7 $\mu g\ l^{-1}$ P.

Sediment samples were collected on one occasion (28/04/1994) and analysed for organic matter, N and P. N levels were much lower than P.

As part of a 'base-line' survey of photosynthetic organisms, and to gain an insight into the role that algae and aquatic macrophytes play in nutrient cycling at the Reservoir, algal samples were taken to coincide with the water samples. 210 algal taxa were recorded between April 1993 and June 1994 at 6 sites. These included 30 blue-green algae, 1 Rhodophyta, 7 Euglenophyta, 2 Cryptophyta, 4 Pyrrophyta, 4 Chrysophyta, 3 Xanthophyta, 114 Bacillariophyta (diatoms), and 45 Chlorophyta. 41 species of macrophytes were found at the sites including 2 lichens, 1 species of aquatic moss and 38 vascular plants. The algal samples were classified using TWINSPAN.

To investigate whether organisms are P-limited, eight species were chosen for a preliminary study of their "surface" phosphatase activities. Five were algae: *Ulothrix zonata*, *Stigeoclonium tenue*, locally frequent in spring and early summer, *Draparnaldia glomerata* and *Chaetophora incrassata*, locally frequent in late summer and autumn, and *Nitella flexilis*, found in great abundance in May and June 1994 in a shallow bay at the Reservoir.. The other three were macrophytes: *Potamogeton berchtoldii*, *P. obtusifolius*, and *P. natans*. Phosphatase activity was associated with all eight species suggesting that the ability to hydrolyse organic phosphate is important for species at Bakethin Reservoir. The extent to which the phosphatase activity was attributable to the epiphytes is unclear.

Acknowledgements

Many people gave help and assistance throughout this project and I gratefully acknowledge their contributions.

Firstly I would like to give my thanks to my supervisor Dr B.A.Whitton for his guidance throughout the project. Research facilities were provided in the Department of Biological Sciences by Professor P.R.Evans. The project was funded by Northumbrian Water Ltd.

My especial thanks go to N.J.Patten for his infectious enthusiasm and invaluable practical assistance.

My sincere thanks go to all the members of the Algal research group notably G.M.M.Baloch, E.M.Bresnan, G.J.Brown, G.Y.Chan, G.M.Chandio, Dr M.G.Kelly, H.L.Luff, A.L.Milligan, S.N.Pattinson, P.A.R.J. Stevenson and J.M.Yelloly for their friendship and support throughout the project. In particular I would like to thank E.M. Bresnan, H.L. Luff and A.L. Milligan for their lovely company at Bakethin.

I am very grateful to, A.J.Cross, G.Lewis and M.J.Metcalfe for assistance in sample collection, especially in the winter and also to M.J.Jubb and J.E.Markham.

I am grateful to J. A. Moore of the Natural History Museum for identification of *Nitella flexilis*.

I am grateful to S. Juggins of the University of Newcastle for the loan of a program to convert data from Paradox to TWINSPAN.

I am grateful to the Department of Biological Sciences photographers, for their help with the photos.

I would like to thank my family very much for their continued interest and encouragement.

CONTENTS

Abbreviations

EU	European Union
Pi	orthophosphate
FRP	filtrable reactive phosphorus
TFP	total filtrable phosphorus
FOP	filtrable organic phosphorus
PMEase	phosphomonoesterase
pNPP	para-nitrophenylphosphate
pNP	para-nitrophenol
4-MUP	methylumbelliferyl phosphate
CAPS	3-(cyclohexamino)-1-propanesulphonic acid
DMG	3,3-dimethyl glutaric acid
EDTA	ethylenediamine-tetra-acetic acid
HEPES	N-2-hydroxymethylpiperazine-N'-2-ethanesulphonic acid
pKA	dissociation constant
STW	sewage treatment works
USTW	upstream of the STW
WTW	Wissenschaftlich Technische Werkstätten
TWINSPAN	Two-Way Indicator Species Analysis

List of Tables

List of Figures

1 INTRODUCTION

1.1 The project

This project, funded by Northumbrian Water Ltd, arose out of an interest in the ecology and conservation of Bakethin Reservoir. This Reservoir was created in 1979 by damming the North Tyne and has been developed as an area of nature conservation within the larger Kielder Water scheme. Other research funded by Northumbrian Water Ltd and carried out at Bakethin includes investigations of the effect of recreation on bird numbers and water quality and invertebrates. Research into the conservation of otters is being funded in collaboration with other local conservation organisations.

Bakethin Reservoir is a small upland water body which serves as a feeder for Kielder Reservoir and is located in the middle of Kielder forest, Northumberland, England. A weir between Bakethin and Kielder prevents the water level in Bakethin falling below 183.5 m; however, water can rise up to 1.7 m above the Bakethin weir.

A review of the relevant literature showed that although a large number of studies exist on nutrients in freshwater, only a limited amount of literature exists on the nutrient regimes of reservoir environments. Reservoirs are often termed 'man-made lakes'; however, certain features exist that are characteristic of reservoirs and distinguish them from lakes. The remainder of this introduction gives an overview of nutrients in freshwater, an introduction to reservoir environments and how they differ from naturally occurring lakes, how nutrients are cycled in freshwater environments and how specific features of reservoirs are likely to effect nutrient cycling. The aims of the project are then outlined at the end of the introduction.

1.2 Nutrients in freshwater

1.21 Introduction to N and P

A large number of studies have investigated nutrients in freshwater and established the dominant role of nutrient availability, primarily of N and P, in controlling the growth and abundance of phytoplankton (Hecky & Kilham, 1988), macroalgae (Lapointe & O'Connell, 1989), and freshwater angiosperms. Both N and P are of major importance as metabolites, and their concentrations should always be considered first in determining possible limitations in primary production. Inputs of nutrients can either be allochthonous or autochthonous.

1.22 N in freshwater

N occurs in natural waters in many phases including dissolved N_2, NH_4^+, NO_2^-, NO_3^- and a large number of organic compounds (e.g. amino acids, amines, nucleotides, proteins, and refractory humic compounds of low N content).

Allochthonous inputs include those contained in particulate matter and precipitation falling directly on the surface of the reservoir and inputs of N from surface and groundwater drainage. Autochthonous inputs include N_2 fixation both in the water and in the sediments. Losses of N occur by outflow from the basin; reduction of NO_3^- to N_2 by bacterial denitrification with loss of N_2 to the atmosphere; and sedimentation of inorganic and organic N-containing compounds. The N cycle in naturally occurring lakes is mainly microbial; bacterial oxidation and reduction of N compounds are coupled with photosynthetic assimilation and utilisation by algae and aquatic macrophytes. The direct role of animals in the N cycle of freshwater is very small, under certain conditions; however; their grazing activities can influence

microbial populations and N transformation rates, as well as N utilization rates by photosynthetic organisms (Wetzel, 1983).

1.23 P in freshwater

P occurs in natural waters almost always as phosphates (Corbridge, 1991). These are classed as orthophosphates, condensed phosphates and organically-bound phosphates. They can occur in particles, colloids, in solution or in the bodies of aquatic organisms. Phosphates can also occur in bottom sediments both in inorganic forms and incorporated into organic compounds (American Public Health Association, 1989). P absorbed into a cell becomes part of the structural component of the cell (e.g. in poly-P-RNA) and is continually turned over in the energetic processes of organisms (e.g. as adenosine di- and tri-phosphate)

Allochthonous inputs of P are similar to those for N and include precipitation, surface and groundwater drainage. Rainfall can affect the level of P in a water system in two ways, by direct input into the water system itself and by leaching of nutrients from the surrounding soil area (Ahl, 1988; Holten *et al.*, 1988). Internal P cycling includes uptake and excretion of P by bacteria and algae, and exchange of P between the water and sediments. Invertebrate fauna also play a role in the cycling of P through the system. Ingestion of food particles by zooplankton at certain times of the year can be quite large. Through egestion, these creatures can release nutrients in the form of soluble P ions and some organic P compounds into the environment, which can then be reassimilated (Lehman, 1980).

P is readily hydrolysed from organic compounds by phosphatases which are hydrolytic enzymes present in many bacteria and on the surface of some

phytoplankton, particularly those from environments low in inorganic phosphate (Parsons *et al.*, 1984).

1.3 Reservoir environments

1.31 Comparison with lakes

Taub (1984) identified the following points that indicate differences between reservoirs and lakes.

1. The ratio of the drainage area to lake surface is much larger for reservoirs
2. Water retention time is much shorter for reservoirs
3. The depths of reservoirs, relative to their surface areas, are less than the relative depths of natural lakes. Wind prevents thermal stratification and currents can maintain fine particulate material in suspension.
4. Allochthanous materials such as inorganic particles, detritus, benthic algae, enter reservoirs from a main tributary, rather from several smaller ones as is typically the case in natural lakes.

1.32 Margins

Wetzel (1990) stated that: "As the large majority of lakes all over the world are small and shallow the littoral zone covers a considerable part of water bodies and the littoral plays an important role in lake ecosystem functioning".

Pieczynska (1986) summarised the properties of littoral zones as follows:

1. The interaction between lake water and bottom sediments takes place most actively in the littoral zone. A large proportion of suspended matter, carried into the reservoir precipitates on the bottom of the littoral zone and various kinds of soluble substances are released from bottom sediments into the water.

2. Wind and wave action cause the agitation and re-suspension of fine particulate matter, both organic and inorganic, in the surface layer of the sediments, enhancing the interaction.
3. Water level fluctuations, distribution of macrophytes, the influence of the land and waterbody, favour the formation of specific and mosaic habitats partly isolated from surrounding habitats.
4. The littoral zone is colonised by rich plant and animal communities. Macrophytes are especially important for spatial organisation of the environment, substratum formation for other organisms and nutrient cycling.
5. Organic matter produced and accumulated in the area has a number of fates but is most likely to be broken down by microbial decomposition
6. Emergent macrophyte stands and submerged macrophyte/periphyton complexes can be a protective barrier for nutrients entering the reservoir.

Reservoir margins have similar properties to the littoral zone of lakes, but from the points in Section 1.32 highlighting the differences between reservoirs and lakes it is likely that the role of reservoir margins in nutrient cycling is even greater than the littoral zone in lakes.

Background information on Bakethin Reservoir is given in Chapter 3.

1.4 Role of sediments in nutrient cycling

1.41 General properties of sediments

The effect of sediments on water quality differs greatly depending on their particle size. Sediments are sorted by water movement into particle size gradients. Bacterial populations are several orders of magnitude higher in sediments than the overlying water. The sediments are therefore considered as a major site of microbial

degradation of detrital organic matter and biogeochemical recycling of nutrients. Sediments are composed of organic matter, particulate mineral matter and inorganic compounds from biogenic origin such as diatom frustules. Most freshwater sediments contain significant amounts (10 to 90% or more) of organic matter (Wetzel, 1988).

In all but well-flushed coarse sediments, concentrations of biologically important nutrients (silicate, nitrate, ammonia and phosphate) increase with depth to levels which are high, relative to those in overlying water. The major transport process for exchange across the sediment surface is thought to be diffusion and models for diffusive flux have been proposed (Berner, 1980). There are several other processes which transport soluble nutrients and gases out of sediments in addition to diffusion *i.e.* active transport. Whatever the mechanism of release, where vertical transport is sufficient and regenerated nutrients enter the euphotic zone, they may be used for biological production.

1.42 Sediment N

N concentrations in sediments are controlled by microbially mediated transformation processes such as ammonification (breakdown of organic matter to NH_4^+), nitrification (oxidation of NH_4^+ to NO_3^-) and denitrification (reduction of NO_3^- to N_2).

1.43 Sediment P

The role of sediments in P cycling in lakes has been known for many years. The capacity of bottom sediments to retain or release P depends on physico-chemical properties of sediments and oxidation-reduction conditions at the sediment-water interface (Istanovics *et al.*, 1989). High P binding capacity of non-calcareous sediments has often been associated with Fe and Al components (Ku *et al.*, 1978). In calcareous sediments; however, P sorption has been attributed to adsorption of P onto

$CaCO_3$ or co-precipitation with $CaCO_3$ (Freeman & Rowell, 1981). Nixon *et al.* (1980) measured a seasonal release of inorganic P from sediments in Narragansett Bay which was strongly correlated with temperature. Rates were high in summer which on an annual basis was enough to support about 50% of the P required for phytoplankton production.

1.44 Sediments at Bakethin

The sediments at Bakethin Reservoir originally derived from the terrestrial sediments of the area and have since been supplemented with sediments from the catchment area and sediments produced *in situ*.

1.5 Algal communities

1.51 Introduction

In most aquatic environments the algal populations change temporally. The factors responsible for these changes are many including: temperature, light, turbulence, inorganic nutrients, organic materials, grazing, parasitism. Change is continuous, but certain times of the year such as spring and autumn are 'shock' periods for the algae.

The occurrence of two main ecological groups of algae are expected in lake and reservoir environments. These are planktonic algae, including phytoplankton and picoplankton, and periphyton, algae that grow on hard surfaces. Periphyton includes episammic (growing on sediments), epiphytic (growing on macrophytes), epizooic (growing on the surface of animals) and epilithic (growing on the surface of rocks and stones) (Wetzel, 1983).

A large bibliography of literature on the ecology of algae up to 1981 can be found in Round (1981).

Phytoplankton biomass seems to be determined not only by 'bottom-up' mechanisms (physical or nutrients) but also by 'top-down' processes, such as predation and parasitism (Carvalho, 1994).

1.52 Nutrient cycling by algae

Nutrients can appear in several different compartments: inorganic nutrients, dead organic matter, phytoplankton, grazers, bottom algae, detritus at the sediment-water interface, bottom detritus, inorganic bottom nutrients (Van der Molen *et al.*, 1994). Phytoplankton and periphyton take up nutrients from the water and these nutrients are subsequently recycled by grazing or sedimentation.

1.6 Role of macrophytes in nutrient cycling

1.61 Introduction

Aquatic macrophytes include a range of flowering plants, ferns, mosses, liverworts, lichens and larger algae such as stoneworts. Aquatic species are regarded as plants which spend the majority of time submerged in water, floating on the surface, or rooted beneath the water but with shoots emerging from it. Lake macrophytes are commonly distributed in a distinct zonation from land to progressively deeper water. Species distribution is determined by many factors including wave action, sediment composition and light climate (Spence, 1982), morphometry, in particular average slope (Duarte & Kalff, 1986).

Dense stands of emergent, floating or submerged plants reduce water movement and accelerate the deposition of suspended solid particles. Emergent plant communities of wetlands are as productive as, or even more productive than, upland forests or grasslands (Wetzel, 1983). Submersed macrophytes are also efficient collectors of nutrients. However, uptake by plants does not necessarily mean the removal of

absorbed nutrients because sooner or later they are released into the reservoir again by the death and subsequent decomposition of plant biomass. A certain amount of nutrients may be lost from their normal cycle in the lake system due to biomass buried deeply by rapid sedimentation, and partial loss of N by denitrification.

Growth of submerged plants can significantly affect N cycling in sediments (Moriarty & Boon, 1989). Macrophytes are an especially important biotic element in the littoral zone. They are significant for spatial organisation of the environment, substratum formation for other organisms and nutrient cycling. The intensity of nutrient uptake by roots and/or shoots, translocation, release by healthy plants and from decaying plants, exchange of elements within macrophyte/periphyton complexes, all determine the nutrient cycling in the littoral region. The role of macrophytes in the nutrient budget is determined primarily by the site of nutrient uptake. It is highly likely that for emergent plants the sediment is the prime nutrient source, but there are contradictory opinions regarding the factors regulating shoot or root proportion in nutrient uptake by submersed macrophytes.

1.62 Nutrients from water or sediments?

Concentrations of nutrients in the water are generally several orders of magnitude lower than in the interstitial water of sediments. Wetzel (1988) concludes that ion absorption in submersed macrophytes occurs both from the water by foliage and from the interstitial water of sediments by root and rhyzoid systems. Translocation occurs in both directions. In most cases; however, roots serve as the primary site of nutrient absorption from the sediments.

1.7 Use of floristic surveys

Chemical monitoring has limitations associated with temporal and spatial sampling; organisms react to fluctuations in water quality which may be missed by intermittent chemical analysis (Spellerberg, 1993). Most organisations responsible for maintaining river water quality rely largely on fish and macroinveterbrates for biological monitoring. However, the use of algae for monitoring rivers has been documented from 15 European Countries (Whitton *et al.*, 1991), making it clear that many include algae in their surveys. The use of algae for monitoring rivers in the United Kingdom has been documented by Harding and Hawley, (1991).

Floristic surveys and taxonomic records are important for monitoring long term changes. Future studies at Bakethin Reservoir will be able to relate back to the floristic survey undertaken and reported in this dissertation. Diatoms have the advantage that their siliceous walls provide the details from which identification is made and every sample ends up permanently mounted on a microscope slide. Thus the record can be checked , species compared with those from other collections and samples compared over periods of time (Round, 1991). Floristic surveys will be required for implementation of the Urban Wastewater Treatment Directive (UWWTD EU 1991)

1.8 Indications of nutrient status

1.81 N:P ratios

One approach to determine the nutrient limiting algal growth, is to use the "Redfield ratio" which predicts intra-cellular concentration of C: N: P of 106:16:1 (by molarity) in algae growing under conditions where growth is not limited by nutrients (Redfield,

1934; Tett *et al.*, 1985). The supply of nutrients in a balanced atomic ratio is important for photosynthetic organisms especially at low nutrient conditions.

Nutrient sources of lakes have divergent N:P mass ratios, ranging from 20 to >200 for precipitation, groundwater, and export from rural lands and soils and from 10 to <1 for sediments, sewage, urban runoff, and faeces (Downing & McCauley, 1992). In contrast to the range of N:P found in freshwater and nutrient sources, the N:P composition of aquatic organisms is fairly restricted. The disparity between the narrow N:P requirements of aquatic organisms, and the broad range of N:P in nutrient sources means that N:P should influence algal production and biomass in lakes, and as Bakethin has nutrient sources with a wide range of N:P ratios the N:P ratio of the water and sediments is likely to effect biological production.

1.82 Phosphatase enzymes

Photosynthetic organisms can directly assimilate dissolved inorganic P. By using phosphatase enzymes, many micro-organisms and some plant roots have the ability to hydrolyse one or more types of organic P in their environment. These enzymes cleave the phosphate group on the compound and then the free phosphate ion can be taken in by the regular phosphate transport system.

With respect to the hydrolysation of P there are two main types of phosphatases, phosphomonoesterases (PMEases) and phosphodiesterases (PDEases), most work has concentrated on PMEases and these are the enzymes studied in this project.

Phosphatase enzymes are pH specific, and depending on the pH at which maximum activity occurs, phosphatases have been classed as either acid or alkaline (Duff *et al.*, 1994). Alkaline phosphatases require divalent metal ions for their activity, but are inhibited by chelators such as EDTA, whereas acid phosphatases do not require

divalent metal ions and are inhibited by fluoride (McComb *et al.*, 1979) The most widely recognised enzyme in aquatic systems is the non-specific alkaline phosphatase (AP) (Taft *et al.*, 1977; Petterson, 1980).

Enzyme activities can be 'induced' in response to an environmental change or work at a continuous rate within the cell. It is thought that when the organism becomes moderately P limited this induces phosphatase activity. Chróst and Overbeck (1987) failed to observe a correlation between phosphatase activity and the orthophosphate content of water, and thought that phosphatase activity relates more to the internal P content of organisms.

Measurements of this activity in organisms with the ability to form surface phosphatases can be used to assess the P status of the organism and, with due caution, this can be developed as a biological approach to assess whether or not the environment is under P stress. Recent experiments; however, show that cotton roots also form "surface" phosphatases under N limitation (G.M.M.Baloch, 1994, pers. comm.), so care is needed in interpreting the results

Measurement of PMEase activity has been performed either fluorometrically or spectophotometrically with PME substrates. The two most often used are methylumbelliferyl phosphate (MUP) and *p*-nitrophenyl phosphate (*p*-NPP) (Duff *et al.*, 1994; Chróst and Krambeck, 1986). Most studies have been on aseptically grown species to eliminate the possibility that the phosphatase activity is from the micro-organisms. There has been a limited amount of work on species collected fresh from their natural environment.

Initially phosphatase enzymes were considered to contribute almost exclusively to enzymatic P-regeneration in natural waters, this has now been disputed. A recent

paper (Siude & Gude, 1994) documents studies on 5'-nucleotidase and compares the contribution of this enzyme with phosphatases on the regeneration of inorganic P in two lakes of different trophic state. They conclude that regeneration of P may be more significant by the 5'-nase system, in certain cases. These enzymes have not been investigated in this study.

1.83 Storage products

The type of storage products formed by blue-green algae is another indication of nutrient status. Polyphosphate granules form under P-surplus conditions. Cyanophycin granules form under N-surplus conditions (Fuhs, 1973). These storage products can be identified by staining.

1.9 Aims

The aims of the project are:

To determine the significant inputs of nutrients to the Reservoir and if they vary on a seasonal basis.

To investigate the seasonal changes in chemical variables, especially nutrients, and photosynthetic organisms at sites around the Reservoir.

To investigate whether certain organisms are P limited at the sites.

2 METHODS

2.1 Field sampling and measurements

2.11 Physical and chemical variables

Temperature, conductivity, dissolved oxygen and pH were measured *in situ* using WTW equipment, apart from a Russell CE7L pH probe. The various models were: thermistor attached to LF 91 meter; conductivity, LF 91 meter; O_2, OX1 91 meter and electrode model EO 90. Since the electrode required a steady current of 15 cm s^{-1} past the membrane, the electrode was stirred in the water, care being taken to minimise disturbance at the surface of the water. Water samples were collected in acid washed 250-ml polypropylene bottles. Each bottle and its top was rinsed three times in the water from the site before taking the sample.

2.12 Photosynthetic organisms

Epipelic algal samples were sampled using a small metal borer (diam. 1.6 cm) inserted to a depth of 2 cm. The sample was recovered by inserting an old credit card under the corer and transferring the contents straight to a snap cap glass bottle.

Epilithon was brushed or scraped off *in situ* rocks. Periphyton on submerged angiosperms was either gently scraped off, or part of the plant was removed with the epiphyton still in place. That on submerged mosses was either removed by gently squeezing or shaking the moss or again part of the moss was removed. Samples of any algal flocs present at the site were taken. Each sample was placed in a separate labelled snap cap glass bottle with a record of the type of community and a rough estimate made of how much that community represented of the whole algal community at that site.

The aquatic macrophytes present at a site were recorded and their abundance scored as a percentage of surface area covered. Pieces of specimens, which could not be identified immediately, were returned to the laboratory for subsequent identification.

2.13 Use of SCUBA

Scuba diving was used on two occasions firstly to conduct a field survey (25/08/93) to investigate the extent of the cover of *Potamogeton obtusifolius* and *P. berchtoldii*. To determine how far plant cover extended away from the Reservoir margin a steel cable was laid out from Site 03 for 200m across to the other side of the Reservoir. Divers swam along the transect, any plant material seen was recorded, with particular attention to cover of *P. obtusifolius* and *P. berchtoldii*. The results were also recorded on a video camera.

Secondly scuba divers were used to collect sediments from the middle of the Reservoir (28/04/94). Sediments collected in this way were undisturbed.

2.2 Laboratory equipment

2.21 pH meter

The pH measurements in the lab were carried out using an Ingold WTW combination electrode and as EIL metre (model 7050). The probe was calibrated with BDH standard buffer solutions which were prepared in MilliQ water.

2.22 Colourimetric analysis

Colourimetric analysis was performed using a Shimadzu double beam spectrophotometer (model UV-150-02). 1 cm quartz cuvettes were used.

2.23 Fluorimetric analysis

A Baird Atomic Fluoripoint Spectrofluorimeter was used for fluorimetric analysis during the phosphatase assay with 4-MUP as a substrate. Plastic cuvettes with a

path length of 1 cm were used at a wavelength of 440 nm emission and 365 nm excitation.

2.24 Centrifuge

Two centrifuges were used, a bench top Econospin from Sorvall instruments for small samples (up to 30 ml) *i.e.*, for diatom digestion and concentration of water samples for phytoplankton counts. For larger samples a Beckman centrifuge with a JA14 rotor was used.

2.25 Glassware

All glassware for chemical analysis was washed in sulphuric acid (5%). Snap cap glass bottles for phosphatase assays were washed in Decon.

2.3 Water chemistry

2.31 Filtration

Samples for all variables measured in the laboratory, except total alkalinity, Mn and Fe, were filtered with a washed GF/F filter (pore size 0.7μm) immediately on return to the laboratory. Mn and Fe were excluded, because most analyses of filtered samples were near or below the detection limit.

2.32 Optical density

Optical density was measured colorimetrically at a wavelength of 420nm using 1 cm cells and a Shimadzu double beam spectrophotometer (model UV-150-02).

2.33 Total alkalinity

Total alkalinity was measured by a potentiometric titration against 0.02M H_2SO_4 (American Public Health Association, 1989).

2.34 Cation analysis

Metals were analysed by atomic absorption spectroscopy (Perkin-Elmer 5000) using an air-C_2H_2 flame. Standard solutions were used to calibrate and were prepared to contain the same molarity of acid as the sample solutions. Pre-treatment of samples was required for the determination of K (addition of NaCl), Mg and Ca (addition of lanthium chloride).

2.35 N analysis

NO_2-N and NH_4-N were measured colorimetrically according to the method of Stainton *et al.*, (1977). The nitrite was measured by a diazotization method that resulted in a pink azo dye whose absorbance obeyed Beer's Law up to about 500 $\mu g\ l^{-1}$. The ammonia reacted with phenol and hypochlorite under alkaline conditions to form indophenol blue. The colour development was proportional to the concentration of ammonium with in the range 5 to 1000 $\mu g\ l^{-1}$. Sensitivity was high, with a standard deviation of $\pm$ 2 to 5 $\mu g\ l^{-1}$. NO_3-N was measured as NO_2-N after reduction by passing through a column of copperised Cd metal filings.

2.36 P analysis

The method is based on Eisenreich *et al.* (1975). It is a modification of the method of Murphy and Riley, (1962) which is the basis of the Standing Committee of Analysts method (1981). The filtered water sample was allowed to react with a composite reagent of molybdate, ascorbic acid and trivalent antimony. The molybdic acids formed were then converted by reducing agents to a blue coloured complex. The method was applicable in a range of about 1 to 500 $\mu g\ l^{-1}$ with a precision level of $\pm$ 1 $\mu g\ l^{-1}$.

The two P fractions measured were:

TFP All P passing through GF/F filter and present after hydrolysis with potassium persulphate. (This was not the same as total P, which would include P held by the filter.)

FRP All P passing through GF/F and measured directly.

The difference between TFP and FRP is generally regarded in the literature as organic phosphate, FOP. However, detailed studies would be required to establish whether ferric phosphate (complexes) may also contribute to this fraction.

2.4 Sediment analysis

2.41 pH

Using the method of Allen *et al.*, (1978), sediment and distilled water were mixed in a 1:2.5 ratio and shaken periodically for 30 min. The pH of the water was then taken.

2.42 Grain Size Analysis

This method uses two basins and a series of seven sieves of mesh size: 4 mm, 2 mm, 1.7 mm, 500 μm, 250 μm, 150 μm, and 63 μm.

1. A large amount of wet untreated sediment was placed on the largest sieve (4 mm)
2. The sieve plus contents were 'puddled' gently in a basin of distilled water fitting the diameter of the sieve
3. The material that passed through the 4 mm sieve was then transferred with the water to the next finest sieve (2 mm) in a second basin
4. The process was repeated for the remaining 5 sieves
5. Each sieve and the material contained on the mesh was placed in an oven at 70°C overnight. A piece of shiny paper was placed under each sieve to collect any material falling through the mesh as a result of size reduction during the drying process

6. The samples were removed from the oven and allowed to cool in a desiccate before weighing
7. The percentage by weight that each fraction contributes to the whole was then calculated

2.43 Determination of organic matter

The method used in this project was the weight loss on ignition at 550°C. 4 replicates were used for each sample.

1. Crucibles were pre-treated by placing in a muffle furnace at 550°C to remove any organic matter. The crucibles were then allowed to cool in a desiccator before weighing (C)
2. The sediment was passed through a 500 μm sieve to remove large organic particles, inorganic fragments and macrofauna, and then dried overnight at 70°C
3. The crucibles were half filled with dry sediment and re-weighed (A)
4. The crucibles with dry sediment were then placed in a muffle furnace at 550°C for 1 h. They were then removed and allowed to cool in a desiccator before reweighing (B)
5. Calculation

$$\text{Total organic matter} = \frac{A-B}{A-C} \times 100\%$$

2.45 P analysis

The P content of the sediment was extracted with HCl after ignition at 550°C (Andersen, 1976).

1. 0.2g ash free dry sediment (from the determination of total organic matter) was placed in an Erlenmeyer flask with 25 ml 1 M HCl and boiled on a hot plate for 15 min

2. The sample was then diluted to 100 ml with distilled water, centrifuged and filtered through a Whatman GF/F filter
3. 25 ml of the sample was then used to determine the total P as before

2.44 N analysis

Following the method in the handbook of analytical procedures (Anon., 1986). The N fractions of the sediment were extracted with KCl, filtered and then determined as for the N fractions of the water column.

1. The sediment was passed through a 500 μm sieve to remove large organic and inorganic fragments
2. 20 g moist sediment was placed in an Erlenmeyer flask with 100 ml 2 M KCl, capped and placed on the shaking machine for 2 h
3. The samples were centrifuged and the supernatant filtered using a GF/F filter
4. The filtrate was analysed for NO_3-N, NO_2-N and NH_4-N as before using KCl as a blank

2.5 Algal populations and biomass

2.51 Sub-sampling

Water collected from a depth of 0.5 m (at the same time as water collected for chemical analysis) at the mid-Reservoir site was used for estimates of phytoplankton density. One aliquot was analysed for picoplankton. Another aliquot was centrifuged to concentrate the cells. Counts using a haemacytometer were made for the plankton above the picoplankton size range. In the case of colonial organisms, cell numbers were recorded.

2.52 Autotrophic picoplankton

Picoplankton counts were made as follows:

1. Each sample was prefiltered under gravity through a 3.0-μm Nuclepore polycarbonate membrane and then preserved with buffered formalin (pH 7.0, 2% final concentration) and stored in the dark in a refrigerator
2. A glass-fibre filter (Whatman GF/C) was placed between the polycarbonate membrane and the filter holder as a backing filter
3. 50 ml sample was then drawn under partial vacuum through a 25-mm diameter 0.2-μm Nuclepore filter, which had previously been stained for 20 min with Irgalan black in 2% acetic acid
4. The filter was mounted on a glass microscope slide, which had been pre-chilled at 0^0C for twenty min
5. A drop of non-fluorescent immersion oil was placed onto the filter and a coverslip gently put on top, sandwiching the oil evenly between the filter and coverslip
6. The preparation was then enumerated under epifluorescence microscopy (Nikon Fluophot) using a x 100 oil immersion objective. Autofluorescent cells in the picoplankton size range were detected using green (ca. 420-490 nm) and blue (ca. 500-550 nm) excitation filter sets
7. Estimate cell density

2.53 Larger phytoplankton:

1 6 samples each 30 ml were centrifuged at 1500 rpm for 30 min

2 The top 90% was removed using a pipette, taking care not to disturb the deposited algae

3 The remaining liquid was then mixed thoroughly to resuspend the cells uniformly

4 A drop of sample was placed on the ruled area of the haemacytometer

2.54 Preparation of diatoms:

1. Large pieces of silt and sand were removed from the sample which was then placed into a centrifuge tube
2. A small amount of dilute HCl was then added to each sample to dissolve any calcareous material present
3. Distilled water was added and the sample centrifuged
4. The supernatant was poured off and 5 ml conc. H_2SO_4 added, followed by two crystals potassium permanganate. The tube was agitated and then left for 20 min
5. 10 ml saturated oxalic acid was then added slowly, and the mixture left to sediment overnight
6. Distilled water was then added and the sample centrifuged. The supernatant was poured off and the process repeated until a neutral pH was reached
7. The neutral material was then transferred to a small vial using a clean Pasteur pipette
8. The vial was shaken and then a few drops of the material was transferred to a coverslip. This was heated gently until all the liquid has evaporated leaving a thin grey film over about two thirds of the coverslip
9. A drop of Napthrax was warmed gently on a microscope slide and the coverslip inverted gently onto this. The slide was then allowed to cool before viewing under the microscope using a x 100 oil immersion objective

2.55 Assigning numerical scale

For all algal groups the proportions of each taxa within a sample were recorded on a numerical scale from 0 to 5, known as the short log scale (Causton, 1988).

scale	abundance	percentage of cells
0	absent	0
1	rare	0-2
2	occasional	3-11
3	frequent	12-25
4	abundant	26-50
5	very abundant	>50

2.56 Chlorophyll a analysis

Extraction followed that of Marker and Jinks (1982). The samples were placed in a universal bottle and 5 ml 90% methanol added, the bottle was then covered in aluminium foil to extrude light. The bottles were placed in a water bath at 70^0C, the boiling point of methanol and left for 10 min or until the chlorophyll a had been extracted. The sample fragments were then removed and the methanol topped up to 5 ml. The solution was then read on a spectrophotometer at 665 nm and at 750 nm, the spectrophotometer had previously been blanked on 90% methanol.

2.6 Phosphomonoesterase activity

2.61 Collection of material

Phosphatase assays were carried out as a measure of probable P limitation. The samples were collected in the field, and then transported back within plastic bags in an ice box and then kept at 4^0C for a maximum of 24 h. Large amounts of *Chaetophora incrassata* were collected in autumn 1993 and material that was not analysed fresh

(within 24 h) was kept in a cool room at 5^0C in flasks of growth medium, which was changed weekly. This is refered to as "old". Touching or handling of all specimens was avoided. Higher plants were collected by loosening the sediments of the surrounding area and carefully digging around the plant to remove the plant with the roots still intact.

2.62 Choice of substrate

Two substrates were adopted. p-nitrophenolphosphate (pNPP) was chosen as this has been used in numerous biological studies. However, it probably measures a range of hydrolytic activities, not just the hydrolysis of organic phosphate; the method was also rather insensitive. 4-methylumbelliferyl phosphate (4-MUP) is much more sensitive (Jansson, 1988). Assays were carried out using 71μM pNPP and both 100 and 1 μM 4-MUP.

2.63 Medium

The medium used for routine assays is shown in Table 2.1.

Table 2.1 Concentrations of elements in the assay medium

Major salts	**conc. (mg l^{-1})**	**μM**
$NaHCO_3$	15.85	188.6
KCl	4.28	57.38
$MgSO_4.7H_2O$	25.00	101.4
$CaCl_2.H_2O$	35.83	243.7
Stock added as Fe chelate	**salt (mg l^{-1})**	**μM**
$FeCl_3.6H_2O$	1.21	
Na_2-EDTA.$2H_2O$	1.67	4.17
Trace elements	**salt (mg l^{-1})**	**μM**
$MnCl_2.2H_2O$	0.04	2.28
$CuSO_4.5H_2O$	0.02	0.078
$CoSO_4.7H_2O$	0.01	0.035
$NiSO_4.7H_2O$	0.038	0.030
$ZnSO_4.7H_2O$	0.056	0.019
Na_2MoO_4	0.007	0.028
H_3BO_3	0.72	11.56

Stocks of buffered medium were prepared so that the final concentration of buffer during the assay was 50 mM. The choice of medium was influenced by several factors. The response of different phosphatases to various ions differs, but the concentration included in the medium was one which has been found to permit near maximal activity, without the risk of inhibition. Most microbial and algal phosphatases include Zn and many other metals such as Co. As the status of these metal ions in the enzyme was uncertain, the concentration of the chelating agent was therefore kept low.

2.64 Buffers

There is some indication that the choice of buffer used during the assay will influence the result. Table 2.2 shows the buffers used for each pH value, together with their effective buffering range.

Table 2.2 Range of buffers and their buffering capacity used in the phosphatase assay.

pH	buffer	buffering capacity
3	DMG-NaOH	3.2-7.6
4	DMG-NaOH	3.2-7.6
5	DMG-NaOH	3.2-7.6
5.5	DMG-NaOH	3.2-7.6
6	DMG-NaOH	3.2-7.6
7	HEPES-NaOH	6.8-8.2
8	HEPES-NaOH	6.8-8.2
9	glycine-NaOH	8.6-10.6
10	glycine-NaOH	8.6-10.6
10.5	glycine-NaOH	8.6-10.6

Rates were expressed as activity per unit biomass per unit time, with biomass measured as dry weight or as chlorophyll a. All the rates were then converted to μmol substrate hydrolysed per unit time.

2.65 Pre-assay treatment of organisms

Material was thoroughly washed in assay medium prior to the assay, and a sample of the experimental organism was then checked under a high powered microscope for the presence of micro-organisms.

2.66 Experimental procedure

Reagents were allowed to equilibrate to room temperature before the assay was performed. The assay was carried out in sterile snap cap glass bottles.

Using p-NPP as a substrate:

1. Snap cap bottles were labelled
2. Samples were thoroughly washed in assay medium
3. To each snap cap bottle 1.5 ml buffer (of required pH), 1.4 ml assay medium, was added followed by the organism
4. The samples were immediately transfered to a 25 ^{o}C water bath with shaker to equilibrate
5. 0.1 ml substrate was added and incubate for 30 min (the reaction was observed to be linear with time up to 60 min).
6. The reaction was terminated with 3.0 ml of 5M NaOH after the sample has been removed to prevent the terminator lysing the cells
7. The organism was then used to determine biomass (dry weight)
8. Controls were assayed as above with no sample
9. The solution was read spectrophotometrically at a wavelength of 405 nm using 1 cm cells
10. The enzyme activity was calculated according to the following equation:

$$\frac{reading \times 0.0033}{0.057 \times time(h) \times biomass}$$

Where 0.0033 was the total assay volume in litres

0.057 was the reading for 1μmol product

Using 4-MUP as a substrate:

The procedure for pNPP substrate was followed except that the reaction was initiated with 0.1 ml 4-MUP substrate. The reaction was again observed to be linear and the reaction was terminated with the following

pH 3 - 5.5 0.3 ml of a solution containing 110 mM NaOH, 110 mM K_2HP and 27.5mM EDTA in assay medium.

pH 6 - 10.5 0.3 ml of a solution containing 2.5 M EDTA, 50 M NaOH and 50 M K_2HPO_4.

The assay solutions were read on the fluorimeter straight away.

The enzyme activity was calculated according to the following formula:

$$\frac{reading \times 0.0033}{58 \times time(h) \times biomass}$$

Where 0.0033 was the total assay volume (in litres)

58 was the reading for 1μmol product

2.66 Development of experimental procedure

The phosphatase activity of whole plants of *Nitella flexilis* and *P. obtusifolius* was investigated. The assay procedure (using 1 μm 4-MUP only) used whole plants *of N. flexilis* and either all the roots or all the leaves of *P. obtusifolius*. The size of the organism determined the scale of the assay and the amount of reactants used. Assaying the whole plant reduces the possibility that we are looking at enzyme activity

within the cytoplasm, instead of surface phosphatase activity, as no cells are damaged. This; however, does not rule out the possibility that the substrate can enter the cell.

2.67 Staining

5-bromo-4-chloro-3-indolyl phosphate (BCIP), an organic P substrate, was used to indicate and locate PMEase activity (Coston & Holt, 1958; Holt & Withers, 1958). The procedure of the assay follows that of the pNPP assay except that BCIP was used as the organic phosphorus substrate, rather than pNPP, and the reaction was not terminated.

2.68 P content of assay organisms

This method involves digestion of the material as follows:

1. A known amount of dried material was placed in a snap cap.
2. 10 ml of 8M HNO_3 was added.
3. The snap cap was then placed on a boiling rack in the fume cupboard for 1 hr.
4. Bubble flasks were placed on snap caps after 10 min to reduce evaporation.
5. Solution was then transferred to an Erlenmeyer flask and diluted to 35 ml with distilled water.
6. Adjust pH to 7 with NaOH
7. P content analysed as for water chemistry (see above)

2.7 Safety

COSH regulations were followed for all experiments. Specific hazards were the use of Cd columns for nitrate analysis and concentrated acids used in several methods. Unaccompanied field work was avoided as much as possible. If unavoidable, a mobile phone was taken. The Operations Centre at Kielder was always notified.

2.8 Computing and statistical treatments

2.81 Software packages used

Microsoft Word for Windows$_{TM}$

Microsoft Excel

Paradox 4.0 for DOS

SigmaPlot for Windows

2.82 Statistical analysis

. A value for mean pH has been given (Section 4.1) although as pH is based on a log scale, a mean cannot strictly be calculated without normalisation of the values first.

The data collected has been analysed to determine correlations between variables measured. Multivariate analysis deals with the examination of numerous variables simultaneously. A value that gives a measure of dependence between two random variables X and Y is the correlation coefficient defined as:

$$\rho_{XY} = \frac{E\{(X-\mu_X)(Y-\mu_Y)\}}{\sigma_X \sigma_Y}$$

Where μ_X is the mean of a random variable X

σ_X is the standard deviation of a random variable X

If X and Y are statistically independent, then $\rho_{XY} = 0$ and when X and Y are linearly dependent (i.e., when Y = (b + kX), then $|\rho_{XY}| = 1$

A computer program was written in C (by M.J.Jubb) to obtain correlation coefficients in order to characterise the relationships within and between the environmental variables measured (including rain) and the quantitative biological data *i.e.*, picoplankton density and number of phytoplankton cells per ml.

Twinspan was used to analyse algal data.

2.83 Coding system

Each taxon found at Bakethin can be assigned a 6-figure code, based on a system developed by Whitton *et al.*, (1978) from Maitland (1977)'s system for freshwater animals. If an organism cannot be identified to species then taxonomic features such as filament width were coded for instead.

2.84 Database management

The advantages of the coding system was that results of the floristic survey at Bakethin can be added to a larger database started in Durham in the 1970's. The main file contains counts of the coded organisms on a semi-quantitative scale. Information on the sampling dates and sites were in separate files. A separate file contains the names of all the coded items. Data implementation was by way of 'scripts' written in the language PAL (Paradox Applications Language).

3 BAKETHIN RESERVOIR

3.1 The site

3.11 Introduction

Bakethin and Kielder form a two reservoir series in Northumberland. Bakethin is a shallow feeder reservoir to Kielder approximately 1.5 km S-E. of Kielder village. The two reservoirs are connected by a dam. The volume of Bakethin when water is flowing over the dam is approximately 650 x 10^6 gallons (= 3170 x 10^6 l = 31.7 x 10^5 m^3). In 1979 the Bakethin Conservation Area Advisory Group was established to advise on the management of the area. In spring 1986 and 1987 ecological surveys were undertaken and a management plan drawn up in the winter of 1987/88. The Reservoir lies at an altitude of 185.2 m a.s.l. It functions as a moderately open system with major inputs from the river North Tyne and Kielder Burn and many smaller inputs including Capon Burn and Bakethin Burn.

3.12 Survey maps

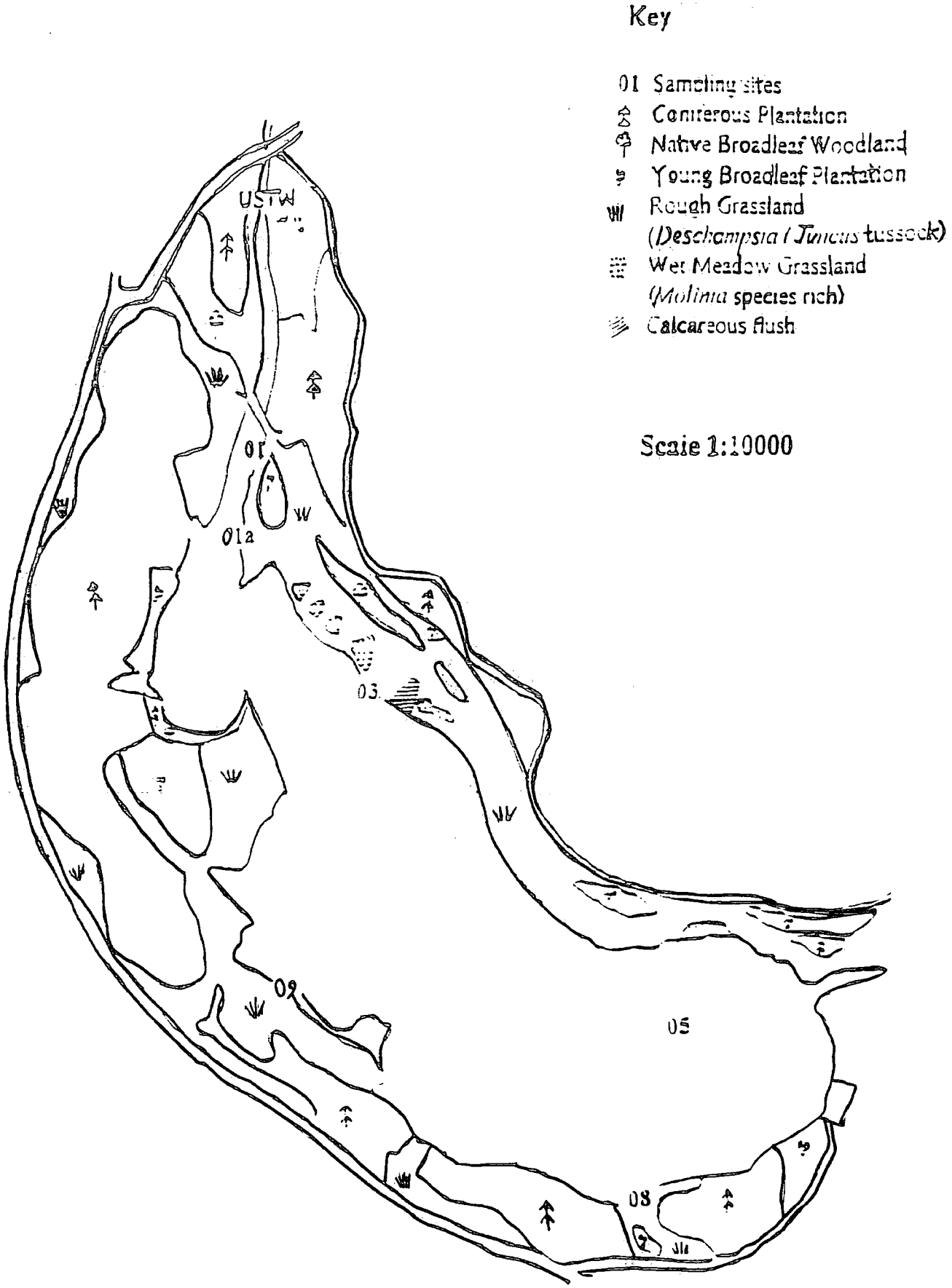
The Bakethin area is covered by the following ordnance survey maps:

1: 63,600 sheet 76
1: 50,000 sheet 80
1: 25,000 sheet 69
1: 10,000
1: 2,500

and the following geological survey maps:

1: 63,600 sheet 7
1: 10,000 sheet 54

FIGURE 3.1 SAMPLING SITES



MODIFIED FROM NORTHUMBERLAND WILDLIFE TRUST (REPORT)

3.2 Nutrient sources

3.21 Identification of potential sources

Sources of nutrients can be allochthonous i.e. those entering the Reservoir from outside, or autochthonous i.e. those arising from internal cycling. Allochthonous inputs are: inflows to the Reservoir, catchment drainage and rainfall. Autochthonous inputs include active exchange with the sediments, and biological cycling. In the Kielder area catchment drainage is likely to be effected by local agriculture and forest management. Known inputs of nutrients to the Reservoir are the Butteryhaugh Sewage Treatment Works (STW), and the Kielder Burn Salmon hatchery. There are two other inputs, Bakethin Burn and Capon Burn.

3.22 Agriculture

There are a number of small farms in the area: Dead Water Farm (J. Hall), East Kielder Farm (L. Finley), Scap Farm (M. Dobson) and a number of small holdings: GowanBurn (R. Nichol) and near Bakethin Dam (W. Steele). These areas are sometimes subjected to a fertiliser regime. The fertiliser used in most cases is 20-10-10, a commercial mix of N, P, K. Approximately 3 t ha^{-1} is applied in the spring.

3.23 Forestry commission

There has been no fertilizer application by the Forestry Commission in the Kielder/Bakethin catchment area since the Reservoir was built (D. Shoredine, pers. comm.).

3.24 The Butteryhaugh STW

The Butteryhaugh STW consents are for a nominal population of 400, and a dry weather flow of 80 m^3 (0.925 l s^{-1}) per day, suspended solids 40 mg l^{-1}, and BOD of 30 mg l^{-1}. The STW regularly achieves suspended solids of 10 mg l^{-1} and a BOD of 8

mg l^{-1}. The STW serves a total of 82 buildings, 16 of which are permanent. There are a total of 129 residents, mainly adults. Also connected to the sewage works are Kielder castle, the campsite, the school and the public toilets. If the Vollenweider (1968) estimates of the average contributions of N (10.8 g) and P (2.18 g) per person per day are applied to a population of 150 people, this equals 1620 g N and 327 g P. On the assumption that all the water is retained in Bakethin, this in turn corresponds to an average value for the Reservoir of 0.51 μg l^{-1} N and 0.1 μg l^{-1} P.

3.25 Kielder Burn salmon hatchery

No flow measurements are made from the hatchery. The inflow pipe has a capacity of 0.25 m^3 s^{-1}, but it is only operated at this flow for test purposes and normal discharges are much less. River quality sampling was carried out by the NRA upstream and downstream from the hatchery and the effluent itself. Water quality parameters measured were: temperature, pH, dissolved oxygen, Biochemical Oxygen Demand (BOD), suspended solids, alkalinity, NH_4-N, NO_3-N and NO_2-N. All apart from BOD and suspended solids were measured at Bakethin Reservoir in this study. Summary statistics for the period 01/01/91 to 31/12/93 are shown in Appendix 3.

There are no significant differences ($P < 0.01$) between the upstream and downstream samples.

3.26 Water movement

The crest of Bakethin weir is at an altitude of 183.5 m . When the water height is greater than 183.5 m the two reservoirs are continuous. Throughout the sampling period this occurred during the following periods:

26/04/93	08/05/93
14/05/93	10/07/93
05/08/93	18/08/93
11/12/93	21/02/94
01/03/94	15/06/94
20/06/94	09/07/94

The true turnover figures for water in Bakethin Reservoir could be determined by using a trace chemical, this has not been done. However, it is likely that the former river course takes much of the flow quite quickly to the weir with water in the perimeter shallows lingering longer.

3.27 Rainfall

Daily rainfall figures are available for Kielder and these have been used to calculate the amount of rainfall between each sampling date.

Table 3.1 Total rainfall (mm) at Kielder between sampling dates

period	**rainfall (mm)**	**no. of days in period**
25/03/93 - 21/04/93	137.0	28
- 20/05/93	107.9	29
- 03/06/93	38.1	14
- 22/06/93	28.5	19
- 19/07/93	47.0	27
- 23/08/93	102.6	35
- 20/09/93	58.6	28
- 18/10/93	86.9	28
- 30/11/93	56.7	43
- 18/12/93	171.7	18
- 17/01/94	160.6	30
- 14/02/94	109.1	28
- 17/03/94	159.8	31
- 18/04/94	99.5	32
- 16/05/94	70.0	28
- 14/06/94	26.0	29
- 18/07/94	62.8	34

3.3 Sample sites

Site 01	**Figure 3.3**
Grid reference	NY 632923
How to find	Just S of the Viaduct on the N shore. The 10 m stretch starts opposite the lifebuoy and goes W
Description	Steep banks, turbid water, some water flow still present
Dominant species	*Glyceria fluitans*, *Phalaris arundinacea*
Site 01A.	**Figure 3.4**
Grid reference	NY 632923
How to find	Approximately 100 m further along the Northern shore than site 01 this site can be identified by a large submerged log.
Description	Shallow banks occur as the river channel broadens out to become the Reservoir. A site of high deposition, indicated by large amounts of leaf litter in the Autumn.
Dominant species	A high diversity of flora. Again *Glyceria fluitans*, *Phalaris arundinacea* are dominant.
Site 03	**Figure 3.6**
Grid reference	NY 634919
How to find	This site stretches 10 m W from the next lifebuoy. It is below the Calcareous Flush
Description	Steep banks, large rocks in the water.
Dominant species	Lack of angiosperms but mosses and lichens present. Dominated by *Chaetophora incrassata* in Autumn 1993 and 1994

Site 05	
Grid reference	NY 641914
How to find	This is the middle of the Reservoir site
Description	Approximately 6 m deep
Dominant species	The plankton is dominated by *Tabellaria flocculosa*
Site 08	**Figure 3.7**
Grid reference	NY 639911
How to find	The slipway W of the Weir. The 10 m site starts from a prominent rock W of the slipway and stretches back to the slipway
Description	A shallow rocky environment, near to the forest
Dominant species	Few angiosperms in the water, the flora is dominated for much of the year by a diatom/blue-green algal assemblage over the rocks.
Site 09	**Figure 3.8**
Grid reference	NY 632914
How to find	From the shore, go as far as possible down the track past the birdhide, cross over the river, site 09 is the 10 m stretch just past a small inlet
Description	An exposed shallow site, often very windy, few rocks, silty substratum
Dominant species	Angiosperms, mosses on the shoreline and lichens on the rocks
Site USTW	**Figure 3.2**
Grid reference	NY 633927

How to find	Next to the forestry commission road from Kielder village along the Northern shore of the Reservoir, is the track leading to the STW. The site is approximately halfway along the track next to a large beech tree
Physical description	This site is characterised by a rocky substratum and shallow fast running water. The sample site is shaded from trees on the riverbank.

Calcareous Flush

Grid reference	NY 635922
How to find	After the 2nd lifebuoy on the Northern shore (Site 03) a boardwalk indicates the bottom of the Flush.
Physical description	Characterised by a slow flow, high concentration of calcium and therefore a high conductivity.
Dominant species	Dominant flora of *Rivularia*, *Nostoc*, *Spirogyra*

Artificial substrate

Algae was also collected from the rope of a buoy in Bakethin Reservoir on the following occasions 16/05/94, 14/06/94, 18/07/94. This site is abbreviated to AS in the following chapters.

3.4 Assay organisms

Ulothrix zonata

Phylum	Chlorophyta
Group	Ulotrichales

Ulothrix zonata is an unbranched filamentous green alga. There are generally 1-8 pyrenoids in each chloroplast and 2-16 zoospores are produced per cell. Sexual

reproduction is isogamous and the cells producing the gametes are often curved, with each cell producing 8-32 gametes. The filaments are free-floating or attached by a basal cell and small rhizoids are occasionally present

Stigeoclonium tenue

Phylum Chlorophyta

Group Chaetophorales

The most widely reported form in Britain with well branched filaments and the main axis 6-15 μm wide. The branches are both opposite and alternate.

Draparnaldia glomerata

Phylum Chlorophyta

Group Chaetophorales

A filamentous green composed of a main axis from which tufts of side branches regularly arise. The cells of the main axis are wide (up to 130 μm) and often barrel-shaped. The side branches are much narrower and the cells are attenuated towards the apices. The chloroplasts are parietal and band-shaped or net-like, with 1-several pyrenoids. The species reach up to 10 cm long. Normally found in clean slow flowing water.

Chaetophora incrassata **Figure 3.9**

Phylum Chlorophyta

Group Chaetophorales

Thalli large and variable in size and shape, containing radiating groups of filaments which are gracefully attenuated to fine points. The filaments often die back towards the base and the entire colony is enveloped in a stiff mucilage. The species occurs

attached to stones, twigs, aquatic plants and even bare sediment in 1994 at the edge of the Reservoir.

Nitella flexilis

Phylum Chlorophyta

Group Charophyceae

Information from Moore, (1986).

N. flexilis is a member of the Characeae (stoneworts). Variable height but approximately 15 cm at Bakethin. Pale green to dark green in colour unless covered in epiphytic diatoms when the plant appears brown.

Potamogeton berchtoldii **Figure 3.10. 3.11**

A small submerged macrophyte with narrow leaves characteristically 1-3 mm wide

Potamogeton obtusifolius **Figure 3.13**

A small submerged macrophyte similar to *P. berchtoldii* but with wider leaves and a blunt end.

Potamogeton natans **Figure 3.12**

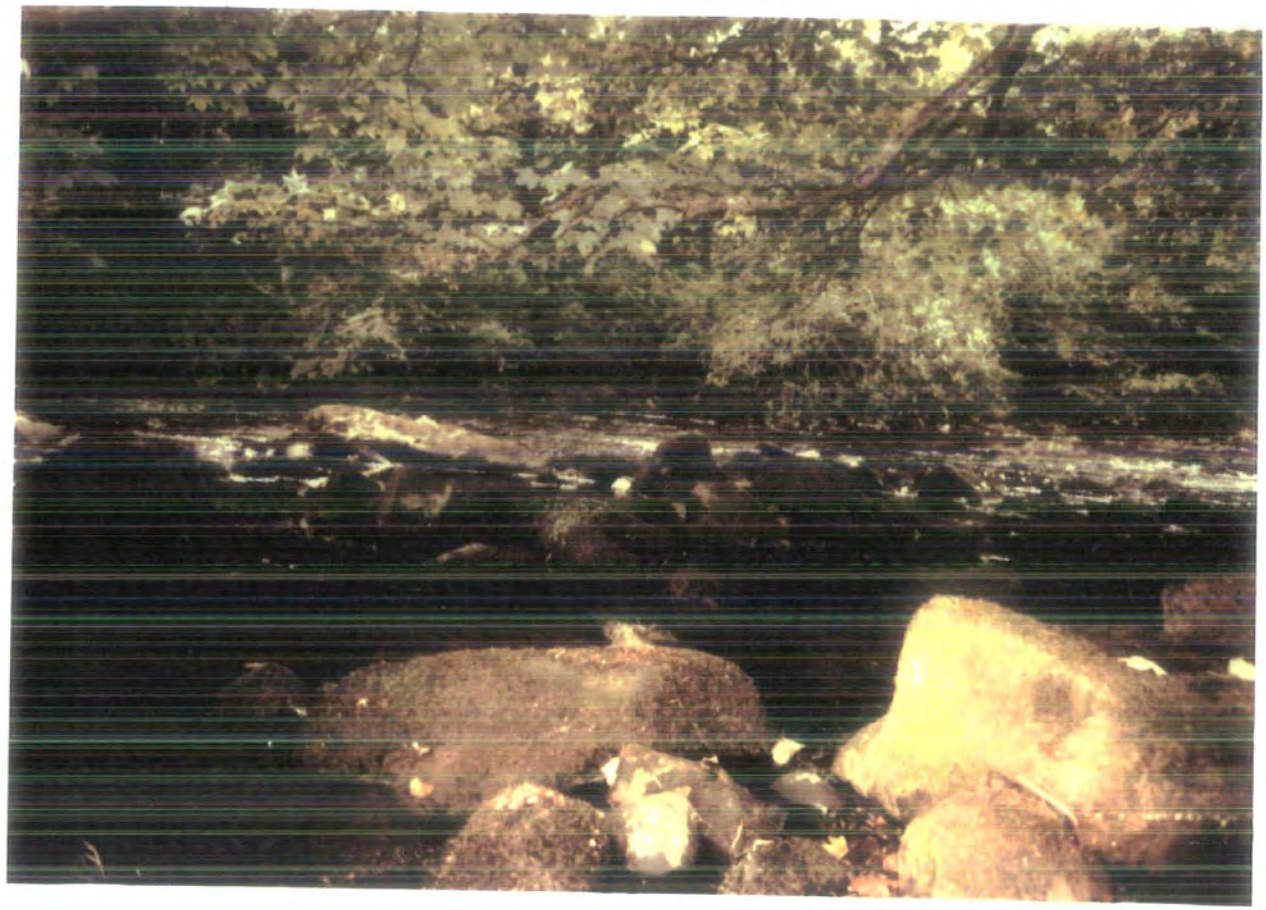
A large, heterophyllous aquatic with long-stalked leathery, floating leaves and narrow translucent, inconspicuous, often short-lived, submerged leaves.

Figure 3.2

A view of the site upstream of the Butteryhaugh Sewage Treatment Works (USTW). Taken 7/9/94.

Figure 3.3

A view taken from the viaduct (7/9/94) at the northern end of the Reservoir. Sites 01 and 01A are on the left hand side of the River. The red life belt that marks Site 01 can be seen.



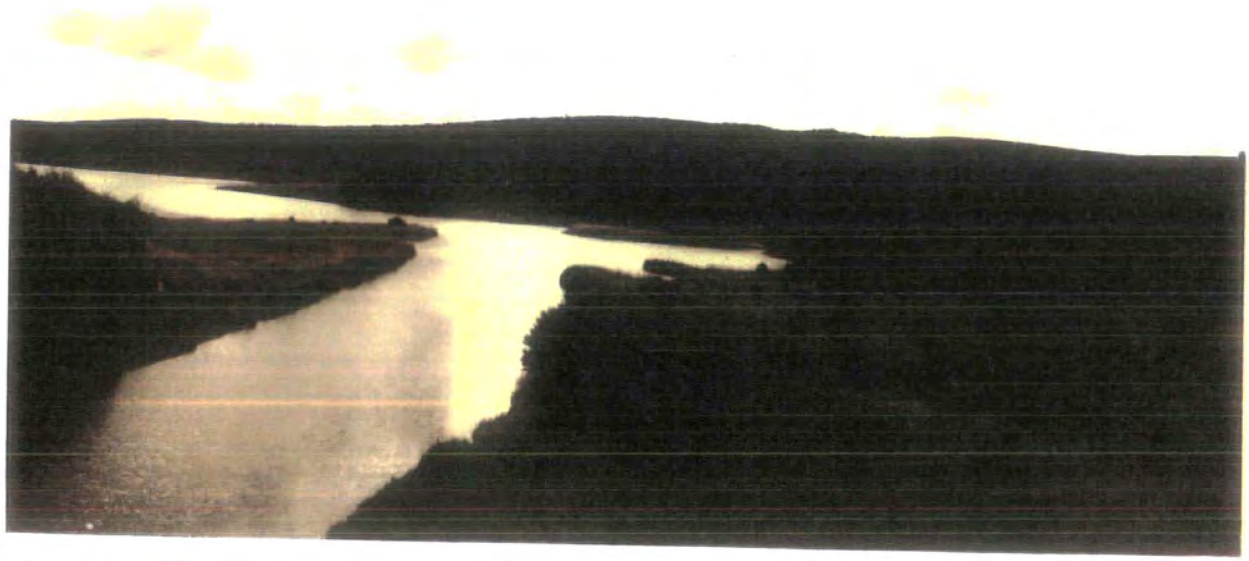


Figure 3.4

Site 01A. Note the *Glyceria fluitans* and *Phalaris arundinacea*.

Figure 3.5

A site between Site 01A and 03. Showing *Carex* sp. and *Equisetum fluviatile*.

The shallow area beyond the *Equisetum* was the site of the *Nitella flexilis* bed.

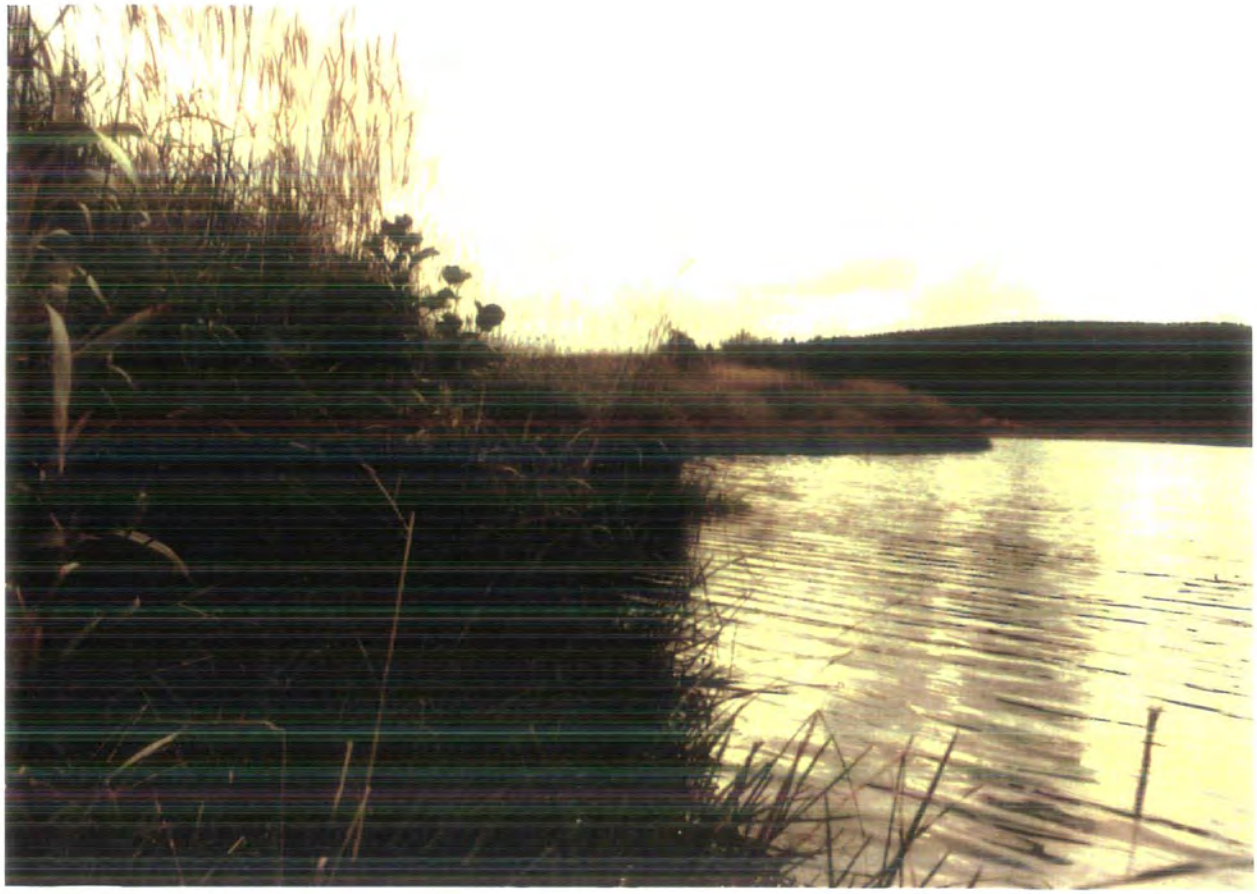


Figure 3.6

Site 03. Taken on 7/9/94. Note the large rocks and sheer banks.

Figure 3.7

Site 08. Taken on 7/9/94 showing the rocky substrate, lack of aquatic macrophytes and the close proximity of the Forest.

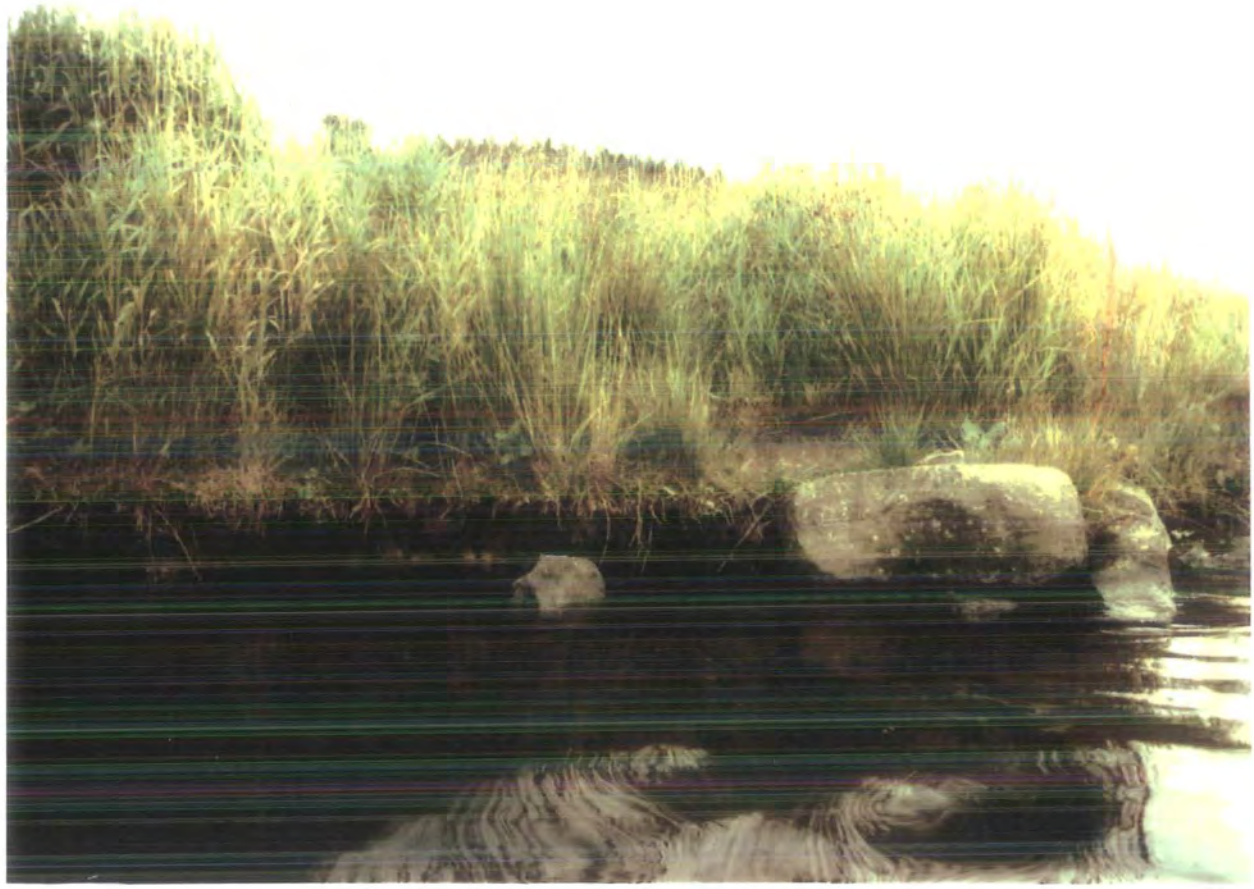


Figure 3.8

Site 09. Taken on 7/9/94. This is a shallow site with a relative abundance of aquatic macrophytes.

Figure 3.9

Showing study organism *Chaetophora incrassata*. The scale bar is 1 cm.

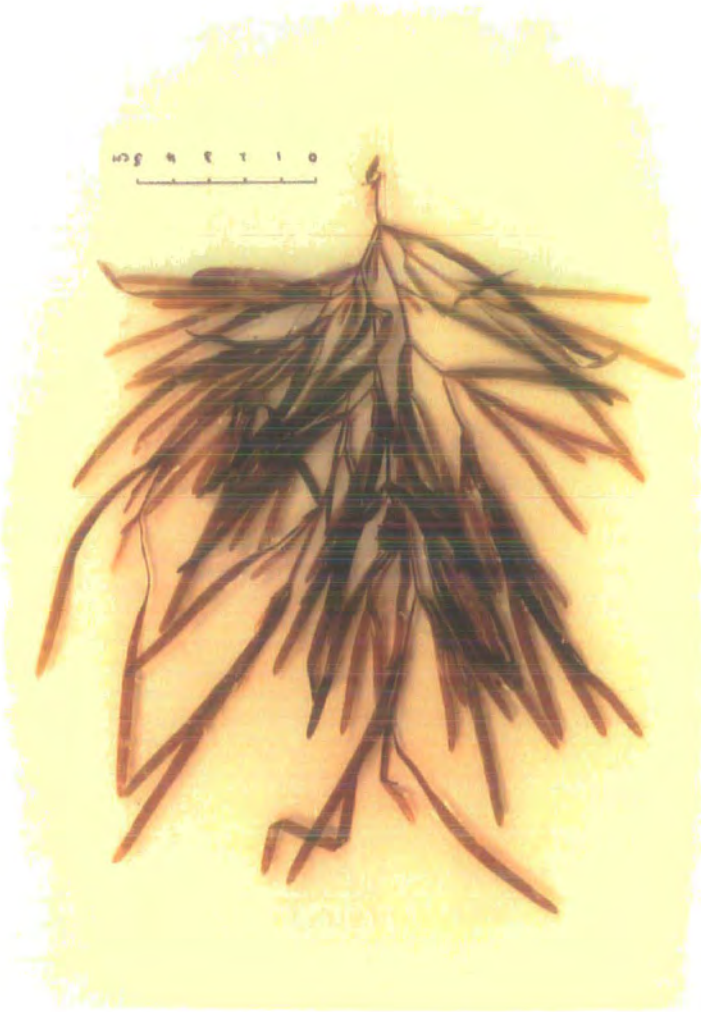

0 1 2 3 4 5cm

4 ENVIRONMENTAL DATA

4.1 Water analysis

Water samples were taken monthly from 25/03/93 to 18/07/94 for Sites 01, 03, 05, 08, 09, and monthly from 21/04/93 to 18/07/94 for Sites 01A and upstream of the STW. Sites 01, 01A, 03, 08 and 09 were sampled a second time in June when the water levels were high. Water samples were taken twice monthly from the Calcareous Flush. See Section 2.1 for the sampling programme.

These data are summarised in Tables 4.11, 4.12 and 4.13. The minimum, maximum and mean values are first given for the twelve month period from 21/04/93 to 14/03/94, not including when the Reservoir was sampled a second time in June. "Mean" pH has been given (Section 2.82). The minimum, maximum and mean values are then given for the whole data set so that comparisons between the two can be made. The full data collected are shown graphically in Figures 4.1 to 4.8 and appendix 4.



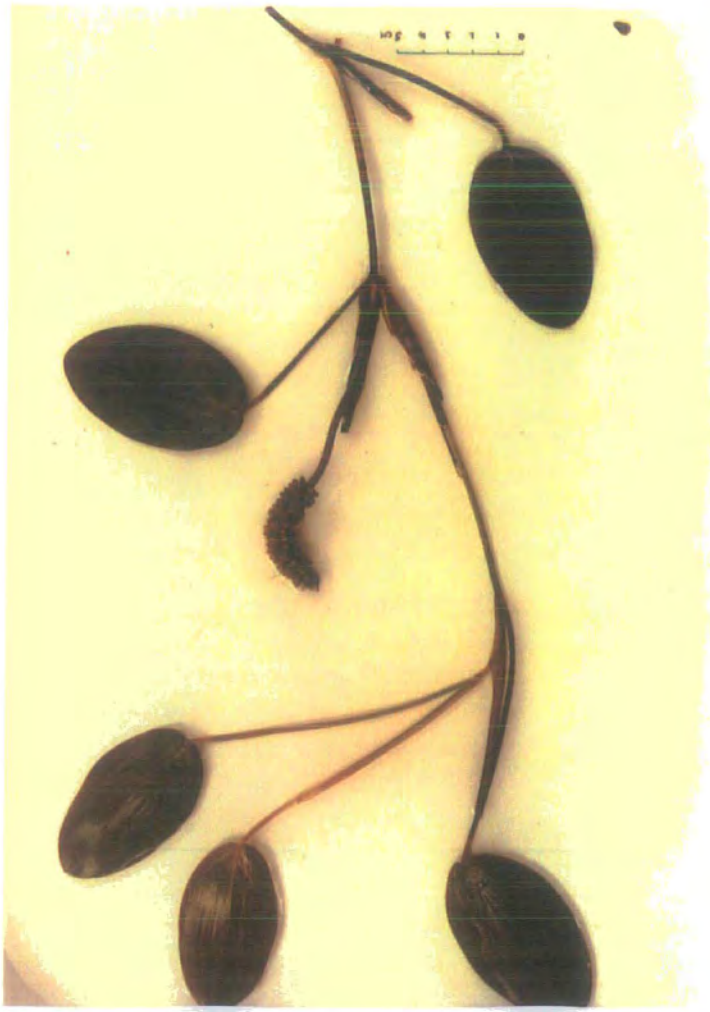


Figure 3.10

Study organisms *Potamogeton berchtoldii* a close up showing inflorescence.

Figure 3.11

Study organism *Potamogeton berchtoldii*. Scale bar is 5 cm in length.

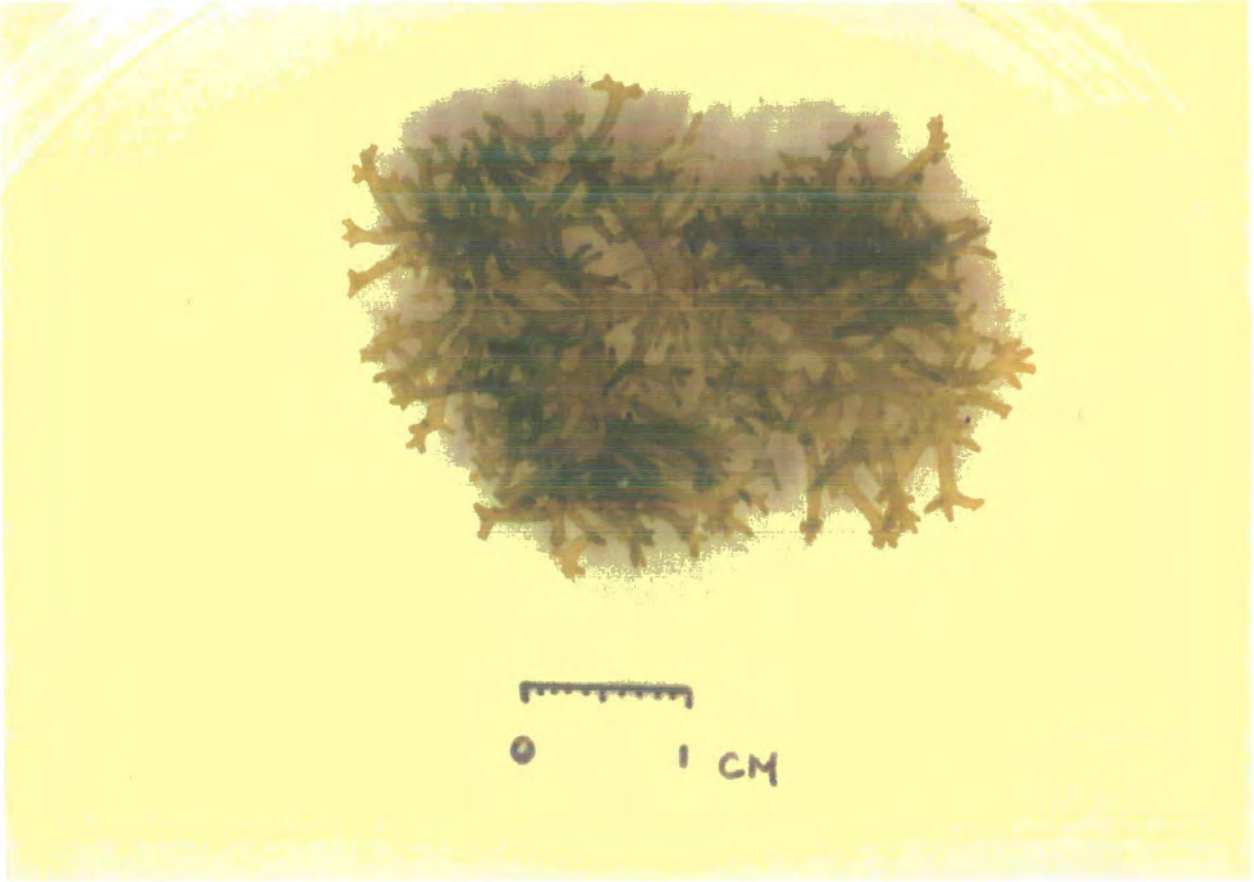

0
1 CM

Figure 3.12

Study organism *Potamogeton natans*. Scale bar is 5 cm in length.

Figure 3.13

Study organism *Potamogeton obtusifolius*. Scale bar is 5 cm.

Table 4.11 Physical and chemical variables at site USTW (upstream of sewage treatment works). n = 12 (12 months) for the first set of columns and n = 16 (16 months) for the second set.

variable	unit	min.	max.	mean	min.	max.	mean
temperature	^{o}C	0.1	15.8	7.2	0.1	16.1	8.4
conductivity	$\mu S\ cm^{-1}$	113.0	193.0	154.0	113.0	298.0	177.6
absorbance	at 420 nm	0.02	0.06	0.04	0.01	0.06	0.0
oxygen	$mg\ l^{-1}$	7.8	13.9	10.8	7.8	14.6	11.4
pH		7.3	8.3	7.8	7.3	8.6	8.0
total alkalinity	$meq\ l^{-1}$	1.5	2.4	1.9	1.5	5.6	2.6
Na	$mg\ l^{-1}$	1.8	13.7	6.8	1.8	13.7	6.9
K	$mg\ l^{-1}$	0.6	1.4	0.8	0.6	2.1	1.0
Mg	$mg\ l^{-1}$	2.5	8.9	5.2	2.5	11.8	6.1
Ca	$mg\ l^{-1}$	8.2	29.0	16.8	8.2	34.9	18.9
Mn	$mg\ l^{-1}$	0.01	0.20	0.04	0.01	0.2	0.0
Fe	$mg\ l^{-1}$	0.06	0.9	0.34	0.06	0.9	0.3
NO_3-N	$\mu g\ l^{-1}$	7.5	95.6	33.7	0.1	95.6	28.6
NO_2-N	$\mu g\ l^{-1}$	1.2	6.7	3.1	0.1	6.7	2.4
NH_4-N	$\mu g\ l^{-1}$	15.2	88.2	51.5	15.2	88.2	53.4
TFP	$\mu g\ l^{-1}$	3.8	16.9	8.8	3.8	16.9	8.9
FRP	$\mu g\ l^{-1}$	<1.0	7.7	3.6	0.5	7.7	3.8

Variables with values outside the range for the 12 month period can be identified from Table 4.11. Variables with values above the previous maximum were: temperature, conductivity, O_2, pH, total alkalinity, K, Mg and Ca. Variables with values below the previous minimum were: NO_3-N and NO_2-N.

Table 4.12 Physical and chemical data for the main Reservoir, based on data from all six sites. n = 72 (12 months) for the first set of columns and n = 107 (17 months and a second time in June) for the second set.

variable	unit	min.	max.	mean	min.	max.	mean
temperature	^{o}C	0.2	16.2	6.9	0.2	19.4	8.5
conductivity	$\mu S\ cm^{-1}$	57.0	177.9	116.3	57.0	241.0	128.9
absorbance	at 420 nm	0.02	0.10	0.05	0.02	0.10	0.05
oxygen	$mg\ l^{-1}$	6.0	13.5	10.6	6.0	13.5	10.7
pH		7.0	8.2	7.5	6.5	8.2	'7.6
total alkalinity	$meq\ l^{-1}$	0.7	5.0	1.8	0.7	5.4	2.2
Na	$mg\ l^{-1}$	1.21	15.3	5.84	1.21	15.3	6.65
K	$mg\ l^{-1}$	0.29	3.41	0.76	0.29	9.8	1.05
Mg	$mg\ l^{-1}$	0.7	8.20	4.04	0.70	10.8	4.72
Ca	$mg\ l^{-1}$	4.96	27.6	14.59	4.8	28.3	15.36
Mn	$mg\ l^{-1}$	0.01	0.23	0.05	0.01	0.40	0.05
Fe	$mg\ l^{-1}$	0.04	2.10	0.43	0.01	2.10	0.35
NO_3-N	$\mu g\ l^{-1}$	8.5	117.8	44.9	5.1	117.8	41.5
NO_2-N	$\mu g\ l^{-1}$	0.1	8.2	3.8	0.1	8.2	3.5
NH_4-N	$\mu g\ l^{-1}$	16.3	106.8	58.7	15.7	135.5	58.0
TFP	$\mu g\ l^{-1}$	<1.0	43.0	10.0	<1.0	49.7	11.4
FRP	$\mu g\ l^{-1}$	<1.0	29.0	4.8	<1.0	49.7.0	5.6
Secchi depth	m	0.63	1.38	0.94	0.63	2.14	1.13

Variables with values outside the range for the 12 month period can be identified from Table 4.12. Variables with values above the previous maximum were: temperature, conductivity, total alkalinity, K, Mg, Ca, Mn, NH_4-N, TFP, FRP and Secchi depth. Variables with values below the previous minimum were: pH, Ca, Fe, NO3-N, and NH4-N.

To identify which variables have changed significantly between April to July 1993 and April to July 1994 paired T-tests were run on the data. As the number of T-tests run was large a significance levels of P = 0.01 and P= 0.02 were chosen. Significant differences are shown in Table 4.13 (significantly higher) and 4.14 (significantly lower). The number of sites, out of a maximum number of 6, where a significant change has occurred are shown.

Table 4.13 Variables that were significantly higher during April to July 1994 than April to July 1993, and the number of sites where the significant increase occurs.

Variable	No. of sites showing significant increase $\mathbb{P} < 0.01$	Number of sites showing significant increase $\mathbb{P} < 0.02$
conductivity	1	3
total alkalinity	6	6
K	2	2
Mg	1	3
Ca	0	1
Mn	0	1
NH_4-N	1	1

Table 4.14 Variables that were significantly lower during April to July 1994 than April to July 1993, and the number of sites where the significant increase occurs.

Variable	No. of sites showing significant decrease $P < 0.01$	Number of sites showing significant decrease $P < 0.02$
absorbance	0	2
Fe	2	2
NO_2-N	6	6

Table 4.15 Physical and chemical data for the Calcareous Flush. n = 24 (12 months) for the first set of data and n = 33 (17 months) for the second set of data.

variable	unit	min.	max.	mean	min.	max.	mean
temperature	^oC	0.5	17.7	9.76	0.5	20.0	10.7
conductivity	$\mu S\ cm^{-1}$	435.0	560.0	488.9	435.0	589.0	491.2
absorbance	at 420 nm	0.01	0.05	0.01	0.000	0.05	0.00
oxygen	$mg\ l^{-1}$	7.8	12.3	9.7	7.8	12.3	9.7
pH		7.19	8.3	8.04	7.2	8.4	8.1
total alkalinity	$meq\ l^{-1}$	6.97	11.0	9.15	6.97	12.0	9.4
Na	$mg\ l^{-1}$	0.34	9.2	4.55	0.34	21.9	6.4
K	$mg\ l^{-1}$	0.8	2.6	1.49	0.8	3.4	1.8
Mg	$mg\ l^{-1}$	4.74	33.8	20.23	4.74	33.8	20.9
Ca	$mg\ l^{-1}$	9.05	120.4	79.11	9.05	120.4	75.9
Mn	$mg\ l^{-1}$	0.01	0.16	0.03	0.01	0.16	0.02
Fe	$mg\ l^{-1}$	0.01	0.47	0.12	0.01	0.47	0.10
NO_3-N	$\mu g\ l^{-1}$	0.10	58.86	23.64	0.10	58.86	19.8
NO_2-N	$\mu g\ l^{-1}$	0.10	6.5	1.86	0.10	6.5	1.6
NH_4-N	$\mu g\ l^{-1}$	15.8	84.1	41.11	15.8	185.9	46.6
TFP	$\mu g\ l^{-1}$	<1.0	25.0	6.88	<1.0	25.0	7.5
FRP	$\mu g\ l^{-1}$	<1.0	10.10	3.23	<1.0	10.10	3.3

It must be noted that the Flush was sampled twice a month, whereas the other 7 sites were sampled monthly. Note the high conductivity and Ca concentrations.

Variables with values outside the range for the 12 month period can be identified from Table 4.15. Variables with values above the previous maximum were: temperature, conductivity, pH, total alkalinity, Na, and K. Variables with values below the previous minimum are: absorbance.

The data for physical and chemical features of the water are shown graphically for the whole data set from March 1993 to July 1994 inclusive.

FIG 4.1 SITE 1 PHYSICAL AND CHEMICAL DATA

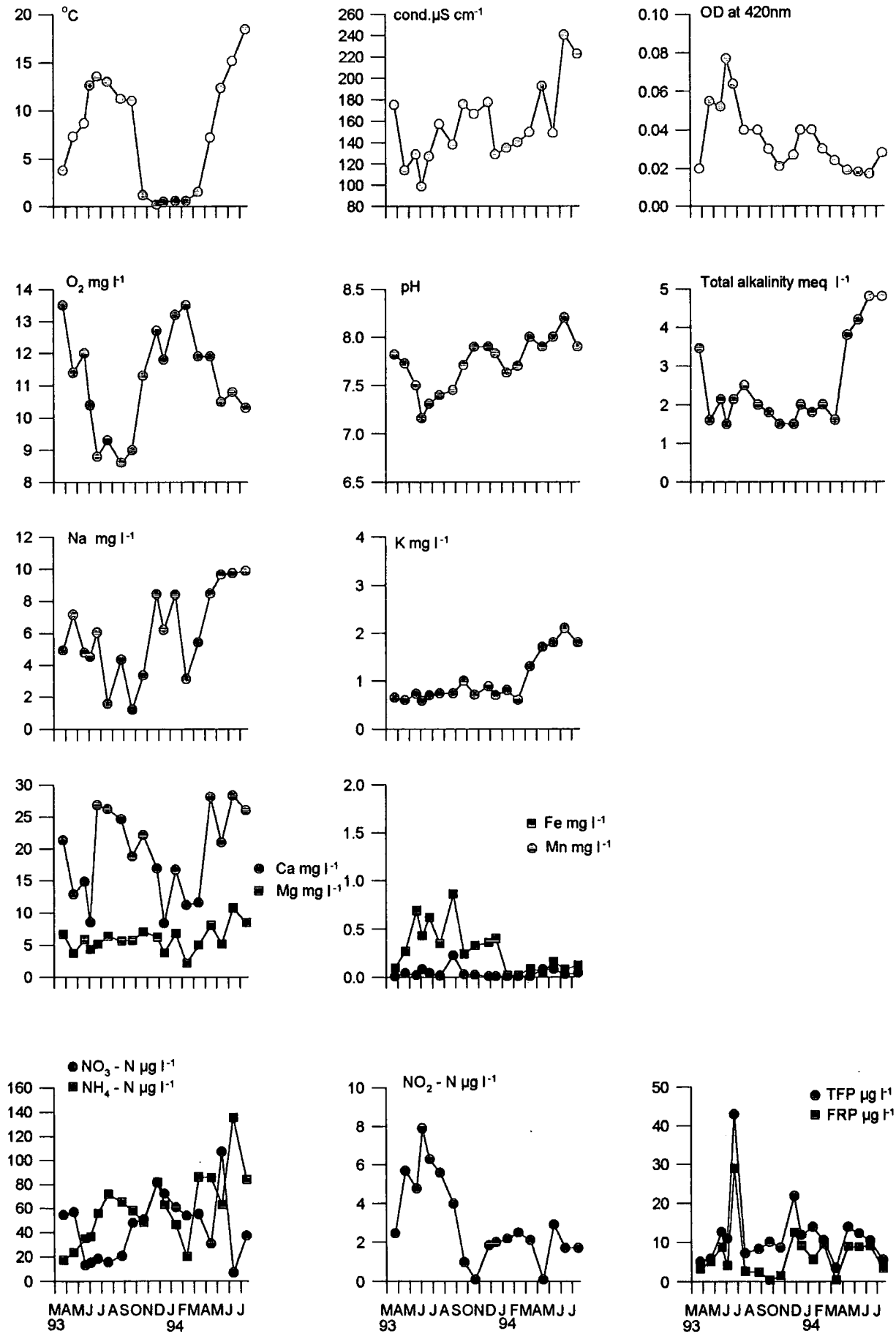


FIG 4.2 SITE 1A PHYSICAL AND CHEMICAL DATA

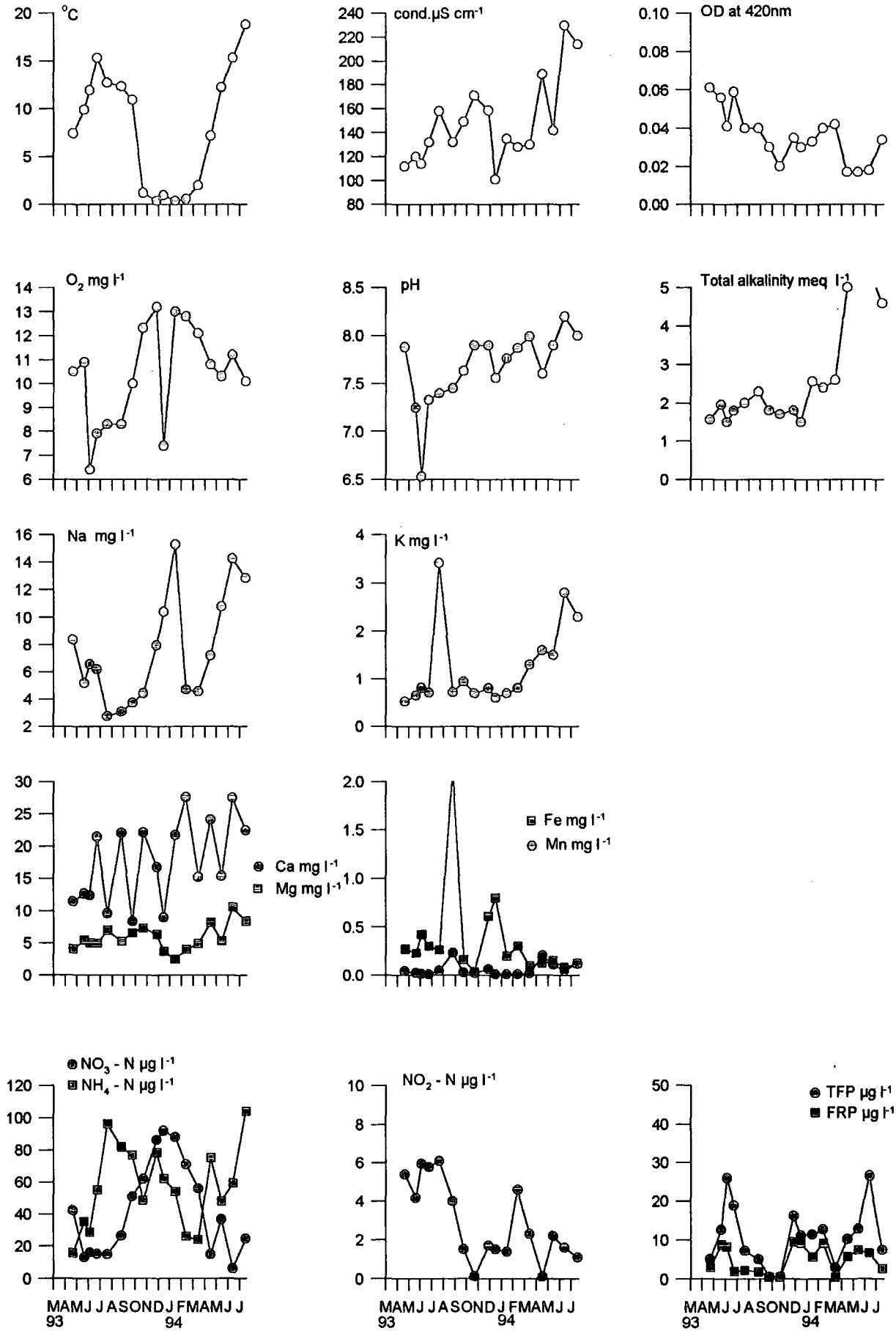
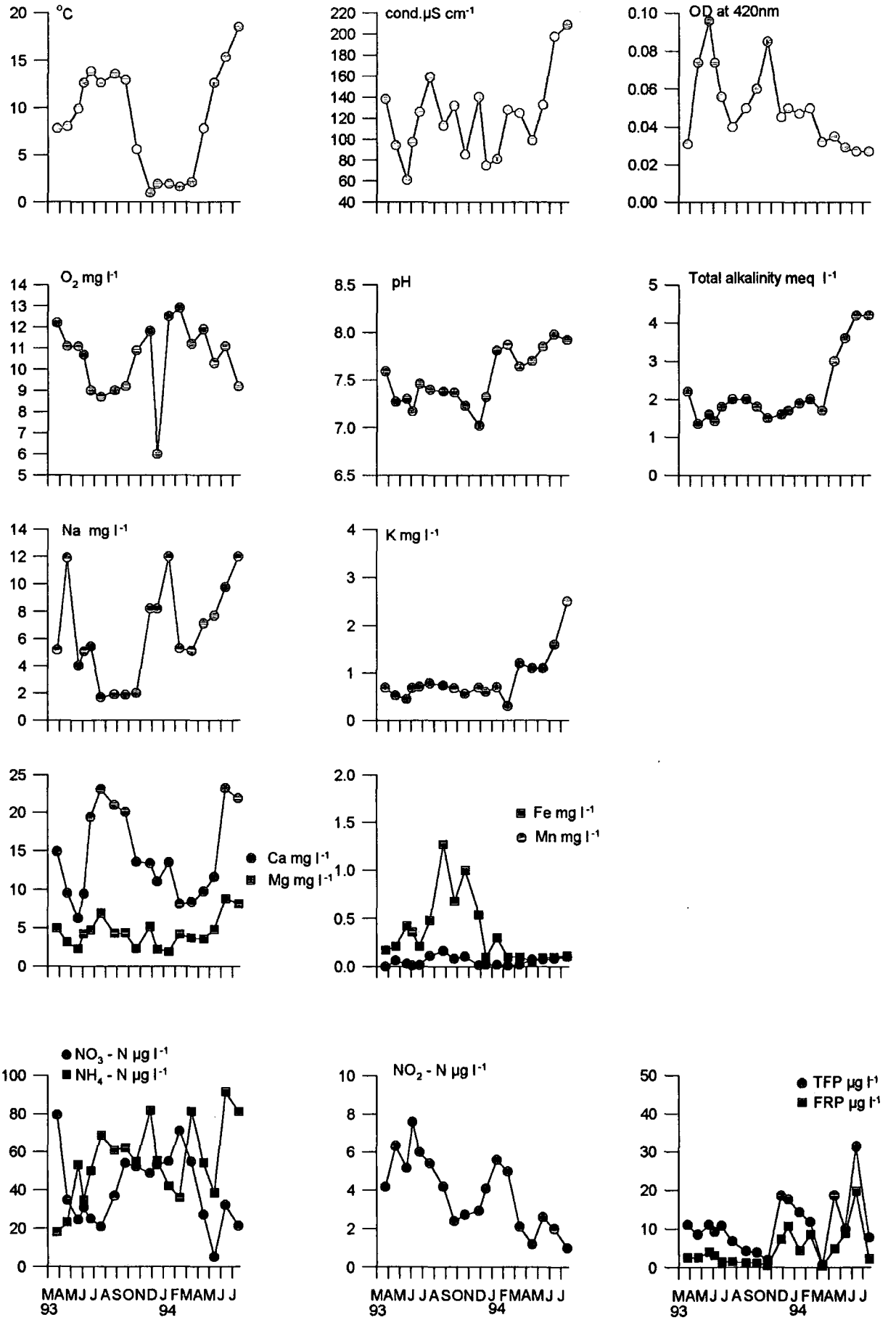
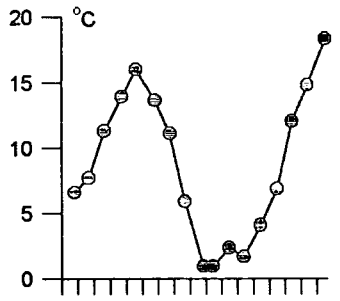
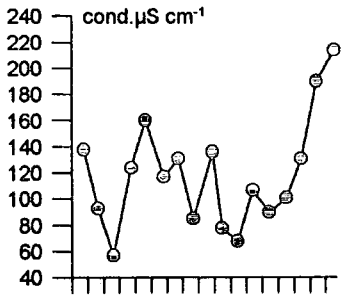
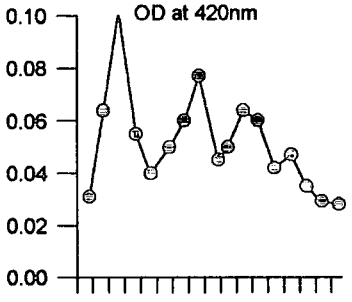


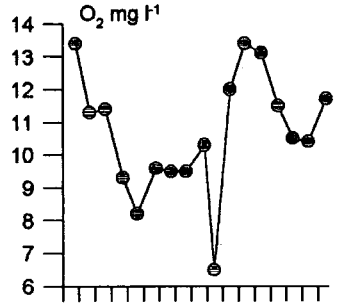
FIG 4.3 SITE 3 PHYSICAL AND CHEMICAL DATA

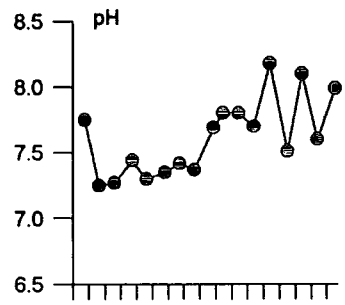


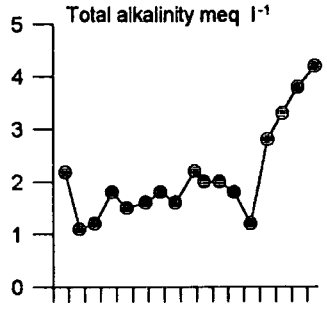

°C
20
15
10
5
0

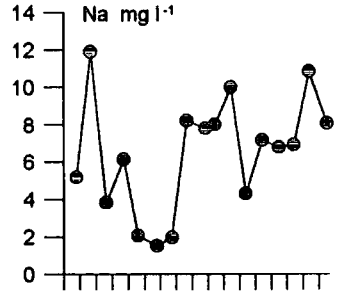

cond.µS cm-1
240
220
200
180
160
140
120
100
80
60
40

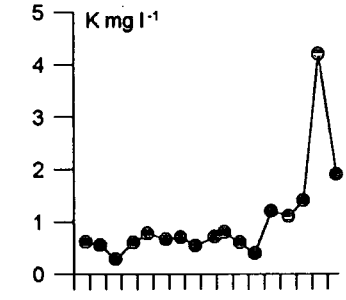

OD at 420nm
0.10
0.08
0.06
0.04
0.02
0.00

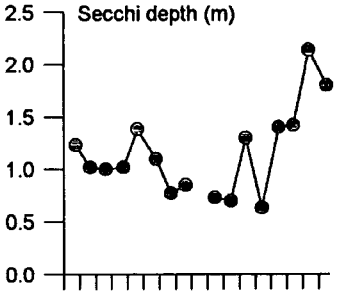

O2 mg l-1
14
13
12
11
10
9
8
7
6

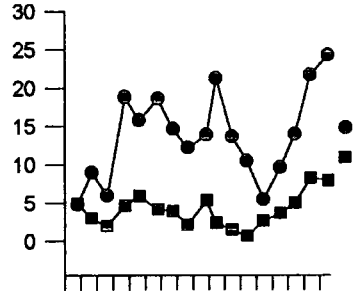

pH
8.5
8.0
7.5
7.0
6.5

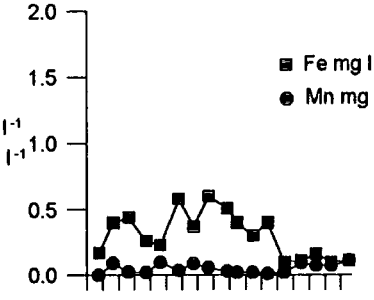

Total alkalinity meq l-1
5
4
3
2
1
0

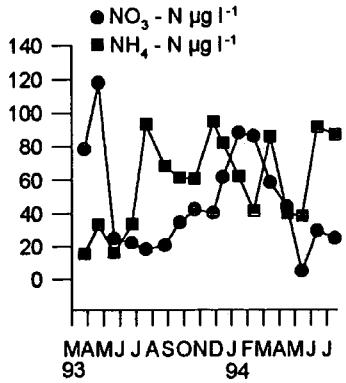

Na mg l-1
14
12
10
8
6
4
2
0

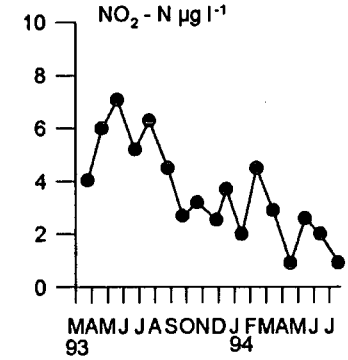

K mg l-1
5
4
3
2
1
0

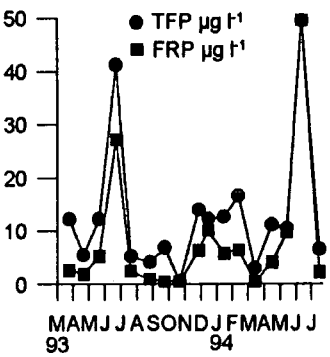

Secchi depth (m)
2.5
2.0
1.5
1.0
0.5
0.0

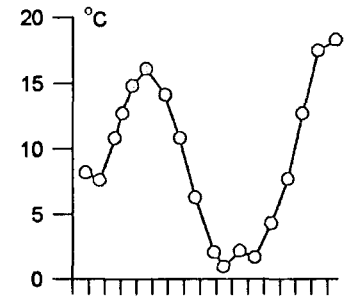

30
25
20
15
10
5
0
Ca mg l-1
Mg mg l-1

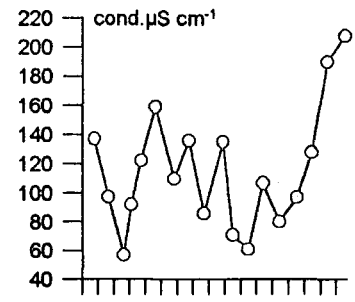

2.0
1.5
1.0
0.5
0.0
Fe mg l-1
Mn mg l-1

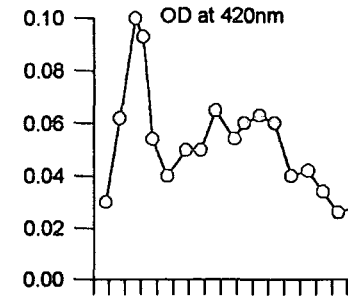

NO3 - N µg l-1
NH4 - N µg l-1
140
120
100
80
60
40
20
0
MAMJ J ASONDJ FMAMJ J
93
94

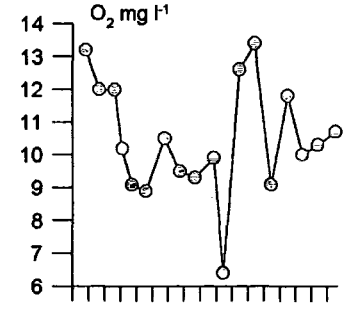

NO2 - N µg l-1
10
8
6
4
2
0
MAMJ J ASONDJ FMAMJ J
93
94

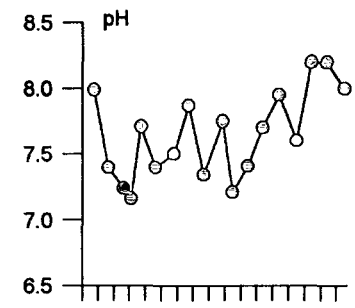

TFP µg l-1
FRP µg l-1
50
40
30
20
10
0
MAMJ J ASONDJ FMAMJ J
93
94

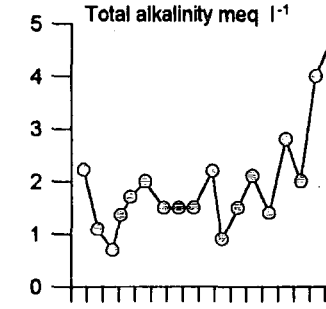

°C
20
15
10
5
0

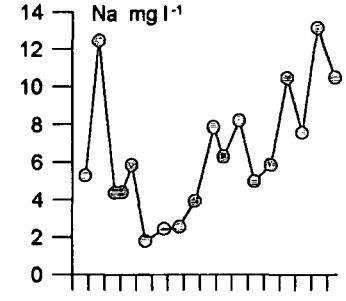

cond.µS cm-1
220
200
180
160
140
120
100
80
60
40

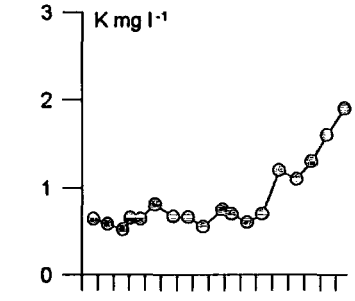

OD at 420nm
0.10
0.08
0.06
0.04
0.02
0.00

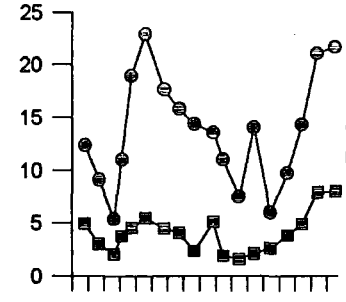

O2 mg l-1
14
13
12
11
10
9
8
7
6

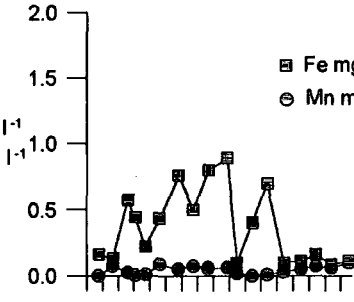

pH
8.5
8.0
7.5
7.0
6.5

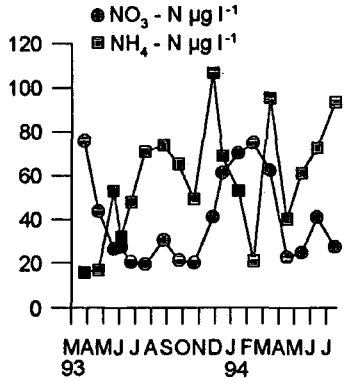

Total alkalinity meq l-1
5
4
3
2
1
0

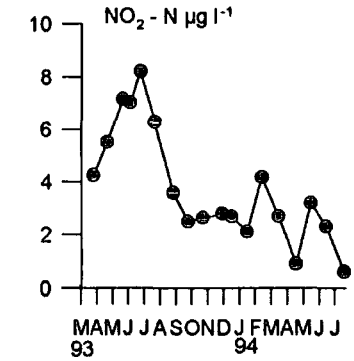

Na mg l-1
14
12
10
8
6
4
2
0

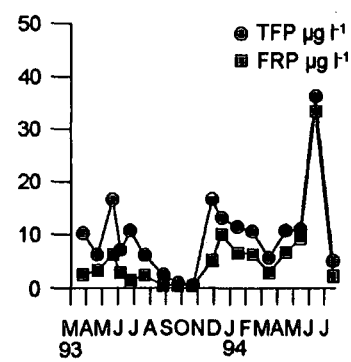

K mg l-1
3
2
1
0


25
20
15
10
5
0
Ca mg l-1
Mg mg l-1


2.0
1.5
1.0
0.5
0.0
Fe mg l-1
Mn mg l-1


NO3 - N µg l-1
NH4 - N µg l-1
120
100
80
60
40
20
0
MAMJ J ASONDJ FMAMJ J
93
94


NO2 - N µg l-1
10
8
6
4
2
0
MAMJ J ASONDJ FMAMJ J
93
94


TFP µg l-1
FRP µg l-1
50
40
30
20
10
0
MAMJ J ASONDJ FMAMJ J
93
94

SITE 9 PHYSICAL AND CHEMICAL DATA

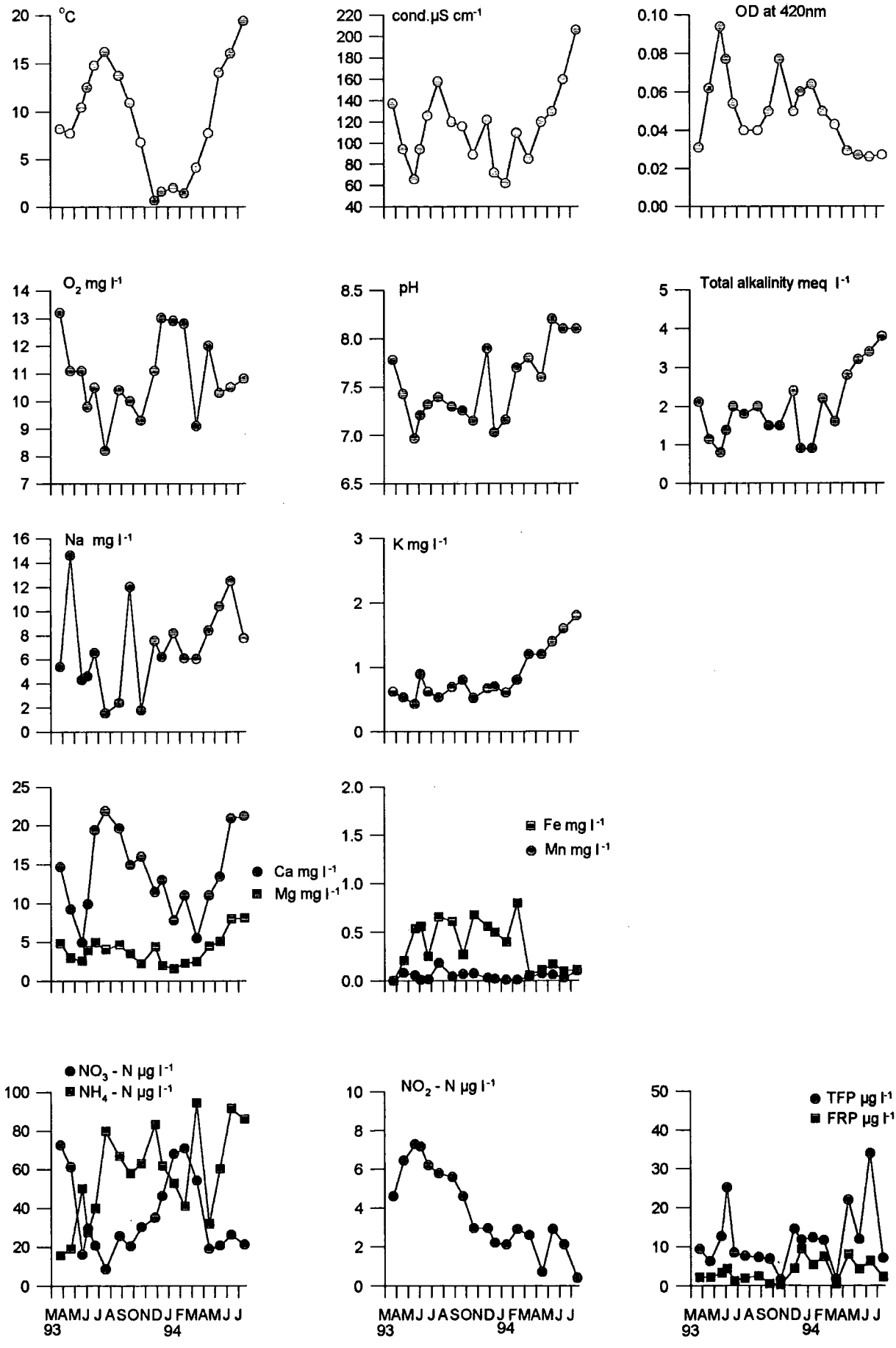


FIG 4.7 SITE USTW PHYSICAL AND CHEMICAL DATA

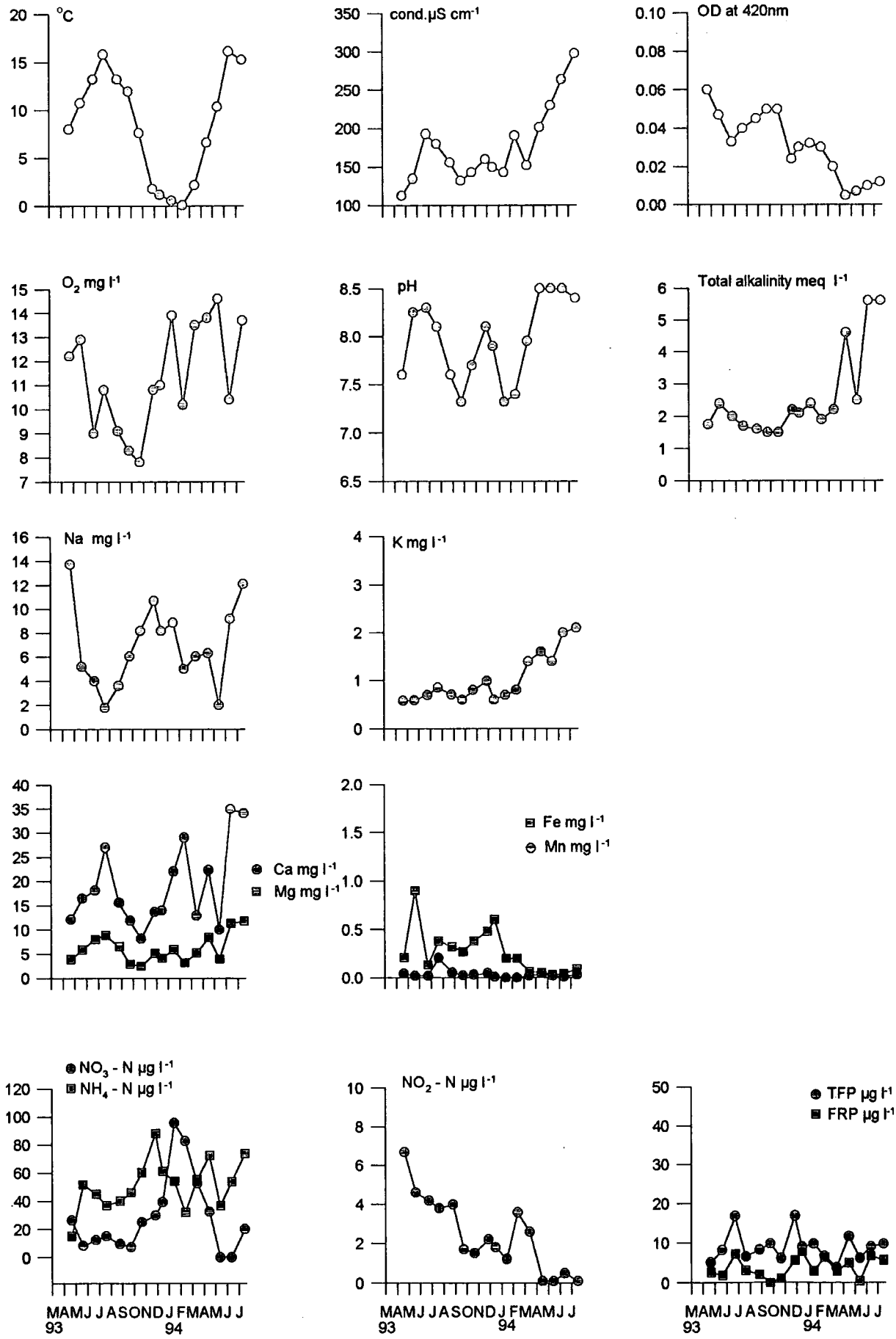
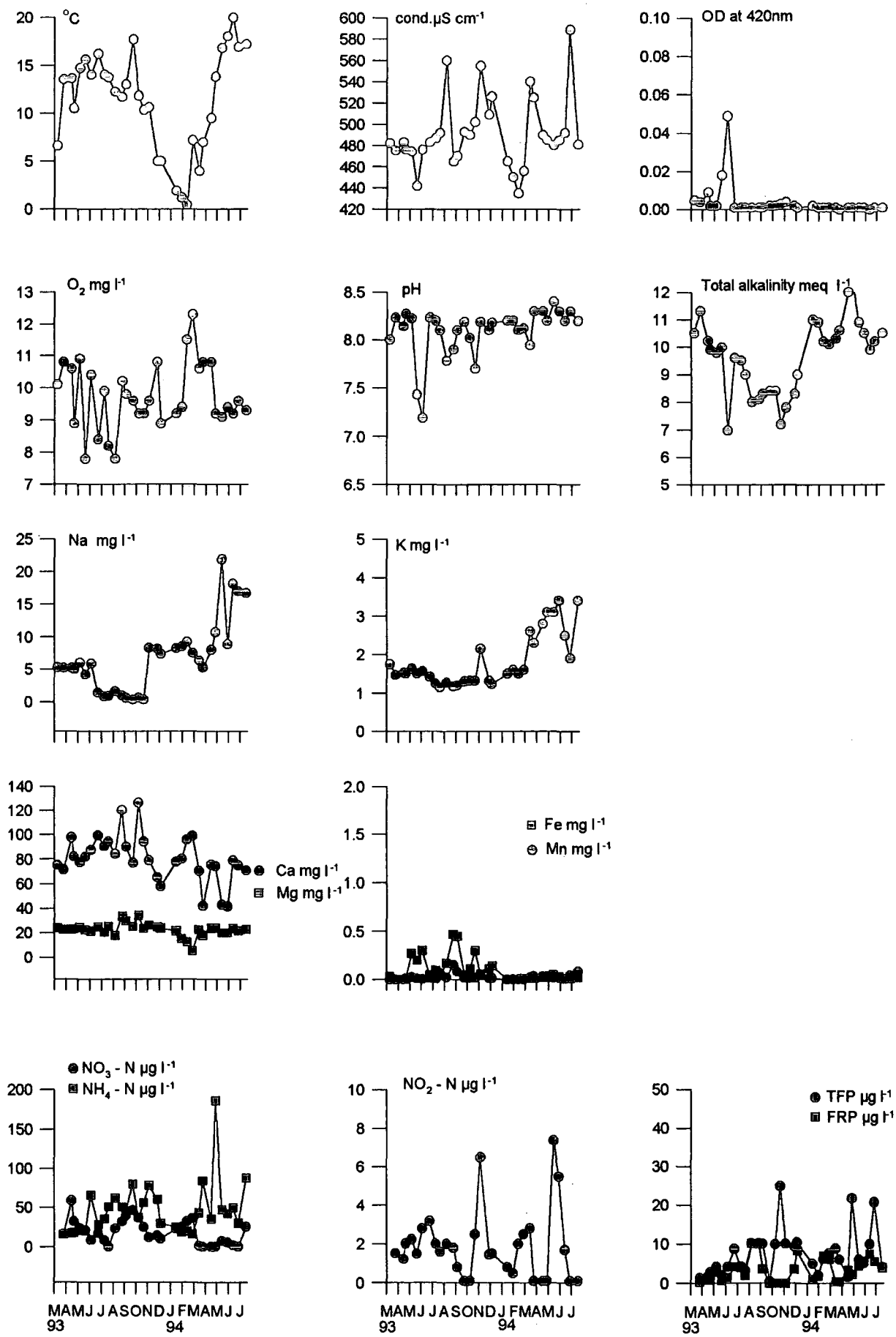


FIG. 4.8 FLUSH PHYSICAL AND CHEMICAL DATA



4.2 N:P ratios

Tables 4.21 to 4.26 give the N:P ratios for all main Reservoir water samples analysed. The N:P ratios are based on mass, and where a sample is below the detection limit the N:P ratio is calculated using a value halfway between the detection limit value and zero. For P the detection limit is 1.0 $\mu g\ l^{-1}$ and so a value of 0.5 is used in the calculation. N:P ratios have been calculated using inorganic N (total of the N fractions measured) and both TFP and FRP.

Table 4.21 Total N, TFP, FRP, FOP,($\mu g\ l^{-1}$) and N:TFP and N:FRP ratios by weight for SITE 01 on each sampling date.

Date	Total N	TFP	FRP	FOP	N:TFP	N:FRP
25/03/93	74.4	4.9	3.3	1.6	15.1	22.7
21/04/93	86.3	5.7	5.1	0.6	15.0	17.0
20/05/93	53.1	12.6	8.7	3.9	4.2	6.1
03/06/93	59.6	10.9	4.1	6.8	5.5	14.5
22/06/93	80.5	43.0	29.0	14.0	1.9	2.8
19/07/93	92.9	7.2	2.6	4.6	13.0	35.6
23/08/93	90.1	8.3	2.4	5.9	10.9	37.5
20/09/93	107.3	10.1	<1.0	9.6	10.6	214.6
18/10/93	99.3	8.6	1.5	7.1	11.5	66.2
30/11/93	164.9	21.9	12.5	9.4	7.5	13.2
18/12/93	137.0	11.9	9.2	2.7	11.5	14.9
17/01/94	109.7	13.9	5.7	8.2	7.9	19.2
14/02/94	76.5	10.5	9.5	1.0	7.3	8.1
17/03/94	143.4	3.4	<1.0	2.9	42.2	286.8
18/04/94	116.3	13.9	8.9	5.0	8.4	13.1
16/05/94	173.1	12.3	8.8	3.5	14.1	19.6
14/06/94	144.1	10.4	9.1	1.3	13.9	15.8
18/07/94	123.2	5.5	3.5	2.0	22.5	35.3

Table 4.22 Total N, TFP, FRP, FOP,(μg l^{-1}) and N:TFP and N:FRP ratios by weight for SITE 01A on each sampling date.

Date	Total N	TFP	FRP	FOP	N:TFP	N:FRP
21/04/93	63.4	4.9	2.9	2.0	12.8	21.7
20/05/93	52.5	12.6	8.7	3.9	4.2	6.0
03/06/93	50.6	26.0	8.2	17.8	2.0	6.2
22/06/93	75.8	18.9	1.9	17.0	4.0	40.1
19/07/93	117.3	7.2	2.2	5.0	16.4	54.1
23/08/93	112.5	5.0	1.8	3.2	22.5	62.5
20/09/93	129.2	<1.0	<1.0	0.0	280.9	258.4
18/10/93	110.5	<1.0	<1.0	0.0	245.5	221.0
30/11/93	166.3	16.2	9.5	6.7	10.3	17.5
18/12/93	155.5	11.2	9.2	2.0	13.9	16.9
17/01/94	143.4	11.4	5.7	5.7	12.6	25.2
14/02/94	101.6	12.7	9.2	3.5	8.0	11.0
17/03/94	126.8	2.9	<1.0	2.4	43.7	253.6
18/04/94	90.1	10.2	5.6	4.6	8.83	16.1
16/05/94	86.7	12.8	7.3	5.5	6.8	11.9
14/06/94	67.2	26.7	6.6	21.1	2.5	10.1
18/07/94	129.8	7.4	2.6	4.8	17.6	49.2

Table 4.23 Total N, TFP, FRP, FOP,(μg l^{-1}) and N:TFP and N:FRP ratios by weight for SITE 03 on each sampling date.

Date	Total N	TFP	FRP	FOP	N:TFP	N:FRP
25/03/93	101.6	11.0	2.6	8.4	9.3	39.7
21/04/93	64.0	8.6	2.6	6.0	7.5	25.0
20/05/93	82.8	11.0	4.0	7.0	7.6	20.7
03/06/93	73.1	9.2	3.1	6.1	8.0	23.7
22/06/93	81.0	10.8	1.5	9.3	7.5	55.5
19/07/93	95.0	6.8	1.5	5.3	13.9	62.5
23/08/93	102.0	4.2	1.3	2.9	24.3	78.5
20/09/93	118.6	3.8	1.2	2.6	31.0	98.8
18/10/93	109.6	1.9	<1.0	1.4	57.7	219.2
30/11/93	133.2	18.5	7.3	11.2	7.2	18.3
18/12/93	112.4	17.7	10.7	7.0	6.4	10.5
17/01/94	102.7	14.3	4.5	9.8	7.2	22.8
14/02/94	112.0	11.8	8.5	3.3	9.5	13.2
17/03/94	138.3	<1.0	<1.0	0.0	276.6	276.6
18/04/94	82.7	18.6	4.9	13.7	4.5	16.9
16/05/94	46.2	9.9	8.8	1.1	4.7	5.2
14/06/94	125.8	31.5	19.8	11.7	4.0	6.4
18/07/94	103.6	7.8	2.2	5.6	13.2	46.7

Table 4.24 Total N, TFP, FRP, FOP,(µg l^{-1}) and N:TFP and N:FRP ratios by weight for SITE 05 on each sampling date.

Date	Total N	TFP	FRP	FOP	N:TFP	N:FRP
25/03/93	98.2	12.2	2.6	9.6	8.1	38.3
21/04/93	156.9	5.3	1.8	3.5	29.4	85.8
20/05/93	48.3	12.2	5.1	7.1	4.0	9.5
22/06/93	60.9	41.2	27.2	14.0	1.5	2.2
19/07/93	117.9	5.1	2.4	2.7	23.3	49.3
23/08/93	93.6	4.0	<1.0	3.5	23.4	187.2
20/09/93	98.5	6.7	<1.0	6.2	14.7	197.0
18/10/93	106.1	<1.0	<1.0	0.0	212.2	212.2
30/11/93	137.6	13.9	6.2	7.7	9.9	22.2
18/12/93	147.2	12.3	10.0	2.3	12.0	14.7
17/01/94	152.2	12.6	5.7	6.9	12.1	26.7
14/02/94	132.1	16.6	6.3	10.3	8.0	21.0
17/03/94	147.2	2.9	<1.0	2.4	50.8	294.4
18/04/94	85.1	11.1	4.0	7.1	7.7	21.3
16/05/94	46.2	10.4	9.6	0.8	4.4	4.8
14/06/94	123.2	49.7	49.7	0.0	2.5	2.5
18/07/94	112.8	6.4	2.2	4.2	17.6	50.8

Table 4.25 Total N, TFP, FRP, FOP,(µg l^{-1}) and N:TFP and N:FRP ratios by weight for SITE 08 on each sampling date.

Date	Total N	TFP	FRP	FOP	N:TFP	N:FRP
25/03/93	95.8	10.2	2.6	7.6	9.4	37.4
21/04/93	66.1	6.2	3.3	2.9	10.7	20.1
20/05/93	86.9	16.6	6.2	10.4	5.2	14.1
03/06/93	66.4	7.1	2.8	4.3	9.4	23.7
22/06/93	76.7	10.7	1.5	9.2	7.2	52.5
19/07/93	96.8	6.1	2.4	3.7	15.8	40.5
23/08/93	108.2	2.4	<1.0	1.9	45.1	216.4
20/09/93	89.1	<1.0	<1.0	0.0	178.2	178.2
18/10/93	71.8	<1.0	<1.0	0.0	143.7	143.7
30/11/93	150.7	16.6	5.1	11.5	9.1	29.6
18/12/93	132.9	13.1	10.0	3.1	10.2	13.3
17/01/94	125.6	11.4	6.5	4.9	11.0	19.3
14/02/94	100.2	10.5	6.3	4.2	9.5	15.9
17/03/94	157.9	5.6	2.9	3.7	28.2	54.5
18/04/94	63.7	10.7	6.6	4.1	6.0	9.7
16/05/94	89.1	10.9	9.2	1.7	8.2	9.7
14/06/94	116.1	36.3	33.5	2.8	3.2	3.5
18/07/94	121.4	5.0	2.2	2.8	24.3	54.7

Table 4.26 Total N, TFP, FRP, FOP,($\mu g\ l^{-1}$) and N:TFP and N:FRP ratios by weight for SITE 09 on each sampling date.

Date	Total N	TFP	FRP	FOP	N:TFP	N:FRP
25/03/93	92.9	9.4	2.2	7.2	9.9	42.4
21/04/93	86.9	6.2	2.2	4.0	14.1	39.7
20/05/93	73.7	12.6	3.3	9.3	5.9	22.5
03/06/93	64.4	25.2	4.3	20.9	2.6	15.1
22/06/93	66.8	8.4	1.3	7.1	7.9	51.O
19/07/93	94.3	7.5	2.0	5.5	12.6	48.1
23/08/93	98.2	7.2	2.4	4.8	13.6	40.9
20/09/93	83.1	6.7	<1.0	6.2	12.4	166.2
18/10/93	96.3	1.4	<1.0	0.9	67.8	192.6
30/11/93	121.4	14.4	4.3	10.1	8.4	28.2
18/12/93	110.4	11.6	9.3	2.3	9.5	11.9
17/01/94	123.2	12.2	5.3	6.9	10.1	23.3
14/02/94	114.9	11.5	7.4	4.1	10.0	15.5
17/03/94	148.8	1.6	<1.0	1.1	93.0	297.6
18/04/94	52.1	21.9	7.9	14.0	2.4	6.6
16/05/94	83.8	11.8	4.2	7.6	7.1	19.8
14/06/94	119.9	33.9	6.2	27.7	3.5	19.3
18/07/94	107.6	6.9	2.2	4.7	15.6	48.5

Experimental studies indicate that in practice, N:P ratios (using N:FRP) (by weight) in water of less than 10 indicate that N is limiting and a ratio greater than 17 indicates that P is limiting (Chiudani & Vighi, 1974; Heathwaite, 1993). The number of occasions where there was N- or P-limitation has been calculated and summarised in Table 4.27

Table 4.27 The number of water samples from each site that have shown either P-limitation, N-limitation or neither.

Site	P limitation	N-limitation	Neither
01	10	3	7
01A	10	2	5
03	13	2	3
05	12	3	2
08	12	1	5
09	14	1	3

From Table 4.27 we can see that the samples are most frequently P-limited and least frequently N-limited. Occasions when the N:FRP ratios of the water analysed falls to a level that we infer N is limiting with respect to P are mostly in Spring and early summer, the dates are shown below:

Site 01 20/05/93, 22/06/93, 14/02/94,

Site 01A 20/05/93, 03/06/93,

Site 03 16/05/94, 14/06/94

Site 05 20/05/93, 22/06/93, 14/06/93

Site 08 18/04/94, 16/05/94, 14/06/94

Site 09 18/04/94

A trend shown at all the sites is high N:P ratios found in August, September, October 1993 and March 1994. N:P ratios were not high in March 1993.

Differences between the N:TFP and N:FRP ratios can only occur when there are differences between TFP and FRP, i.e., when there is a significant amount of organic P (or colloidal). Broadly at sites 01A, 05 and 08 there are small differences between the ratios and at sites 01, 03 and 09 there are large differences between the ratios.

N:P ratios of the Calcareous Flush and upstream of the STW have also been calculated. At USTW the N:FRP ratios range from 8.16 to 110.2. Twelve occasions indicated P limitation, two occasions indicated neither nutrient was limited with respect to the other and two occasions indicated N limitation and these were: 22/06/93 and 14/06/94. At the Flush the N:FRP ratios range from 7.55 to 234.6. N:P ratios in the water analysed from the Calcareous Flush fell to a level where we infer that N is limiting with respect to P on the following 6 occasions:

08/07/93, 05/08/93, 23/08/93, 08/12/93, 14/02/93, 17/03/93, 14/06/94

4.3 Sediments

4.31 Grain size analysis

Sediments from 7 sites were analysed for grain size distribution and N content and sediments from 31 sites (including the above 7) were analysed for organic matter and P content. These data have been summarised in Tables 4.1 to 4.5.

Table 4.31 Grain size analysis (g d wt^{-1}) shown as a percentage for the 6 main Reservoir sites and the site USTW.

site	gravel (%)	sand (%)	silt + clay (%)
01	4.76	59.86	35.38
01A	5.89	22.74	71.37
03	11.74	70.83	17.43
05 (MOR)	3.22	5.74	91.03
08	25.17	51.47	23.36
09	49.00	24.82	26.18
USTW	16.52	81.72	1.75

Sediment texture is given by references to the proportion of sand, silt and clay Site 05 has the greatest percentage of silt and clay. This sediment sample was taken using SCUBA techniques from the bottom of the Reservoir. Site USTW on the river North Tyne before the Reservoir begins has the lowest percentage by weight of silt and clay. The sediments are sorted by water movement into particle size gradients.

4.32 Organic matter content

The percentage of organic matter (g d wt^{-1}) in sediments from each of the sampling sites was determined. In addition the sites from the middle of the Reservoir were also analysed. The samples from the middle of the Reservoir were collected using SCUBA techniques.

Table 4.32 Percentage organic matter (g d. wt^{-1}) of sediment samples

	site	% OM (g d wt^{-1})
Shore	O1	4.60
	01A	4.97
	03	1.25
	08	1.14
	09	2.75
	USTW	0.89
Middle	01	3.77
	03	12.23
	05	10.02
	BUOY (AS)	11.78
	WEIR	12.98

The sediment from the middle of the Reservoir have a significantly higher percentage of organic matter than the shore sites. Distinction must be made between non-living organic matter (detritus) and that associated with organisms.

4.34 P content

The P content of a number of sediments from the Reservoir have been analysed. Table 4.33 shows the results of sediments from the shore side and middle of the Reservoir sites.

Table 4.33 P content of sediments (µg g^{-1}) from the shore side sites and the middle of the Reservoir sites.

	Site	**P (µg g^{-1})**
shore	01	92.0
	01A	91.0
	03	31.0
	08	38.0
	09	61.0
	USTW	3.0
middle of Reservoir	01	122.0
	03	241.0
	05	392.0
	BUOY (AS)	470.0
	WEIR	260.0

The sediment from the middle of the Reservoir has a significantly higher percentage of P than the shore sites. The sediment from site USTW is notably low in P.

4.34 N content

The N content of sediment samples from around the Reservoir were analysed (Table 4.34).

Table 4.34 N content of sediments ($\mu g\ g^{-1}$)

	site	NO_3-N	NO_2-N	NH_4-N	Total N	N:P ratio
shore	01	2.005	0.043	4.715	6.76	0.074
	01A	0.584	0.058	5.239	5.88	0.065
	03	1.164	0.379	7.518	9.06	0.292
	08	2.164	0.073	2.676	4.91	0.129
	09	2.734	0.023	1.455	4.21	0.069
	USTW	1.126	0.011	1.872	3.01	1.003
MOR	05					

The N:P ratio in the sediments is considerably lower than the ratios found in the water.

In addition to the results shown above an additional 20 samples collected from sites around the Reservoir were analysed for P and percentage (by weight) of organic matter (Table 4.35).

Table 4.35 P (μg g $^{-1}$) and % organic matter (by weight) of sediments from sites around the Reservoir.

site	% OM	P (μg g $^{-1}$)	
1	5.43	132	
2	1.88	255	
3	1.40	1	*
4	4.46	3	*
5	4.93	133	
6	4.08	16	
7	0.93	36	
8	1.87	80	
9	1.22	32	
10	19.32	845	
11	4.75	388	
12	7.59	459	
13	2.05	181	
14	1.43	85	
15	3.41	130	
16	55.22	824	
17	2.20	65	
18	0.52	23	
19	19.12	82	
20	4.38	75	

Two Sites (3 & 4) marked with a * have a P content considerably lower than the other sites.

The correlation between % organic matter and P (μg g $^{-1}$) was analysed and showed the following results:

$R^2 = 0.55$, df = (1, 18), beta = 0.744, significant at the level $p < 0.001$

The analysis was repeated ommiting Sites 3 and 4 the results were similar:

$R^2 = 0.55$, df = (1, 16), beta = 0.744, significant at the level $p < 0.001$

Sediments with high organic matter contents generally have a higher P content.

4.4 Statistical analysis of data

The relationships within and between the environmental variables measured (including rain) and the quantitative biological data collected have been characterised (Section 2.8). Significant positive and negative correlations are shown below.

Significant positive correlations were found between the following environmental variables.

		R^2-value
1. conductivity	total alkalinity	0.10
2. conductivity	Mg^{2+}	0.36
3. conductivity	Ca^{2+}	0.75
4. Ca^{2+}	Mg^{2+}	0.53
5. Ca^{2+}	total alkalinity	0.27
6. TFP	FRP	0.09
7. Picoplankton	phytoplankton	0.49
8. Phytoplankton	temperature	0.61
9. Optical density	Secchi depth	0.25

Significant negative correlations were found between the following environmental variables

10. optical density	pH	0.99
11. phytoplankton	rainfall	0.40
12. phytoplankton	nitrate	0.46

For all other values, no significant correlations were found.

5 FLORISTIC SURVEY

5.1 Algae

Algal samples were collected to coincide with the taking of water samples from 6 sites around the Reservoir, including phytoplankton from Site 05, the mid-Reservoir site. 87 samples were collected in all. 210 taxa were found in the regular sampling programme and these are listed below. The phytoplankton and picoplankton data are summarised in Table 5.1. The 87 algal samples have been analysed using TWINSPAN and the results are shown in Section 5.3. For explanation of the code see Section 2.83. Authorities have not been abbreviated.

CODE	TAXON	
Blue-green algae		
10105	*Anabaena*	*inaequalis* (Kützing) Bornet et Flahault
10131	*Anabaena*	not above <= 4 µm
10150	*Anabaena*	sp.
10192	*Anabaena*	sp. > 4 µm <= 8 µm
10450	*Aphanocapsa*	sp.
10850	*Chroococcus*	sp.
11350	*Dermocarpa*	sp.
12533	*Lyngbya*	not above > 2 µm <= 4 µm
12550	*Lyngbya*	sp.
12592	*Lyngbya*	sp. > 4 µm <= 8 µm
12593	*Lyngbya*	sp. > 1 µm <= 2 µm
12594	*Lyngbya*	sp. > 4 µm <= 6 µm
12595	*Lyngbya*	sp. > 6 µm <= 8 µm
12596	*Lyngbya*	sp. > 8 µm <= 12 µm
13215	*Oscillatoria*	*angusta* Koppe
13235	*Oscillatoria*	not above > 8 µm <= 12 µm
13290	*Oscillatoria*	sp. < 2 µm
13291	*Oscillatoria*	sp. > 1 µm <= 2 µm
13292	*Oscillatoria*	sp. > 4 µm <= 8 µm
13293	*Oscillatoria*	sp. > 8 µm <= 12 µm
13294	*Oscillatoria*	sp. > 2 µm <= 4 µm
13295	*Oscillatoria*	sp. > 4 µm <= 6 µm
13296	*Oscillatoria*	sp. > 6 µm <= 8 µm
13390	*Phormidium*	sp. < 2 µm
13392	*Phormidium*	sp. > 4 µm <= 8 µm
13393	*Phormidium*	sp. > 8 µm <= 16 µm

13396	*Phormidium*	sp. > 4 µm <= 6 µm
13397	*Phormidium*	sp. > 6 µm <= 8 µm
13650	*Pseudanabaena*	sp.
14350	*Tolypothrix*	sp.

Rhodophyta

20250	*Batrachospermum*	sp.

Euglenophyta

30250	*Euglena*	sp.
30201	*Euglena*	*acus* Ehrenberg
30203	*Euglena*	*gracilis* Klebs
30601	*Trachelomonas*	*hispida* (Perty) Stein
30650	*Trachelomonas*	sp.
30690	*Trachelomonas*	sp. <= 16 µm long brown lorica
30692	*Trachelomonas*	sp. <= 16 µm long lorica not brown

Cryptophtya

40201	*Cryptomonas*	*erosa* Ehrenberg
40250	*Cryptomonas*	sp.

Pyrrophyta

50150	*Amphidinium*	sp.
50801	*Peridinium*	*cinctum* (O. F. Müller) Ehrenberg
50850	*Peridinium*	sp.
59991	*Pyrrophyta*	sp. <= 16 µm diameter motile

Chrysophyta

81190	*Ochromonas*	sp. <= 4 µm long
81649	*Mallomonas*	not above
81650	*Mallomonas*	sp.
81950	*Ochromonas*	sp.

Xanthophyta

91350	*Tribonema*	sp.
91391	*Tribonema*	sp. <= 4 µm
91392	*Tribonema*	sp.> 4 <= 8 µm

Bacillariophyta (centrales)

110301	*Coscinodiscus*	*lacustris* Grunow
110450	*Cyclotella*	sp.
110609	*Melosira*	*varians* Agardh
110650	*Melosira*	sp.
110850	*Stephanodiscus*	sp.

Bacillariophyta (pennates)

120109	*Achnanthes*	*hungarica* Grunow
120110	*Achnanthes*	*lanceolata* (Brébisson) Grunow
120114	*Achnanthes*	*minutissima* Kützing

120124	*Achnanthes*	*coarctata* (Brébisson) Grunow
120150	*Achnanthes*	sp.
120410	*Amphora*	*pediculus* (Kützing) Grunow
120450	*Amphora*	sp.
120601	*Asterionella*	*formosa* Hassall
120803	*Caloneis*	*silicula* (Ehrenberg) Cleve
121001	*Ceratoneis*	*arcus* (Ehrenberg) Kützing
121101	*Cocconeis*	*pediculus* Ehrenberg
121102	*Cocconeis*	*placentula* Ehrenberg
121104	*Cocconeis*	*disculis* (Schumann) Cleve
121150	*Cocconeis*	sp.
121308	*Cymbella*	*cymbiformis* (Kützing) van Heurck
121311	*Cymbella*	*gracilis* (Ehrenberg) Kützing
121312	*Cymbella*	*helvetica* Kützing
121329	*Cymbella*	*hebredica* (Grunow) Pantocsek
121330	*Cymbella*	*minuta* Hilse
121337	*Cymbella*	*hungarica* (Grunow) Pantocsek
121339	*Cymbella*	*subcuspidata* Krammer
121340	*Cymbella*	*mesiana* Cholnoky
121341	*Cymbella*	*musicola* Kützing
121349	*Cymbella*	*not above*
121350	*Cymbella*	sp.
121501	*Diatoma*	*elongatum* (Lyngbye) Agardh
121504	*Diatoma*	*vulgare* Bory
121510	*Diatoma*	*tenuis* Agardh
121511	*Diatoma*	*moniliformis* Kützing
121512	*Diatoma*	*ehrenbergii* Kützing
121550	*Diatoma*	sp.
121701	*Didymosphenia*	*geminata* (Lyngbye) M. Schmidt
122001	*Eunotia*	*arcus* Ehrenberg
122002	*Eunotia*	*exigua* (Brébisson) Grunow
122009	*Eunotia*	*septentrionalis* Oestrup
122010	*Eunotia*	*bilunaris* (Ehrenberg) Mills
122011	*Eunotia*	*tecta* Krasske
122101	*Fragilaria*	*brevistriata* Grunow
122102	*Fragilaria*	*capucina* Desmaziers
122103	*Fragilaria*	*construens* (Ehrenberg) Grunow
122104	*Fragilaria*	*crotonensis* Kilton
122106	*Fragilaria*	*pinnata* Ehrenberg
122107	*Fragilaria*	*vaucheriae* (Kützing) Boye Petersen
122118	*Fragilaria*	*incognita* Reichardt
122150	*Fragilaria*	sp.
122203	*Frustulia*	*vulgaris* (Thwaites) de Toni
122301	*Gomphonema*	*acuminatum* Ehrenberg
122311	*Gomphonema*	*olivaceum* (Lyngbye) Kützing
122312	*Gomphonema*	*parvulum* (Kützing) Grunow
122318	*Gomphonema*	*rhombicum* Fricke
122320	*Gomphonema*	*truncatum* Ehrenberg
122323	*Gomphonema*	*grovei* M. Schmidt

122349	*Gomphonema*	not above
122350	*Gomphonema*	sp.
122401	*Gyrosigma*	*acuminatum* (Kützing) Rabenhorst
122402	*Gyrosigma*	*attenuatum* (Kützing) Rabenhorst
122601	*Meridion*	*circulare* Agardh
122707	*Navicula*	*rhynchocephala* (Kützing)
122712	*Navicula*	*gregaria* Donkin
122714	*Navicula*	*lanceolata* (Argadh) Kützing
122723	*Navicula*	*reinhardtii* Grunow
122732	*Navicula*	*lacunolaciniata* Lange-Bertalot & Bonik
122734	*Navicula*	*pusilla* (W. Smith)
122735	*Navicula*	*capitata* Ehrenberg
122749	*Navicula*	not above
122750	*Navicula*	sp.
122753	*Navicula*	*capitoradiata* Germain
122765	*Navicula*	*cruicula* (W. Smith) Donkin
122906	*Neidium*	*productum* (W. Smith) Cleve
122909	*Neidium*	*ampilatum* (Ehrenberg) Krammer
122950	*Neidium*	sp.
123001	*Nitzschia*	*acicularis* (Kützing) W. Smith
123004	*Nitzschia*	*angustata* (W. Smith) Grunow
123006	*Nitzschia*	*dissipata* (Kützing) Grunow
123013	*Nitzschia*	*palea* (Kützing) W. Smith
123013	*Nitzschia*	*paleacea* Grunow
123018	*Nitzschia*	*capitellata* Hustedt
123018	*Nitzschia*	*gracilis* Hantzsch
123024	*Nitzschia*	*recta* Hantzsch
123042	*Nitzschia*	*filiformis* (W. Smith) van Heurck
123050	*Nitzschia*	sp.
123201	*Opephora*	*martyi* Heribaud
123308	*Pinnularia*	*interrupta* W. Smith
123315	*Pinnularia*	*viridis* (Nitzsch) Ehrenberg
123320	*Pinnularia*	*appendiculata* (Agardh) Cleve
123327	*Pinnularia*	*lata* (Brébisson) W. Smith
123328	*Pinnularia*	*cruciformis* Ehreneberg
123350	*Pinnularia*	sp.
123702	*Stauroneis*	*phoenicenteron* (Nitzsch) Ehrenberg
123703	*Stauroneis*	*smithii* Grunow
123704	*Stauroneis*	*legunum* Ehrenberg
123803	*Surirella*	*ovalis* Brébisson
123805	*Surirella*	*robusta* Ehrenberg
123807	*Surirella*	*minuta* Brébisson
123810	*Surirella*	*brebisonii* Krammer & Lange-Bertalot
123811	*Surirella*	*angusta* Kützing
123813	*Surirella*	*splendida* (Ehrenberg) Kützing
123814	*Surirella*	*elegans* Ehrenberg
123901	*Synedra*	*acus* Kützing
123908	*Synedra*	*ulna* (Nitzsch) Ehrenberg
123909	*Synedra*	*nana* Meister

123913	*Synedra*	*pulchella* (Ralfs) Kützing
123914	*Synedra*	*delicatissima* W. Smith
124001	*Tabellaria*	*fenestrata* (Lyngbye) Kützing
124002	*Tabellaria*	*flocculosa* (Roth) Kützing
124003	*Tabellaria*	*ventricosa* Kützing
124101	*Tetracyclus*	*lacustris* Ralfs
124102	*Tetracyclus*	*glans* (Ehrenberg) Mills
124150	*Tetracyclus*	sp.

Chlorophyta

150202	*Asterococcus*	*superbus* (Cienkowski) Scherffel
150602	*Chlamydomonas*	*globulosa* Ehrenberg
150610	*Chlamydomonas*	subgenus *Agloë*
150630	*Chlamydomonas*	subgenus *Chlamydella*
150650	*Chlamydomonas*	subgenus *Euchlamydomonas*
151350	*Haematococcus*	sp.
151501	*Pandorina*	*morum* (Müller) Bory

Chlorophyta

160650	*Chlorella*	sp.
162905	*Pediastrum*	*tetras* (Ehrenberg) Ralfs
162950	*Pediastrum*	sp.
163304	*Scenedesmus*	*arcuatus* Lennerman
163309	*Scenedesmus*	*quadricauda* (Turpin) Brébisson

Chlorophyta

170602	*Chaetophora*	*incrassata* (Hudson) Hazen
170650	*Chaetophora*	sp.
171150	*Cylindrocapsa*	sp.
171301	*Draparnaldia*	*glomerata* (Vaucher) Agardh
171902	*Klebsormidium*	*fluitans* (Gay) Heering
171950	*Klebsormidium*	sp.
172196	*Microspora*	sp. > 24 µm
173153	*Stichococcus*	sp. cells rounded > 2 µm
173203	*Stigeoclonium*	*lubricum* Kützing
173204	*Stigeoclonium*	*subsecundum* Kützing
173205	*Stigeoclonium*	*tenue* Kützing
173401	*Ulothrix*	*aequalis* Kützing
173409	*Ulothrix*	*variabilis* (Kützing) Kirchner
173410	*Ulothrix*	*zonata* (Webb & Mohr) Kützing
173450	*Ulothrix*	sp.
173490	*Ulothrix*	sp. > 6 µm <= 8 µm
173495	*Ulothrix*	sp. > 14 µm

Chlorophyta

180293	*Oedogonium*	sp. > 8 µm <= 12 µm
180294	*Oedogonium*	sp. > 12 µm <= 16 µm

Chlorophyta conjugatophyta

210326	*Closterium*	*parvulum* Nägeli	
210350	*Closterium*	sp.	
210405	*Cosmarium*	*blyttii* Wille	
210450	*Cosmarium*	sp.	
211533	*Mougeotia*	not above > 12 µm <= 16 µm	
211592	*Mougeotia*	sp. <= 8 µm	
211593	*Mougeotia*	sp. > 8 µm <= 12 µm	
211594	*Mougeotia*	sp. > 12 µm <= 16 µm	
211595	*Mougeotia*	sp. > 16 µm <= 24 µm	
211596	*Mougeotia*	sp. > 24 µm	
212305	*Spirogyra*	*varians* (Hassall) Kützing	
212350	*Spirogyra*	> 12 µm <= 16 µm > 1 chloroplast	replicate
212361	*Spirogyra*	> 12 µm <= 16 µm > 1 chloroplast	simple
212362	*Spirogyra*	> 32 µm <= 48 µm > 1 chloroplast	simple
212650	*Staurastrum*	sp.	
212896	*Zygnema*	sp. > 16 µm <= 24 µm	

5.11 Phytoplankton including picoplankton

38 species were found at the middle of the Reservoir site (05) although not all of these are true plankton.

Table 5.1 Monthly cell density in the plankton

Month	**Organisms ml^{-1}**	**No. of taxa**	**Dominant species**	**Picoplankton density (cells ml^{-1})**
April 1993	36	10	*T. flocculosa*	320
May	87	19	*T. fenestrata*	560
June	60	14	no dominant	700
July	125	16	*T. flocculosa*	6120
August	167	4	*T. flocculosa*	10200
September	161	4	*T. flocculosa*	8160
October	25	3	*T. fenestrata*	2040
November	ice	ice	ice	2366
December	19	3	*T. flocculosa*	1632
January 1994	17	3	*T. fenestrata*	2448
February	27	4	*T. flocculosa*	2046
March	51	7	*T. flocculosa*	2132
April	76	10	*T. flocculosa*	426
May	104	12	*T. fenestrata*	760
June	108	10	*T. fenestrata*	828

The total number of cells is higher in the summer. The plankton is dominated by *Tabellaria*. The number of taxa are higher in the spring and summer. The picoplankton density is correlated with the phytoplankton density and is also higher in the summer.

5.2 Aquatic macrophytes

The aquatic macrophytes and other plants found in water at the shoreline sites of the main Reservoir (01, 01A, 03, 08, 09) were recorded to coincide with the water samples and algal samples taken. 41 species were found in total and these are listed below. Certain difficulties were encountered in recording the aquatic macrophytes and other plants due to the fluctuating water level. The list below contains species that are not aquatic macrophytes, and these are marked with a *. Authorities have been abbreviated.

Lichens		
310201	*Bacidia*	*inundata* (Ach.) Ach
313002	*Verrucaria*	*aquatilis* Mudd
Mosses		
321302	*Calliergon*	*cuspidatum* (Hedw.) Kindb.
Vascular cryptogams		
350202	*Equisetum*	*fluviatile* L.
Dicotyledons		
361201	*Caltha*	*palustris* L.
361303	*Cardamine*	*pratensis* L.
361703	*Cirsium*	*palustre* (L.) Scop.
362701	*Filipendula*	*ulmaria* (L.) Maxim.
362803	*Galium*	*palustre* L.
364601	*Mentha*	*aquatica* L.
366101	*Peplis*	*portula* L.
366402*	*Plantago*	*lanceolata* L.
366501	*Polygonum*	*amphibium* L.
366901	*Ranunculus*	*aquatilis* L.
366904	*Ranunculus*	*flammula* L.
367105	*Rorippa*	*nasturtium-aquaticum* (L.) Hayek
367301*	*Rumex*	*acetosa* L.

367505	*Salix*	*caprea* L.
367506	*Salix*	*cinerea* L.
369802	*Veronica*	*beccabunga* L.

Monocotyledons

380203	*Agrostis*	*stolonifera* L.
381801	*Deschampsia*	*caespitosa* (L.) Beauv.
382004	*Eleocharis*	*palustris* (L.) Roem. & Schult.
382502	*Glyceria*	*fluitans* (L.) R. BR.
382901	*Iris*	*pseudacorus* L.
383010	*Juncus*	*effusus* L.
383501*	*Molinia*	*caerulea* (L.) Moench
383701	*Phalaris*	*arundinacea* L.
383801	*Phragmites*	*australis* (Cav.) Trin. ex Steud.
384003	*Potamogeton*	*berchtoldii* Fieb.
384012	*Potamogeton*	*natans* L.
384013	*Potamogeton*	*obtusifolius* Mert. & Koch

Non-coded species

*	*Nardus*	*stricta* L.
*	*Leontodon*	*autumnalis* L.
*	*Trifolium*	*repens* L.
*	*Ranunculus*	*bulbosus* L.
*	*Galium*	*odoratum* (L.) Scop.
*	*Bellis*	*perennis* L.
*	*Festuca*	*rubra* L.
*	*Rumex*	*crispus* L.
*	*Rumex*	*crenulata* L.

5.3 Statistical analysis of algal data

5.31 Introduction

The quantitative biological data (phytoplankton and picoplankton densities) have been analysed to show correlations with environmental variables (Section 4.4) but the semi-quantitative data cannot be treated in the same way. A program written by S. Juggins transfers the algal data from the Paradox data base into TWINSPAN. This is explained below.

5.32 Twinspan analysis

Twinspan (two-way Indicator Species Analysis) is a program designed to perform a divisive cluster analysis on multivariate data, in this case 87 samples taken between 21/04/93 to 14/06/94 from 7 sites around the Reservoir (01, 01A., 03, 05, 08, 09 and AS) which are characterised by the species found. The program uses the concept of the 'pseudospecies' where every record for a species is assigned a pseudospecies code depending on the abundance of the species. *i.e. Tabellaria flocculosa* (code number 124002) found in abundance 5 will be assigned the pseudospecies with code number 1240025. The number of pseudospecies and their quantitative range can be set at the start of the program.

5.33 Results from TWINSPAN

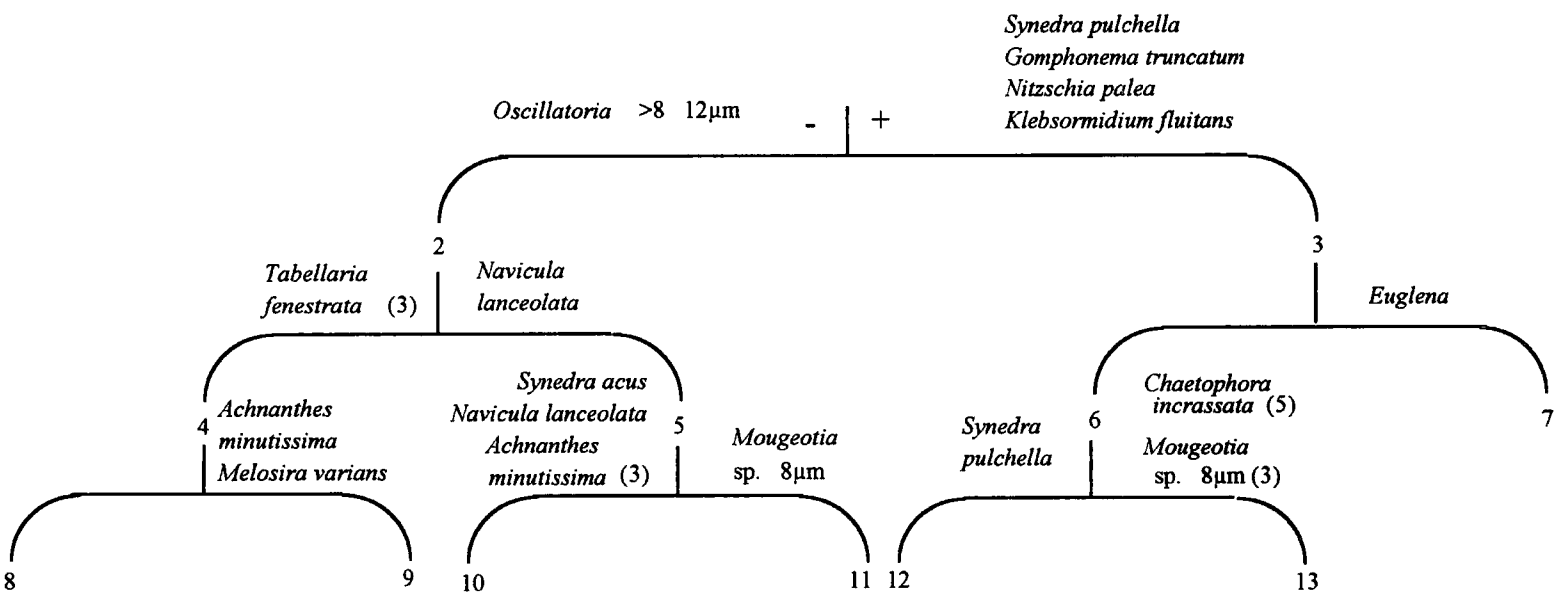
A dendrogram showing how the algal data set has been classified using TWINSPAN is shown in Figure 5.1.

At the third level of the dendrogram 7 end groups are shown (numbered 7, 8, 9, 10, 11, 12, and 13). If we look at the sample numbers that these end groups contain, it becomes clear that end groups 8 and 9 contain only samples taken from site 05 i.e., the phytoplankton. The other end groups seem to have been determined on a seasonal rather than a site basis.

One point to note is that algal samples have been collected monthly from 21/04/93 to 14/06/94 and so the Months April, May and June have been sampled for both 1993 and 1994. Significant differences in the water chemistry of these two periods have been highlighted in Chapter 4. It appears that there are also significant differences between the algal samples too, as they have been placed in different TWINSPAN end groups; end group 10 for April, May and June 1993 and end group 12 for April, May and June 1994.

At each division of the data the TWINSPAN program gives indicator species. One indicator species for group 10 (see above) is *Synedra acus* whereas one indicator for group 11 is *S. pulchella*. The raw data was checked to see if this was a 'real' difference and after this was established the original diatom slides were also checked in case it was an identification error; however, the differences seemed to be 'real'. *Chaetophora incrassata* is an indicator species for group 13 when it is high abundance. Group 13 contains algal samples from Site 03 in the Autumn period. As *C. incrassata* is a 'macro-algae' the high abundance at Site 03 in the Autumn had been noticed and has been used as an experimental organism to identify possible P limitation (see Chapter 6).

Fig 5.1 Dendrogram to show classification of algal data using TWINSPAN



5.4 *Potamogeton* study

A field survey was conducted on 25/08/93 to investigate the extent of the cover of *Potamogeton obtusifolius* and *P. berchtoldii*. The 200 m transect from Site 03 across the Reservoir was swum by SCUBA divers. The Reservoir was found to be shallow, not exceeding a depth of 3 m apart from where the transect crossed the original path of the river North Tyne where the depth was approximately 8 m. No macrophytes were seen at all after 10 m horizontal distance along the transect. There were large areas of the Reservoir that were shallow but had no macrophytic plant life.

Initial investigations of the phosphatase activity and P content of the above species began in 1993. The phosphatase assays were expanded on 1994 including whole plant experiments on *P. obtusifolius* to investigate the contribution to the plant from leaves and roots on a per plant basis. The phosphatase activity of a third species *P. natans* was also investigated. The results of these experiments are found in Chapter 6.

6 PHOSPHATASE ACTIVITIES

6.1 Phosphatase activity assays

A wide range of N:P ratios were found in water samples taken from Bakethin Reservoir (Chapter 4) and to investigate whether certain organisms are P limited phosphatase assays were carried out. The use of physiological indicators to determine nutrient limitation was raised in Chapter 1 (Section 1.8) and the use of phosphatase assays is one such approach.

Experimental methods for the determination of phosphatase activity are outlined in Chapter 2 (Section 2.6) including detailed information on how the material was prepared for the assay. The material was collected fresh from Bakethin Reservoir, and unless otherwise stated the assays were completed within 24 h.

The choice of assay organisms was influenced by abundance, ease of identification in the field, ease of collection, and whether the organisms were considered likely to play an important role in nutrient cycling, at least locally at the Reservoir. The experimental species are *Ulothrix zonata*, *Stigeoclonium tenue*, *Draparnaldia glomerata*, *Chaetophora incrassata*, *Nitella flexilis*, *Potamogeton berchtoldii*, *P. obtusifolius*, and *P. natans*. More information can be found in Section 2.7.

The results obtained from investigating phosphatase activity using different substrates for the same species can be compared directly as they have used sub-samples of the same freshly collected material. The *Potamogeton* species were collected at the same time but not necessarily from the same site.

The assay conditions were standardised rather than trying to simulate field conditions. The temperature was 25^{0}C. The relationship between length of assay and rate of reaction has been found to be linear from 0 to 60 min (Bresnan, 1993; Milligan,

1994). A standard time of 30 min was chosen for all assays. Two substrates were used: p-NPP (71μM) and 4-MUP (1 μM and 100 μM).

The following results refer to the initial experiments to investigate the phosphatase activities of *Chaetophora incrassata*, and the phosphatase activities of the roots and leaves of *Potamogeton obtusifolius* and *P. berchtoldii*. The effect of pH on enzyme activity was investigated (see Section 1.8). Buffers of the following pH values were used: 3, 4, 5, 5.5, 6, 7, 8, 9, 10, 10.5. Tables 6.1, 6.2 and 6.3 below show the results of the initial investigations using the following substrates: 71μM pNPP, 100 μM 4-MUP and 1 μM 4-MUP. The maximum activity is shown and the pH that it occurs at is indicated.

Table 6.1 Maximum enzyme activity (μmol μg chl a^{-1} h^{-1}) or (μmol g d. wt^{-1} h^{-1}) using 71μM pNPP. Algae are abbreviated to generic names. Organisms are shown alphabetically. Rates calculated using chlorophyll a have been converted to dry weight.

Organism	**part**	**max. activity (μmol μg chl a^{-1} h^{-1})**	**max. activity (μmol g d. wt^{-1} h^{-1})**	**pH**
Chaetophora	whole	0.072	720.0	10
P. berchtoldii	roots		9.0	10
P. berchtoldii	leaves		11.0	4
P. obtusifolius	roots		3.0	5
P. obtusifolius	leaves		6.0	5

Table 6.2 Maximum enzyme activity using 100 μM 4-MUP, also see Table 6.1 above.

Organism	**part**	**max. activity (μmol μg chl a^{-1} h^{-1})**	**max. activity (μmol g d. wt^{-1} h^{-1})**	**pH**
Chaetophora	whole	0.0106	106.0	10
P. berchtoldii	roots		6.4	10
P. berchtoldii	leaves		0.5	4
P. obtusifolius	roots		4.8	5.5
P. obtusifolius	leaves		2.5	5

Table 6.3 Maximum enzyme activity using 1 μM 4-MUP, also see Table 6.1 above

Organism	part	max. activity (μmol μg chl a^{-1} h^{-1})	max. activity μmol g d. wt^{-1} h^{-1}	pH
Chaetophora	whole	0.00037	3.70	10
P. berchtoldii	roots		0.05	5
P. berchtoldii	leaves		0.05	3
P. obtusifolius	roots		0.44	10
P. obtusifolius	leaves		0.05	8

The following trends have been observed:

For every case, apart from *P. obtuifolius* roots, the maximum enzyme activity is higher when using substrate pNPP (71μM) than substrate 4-MUP (100 μM). In every case, apart from *P. obtusifolius* roots, the pH at which the maximum enzyme rate occurs is the same for substrates pNPP (71μM) and 4-MUP (100 μM).

By comparing the results in Tables 6.2 and 6.3 it is clear that when the substrate concentration is exactly 100 times greater, the enzyme rates are higher i.e., *Chaetophora* (28.6), *P. berchtoldii* roots (128), *P. berchtoldii* leaves (10), *P. obtusifolius* roots (10.9), *P. obtusifolius* leaves (50) but never exactly 100 times greater, and in 4 cases out of 5 the enzyme rates are less than 100 times greater.

The maximum enzyme activity of *C.incrassata* is always at pH 10, indicating alkaline phosphatase. Both acid and alkaline phosphatases have been found in the roots of the two *Potamogeton* species, whereas the leaves generally showed acid phosphatase activity.

6.2 Phosphatase activities found in "old" *Chaetophora incrassata*

"Old" C*haetophora* incrassata (Section 2.61) has also been analysed to determine enzyme activity. The phosphatase activity of C. *incrassata* kept under various

different conditions was analysed using substrate pNPP only. The rate of activity of fresh material has been added to Table 6.4 so that the rates of activity can be easily compared.

Table 6.4 Phosphatase activity of *Chaetophora incrassata* using pNPP

Lab regime	substrate concentration	max. activity (µmol µg chl a^{-1} h^{-1})	activity (µmol g d. wt^{-1} h^{-1})	pH
Fresh (as Table 6.1)	71 µM	0.072	720	10
Fresh	177 µM	0.210	2100	10
"low P" for 2 weeks	71µM	0.310	3100	10
"high P" for 2 weeks	71µM	0.039	390	10
"low P" for 6 weeks	71µM	1.580	15800	10

The "low P" medium contained 5 µg l^{-1} P. The high P medium contained 1 mg l^{-1}. Maximum phosphatase activity was consistently found to be at pH 10. The maximum activity was slightly more than 2.5 times higher using substrate 177 µM pNPP than 71µM pNPP. A decrease in activity was seen for the material kept in a high P medium for 2 weeks. An increase in activity was seen for the material kept in a low P medium for 2 weeks. The material kept for 6 weeks in a low P medium had very high phosphatase activity. Prior to any experimental work the material was examined under both light and fluorescence microscopy. Large amounts of bacteria were seen on the 6 week old material, but not on any of the other material and this may explain the high activity.

6.3 Phosphatase activity of material collected in 1994

The experimental programme was expanded in 1994 with further species assayed for the presence of phosphatase activity (Section 6.1). The results of the investigations are summarised in Tables 6.5 to 6.7, the maximum rate of enzyme activity is shown

and the pH at which the maximum occurs. The full results showing the mean and standard error for each pH value are depicted graphically in Figures 6.1 to 6.5.

Table 6.5 Maximum enzyme activity found in 1994 studies using 71μM pNPP. See Table 6.1.

Organism	part	max. activity (μmol μg chl a^{-1} h^{-1})	max. activity (μmol g $d.wt^{-1}$ h^{-1})	pH
Chaetophora	whole	0.011	110.0	9
Draparnaldia	whole	0.009	100.0	10
Stigeoclonium	whole	0.047	500.0	10
Ulothrix	whole	0.085	90.0	4

Table 6.6 Maximum enzyme activity found in 1994 studies using 100 μM 4-MUP. Results for *Nitella* are shown at the end of the algae and *Potamogeton natans* at the end of the higher plants. See Table 6.1.

Organism	part	max. activity (μmol μg chl a^{-1} h^{-1})	max. activity (μmol g $d.wt^{-1}$ h^{-1})	pH
Chaetophora	whole	0.010	100.0	4
Draparnaldia	whole	0.011	120.0	10
Stigeoclonium	whole	0.046	480.0	10
Ulothrix	whole	0.003	30.0	9
Nitella	tips		66.6	4
P. berchtoldii	roots		24.0	4
P. berchtoldii	leaves		22.0	3
P. obtusifolius	roots		26.9	5.5
P. obtusifolius	leaves		19.6	5
P. natans	roots		37.2	5.5

Table 6.7 Maximum enzyme activity found in 1994 studies using 1 μM 4-MUP. The order is as for Table 6.6. See Table 6.1

Organism	part	max. activity (μmol μg chl a^{-1} h^{-1})	max. activity (μmol g d.wt^{-1} h^{-1})	pH
Draparnaldia	whole	0.0009	10.0	10
Stigeoclonium	whole	0.0023	24.0	10
Ulothrix	whole	0.0004	5.0	9
Nitella	tips		0.52	9
P. berchtoldii	roots		0.71	7
P. berchtoldii	leaves		0.36	10
P. obtusifolius	roots		1.12	5.5
P. obtusifolius	leaves		0.17	5
P. natans	roots		2.22	5.5

The results summarised in the above tables are also shown in full graphically. Figure 6.1 compares the enzyme activities of the roots of *Potamogeton berchtoldii*, *P. obtusifolius* and *P. natans* when assayed using 100 μM 4-MUP as a substrate. Figure 6.2 compares the enzyme activities of the leaves of *Potamogeton berchtoldii* and *P. obtusifolius* when assayed using 100 μM 4-MUP as a substrate. Figure 6.3 compares the enzyme activities of the roots of *Potamogeton berchtoldii*, *P. obtusifolius* and *P. natans* when assayed using 1 μM 4-MUP as a substrate. Figure 6.4 compares the enzyme activities of the leaves of *Potamogeton berchtoldii* and *P. obtusifolius* when assayed using 1 μM 4-MUP as a substrate. Figure 6.5 compares the enzyme activities of *Stigeoclonium tenue*, *Draparnaldia glomerata* and *Ulothrix zonata* for the following substrates: 71μM pNPP, 100 μM 4-MUP and 1 μM 4-MUP.

From the Tables above and Figure 6.5 it is clear that the algal species are always thus ordered in terms of enzyme activity rates, regardless of the substrate type or concentration used, as:

Stigeoclonium > *Draparnaldia* > *Ulothrix*

The *Potamogeton* species are generally thus ordered in terms of enzyme activity rates, regardless of the substrate type or concentration used.

Potamogeton natans > *P. obtusifolius* > *P. berchtoldii*

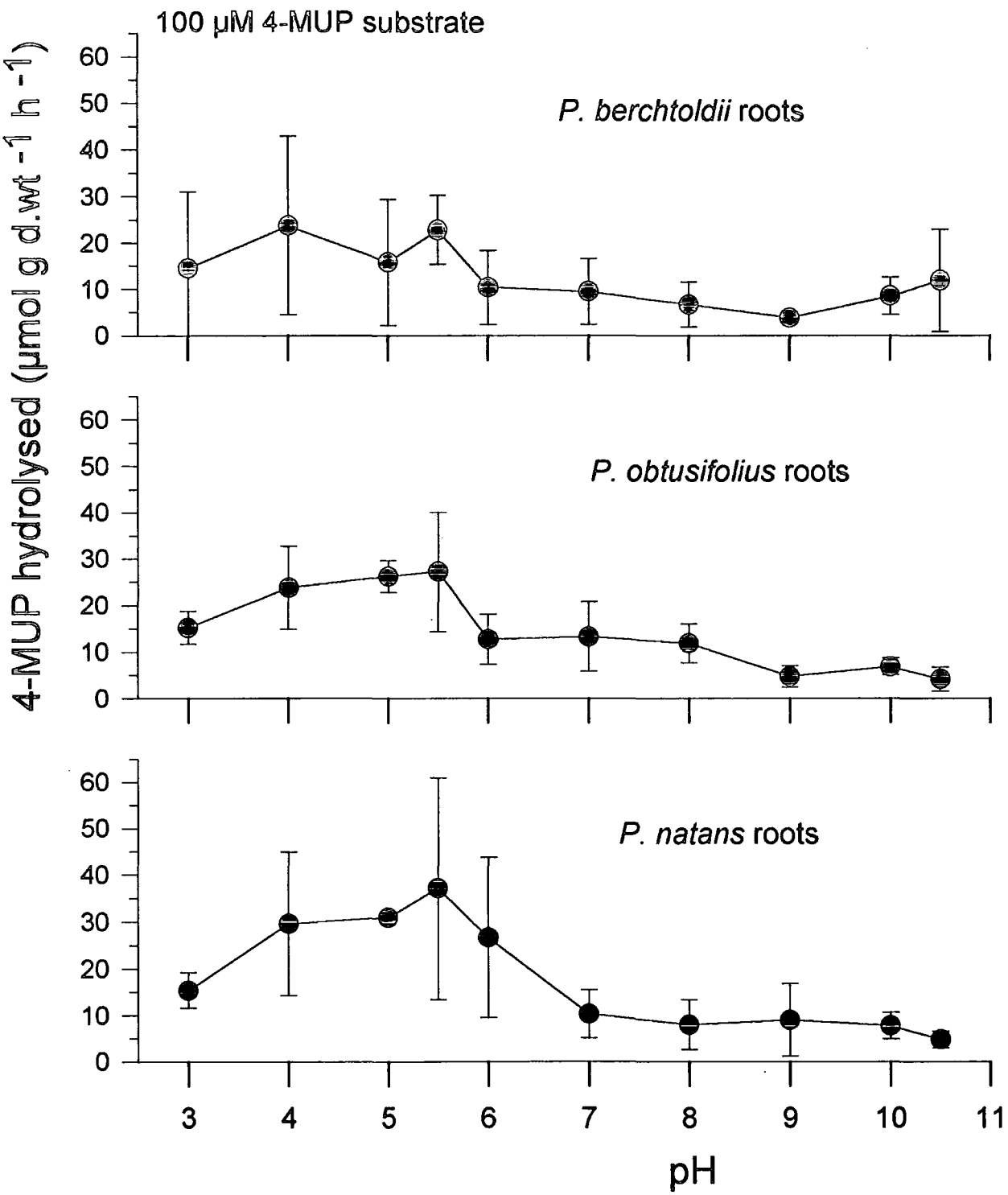
A comparison of the water chemistry (Chapter 4) and algal species (Chapter 5) between 1993 and 1994 has been made. It is more difficult to compare phosphatase activities of species between 1993 and 1994. The phosphatase activities are much lower for the *Chaetophora* collected in 1994, compared to the *Chaetophora* collected in 1993, but explanations for this could lie in the time of year and the collection site. In 1993 *C. incrassata* was collected from the Northern shore of the Reservoir in autumn where it was found in great abundance. The material collected in 1994 (May) was from a small stream entering the Reservoir, so it is from a different environment to that collected in 1993, and a different time of year. The phosphatase activities of the *Potamogeton* species; however, are much higher in 1994. Table 6.8 compares the results from 1993 and 1994.

Table 6.8 Phosphatase activities in *Potamogeton* in 1993 and 1994. The maximum activity rates are given for μmol substrate hydrolysed (g d.wt^{-1} h^{-1}).

Organism	**part**	**substrate**	**max. activity 1993**	**max. activity 1994**	**Ratio 94:93**
P. berchtoldii	roots	100 μM 4-MUP	6.4	24	3.8
P. berchtoldii	leaves		0.5	22	44
P. obtusifolius	roots		4.8	26.9	5.6
P. obtusifolius	leaves		2.5	19.6	7.84
P. berchtoldii	roots	1 μM 4-MUP	0.05	0.71	14.2
P. berchtoldii	leaves		0.05	0.36	7.2
P. obtusifolius	roots		0.44	1.12	2.5
P. obtusifolius	leaves		0.17	0.17	3.4

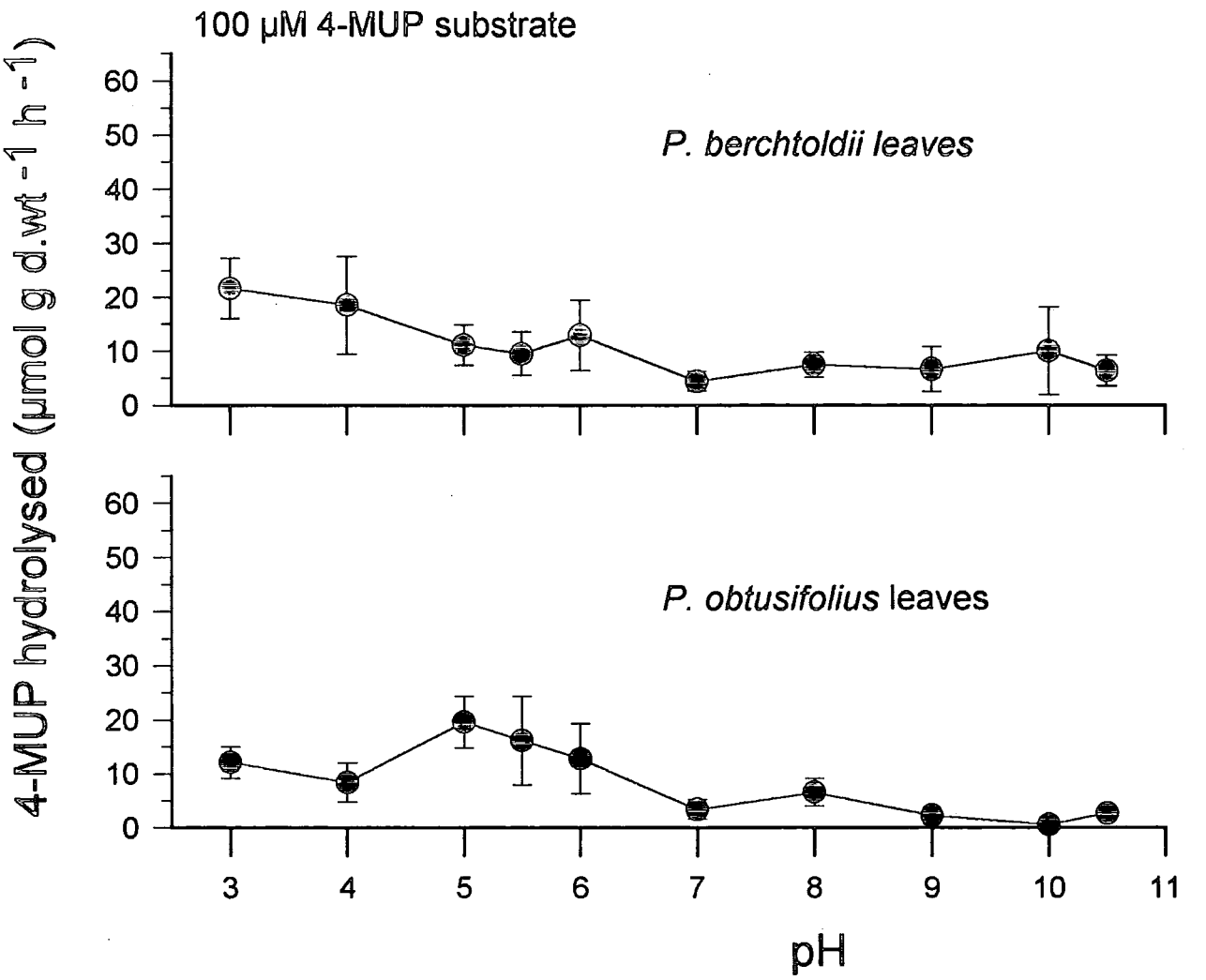
The results of phosphatase assays are shown in full across the pH spectrum in Figures 6.1 to 6.5.

FIG. 6.1

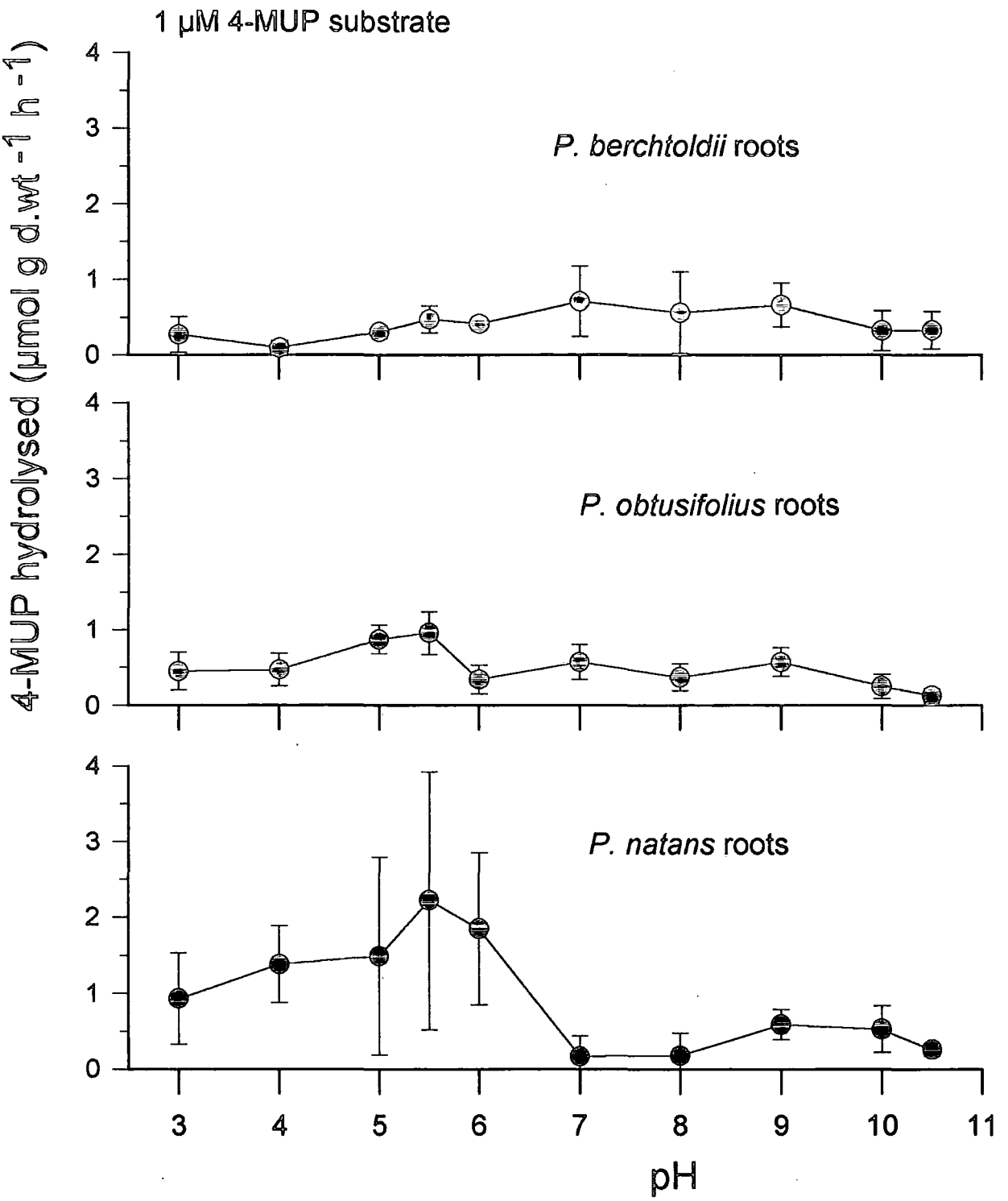


Phosphatase activity of *Potamogeton* roots (4-MUP hydrolysed µmol g^{-1} h^{-1})

FIG. 6.2

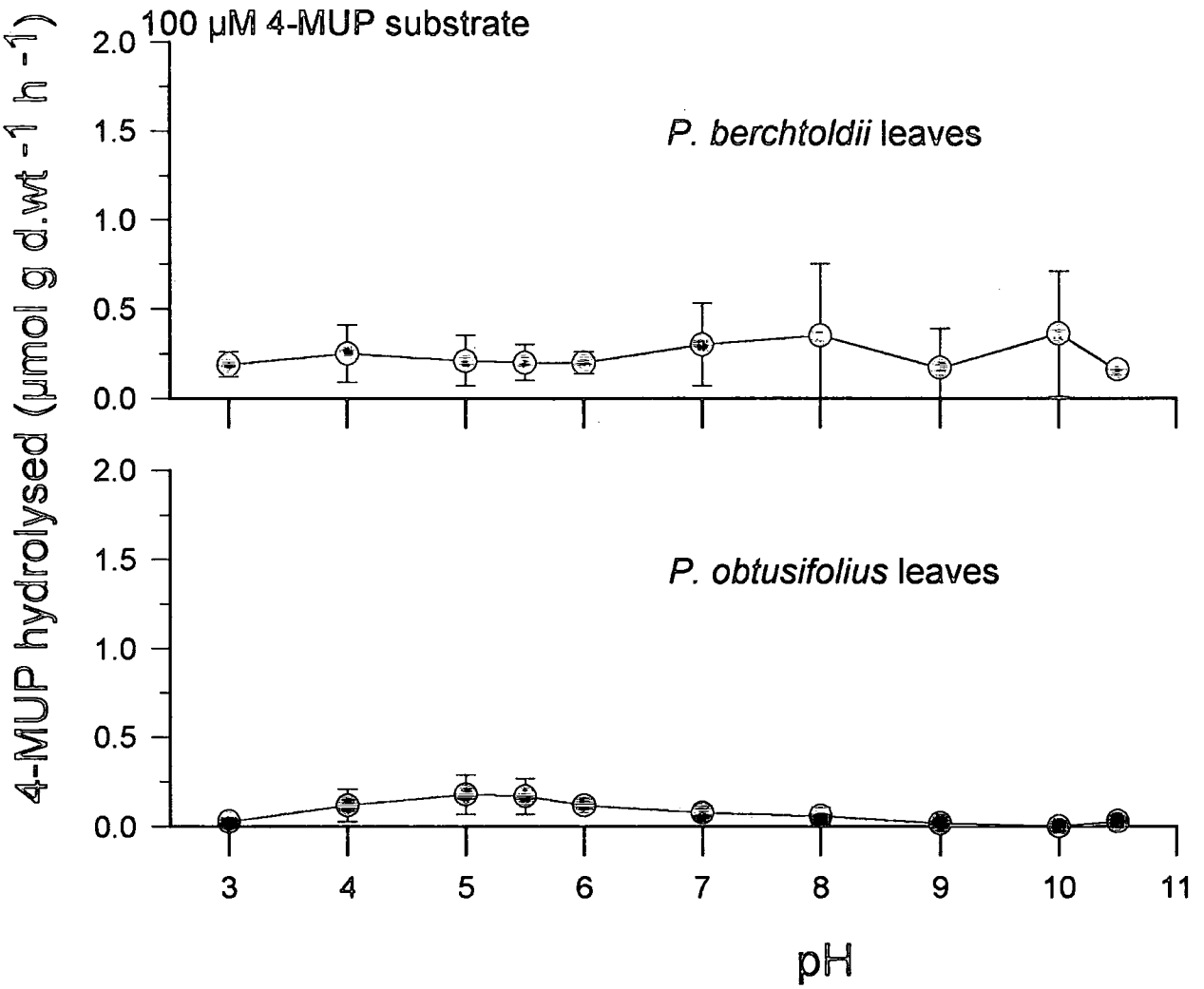


Phosphatase activity of *Potamogeton* leaves (4-MUP hydrolysed µmol g^{-1} h^{-1})



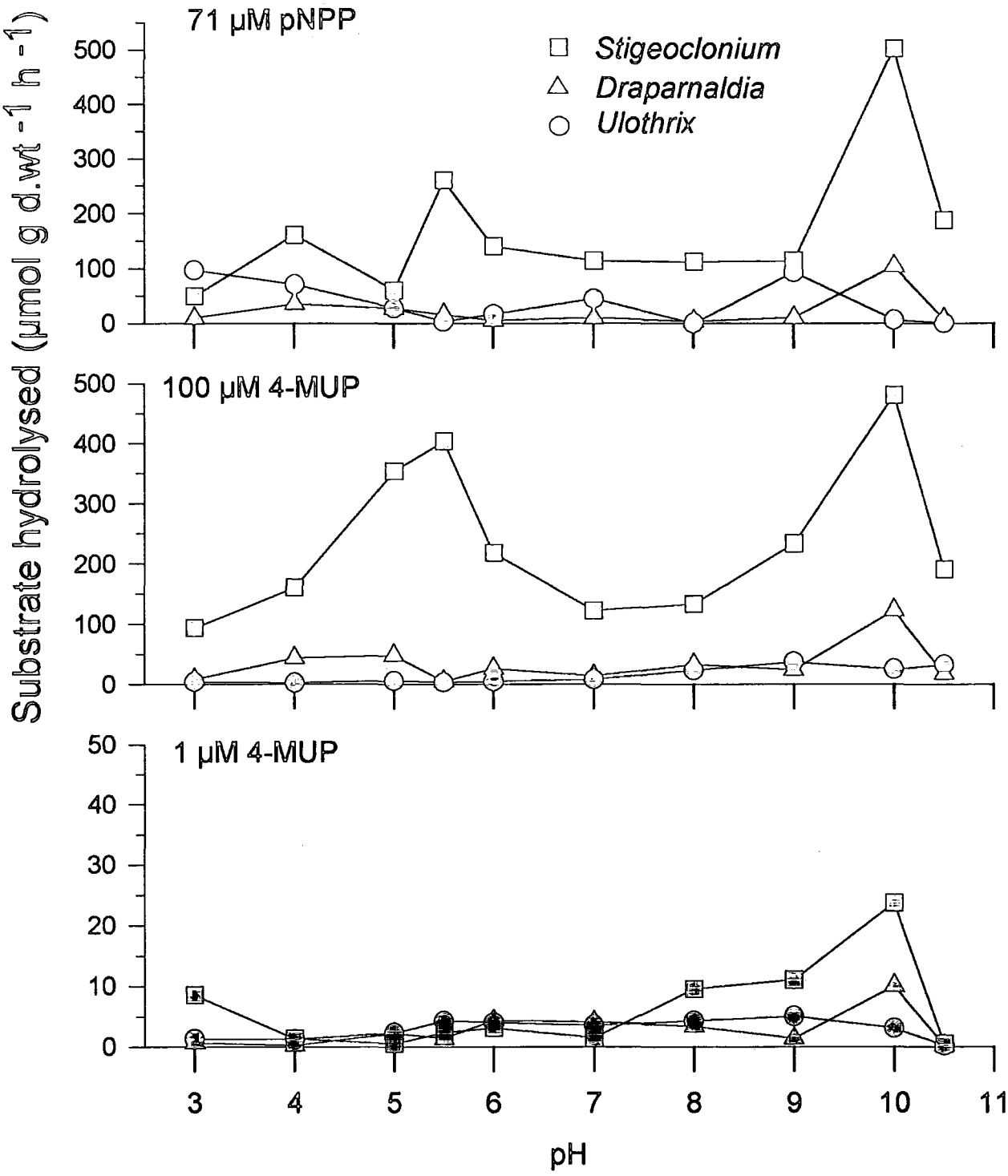
Phosphatase activity of *Potamogeton* roots (4-MUP hydrolysed µmol g^{-1} h^{-1})

FIG. 6.4



Phosphatase activity of *Potamogeton* leaves (4-MUP hydrolysed µmol g^{-1} h^{-1})

FIG. 6.5



Comparison of the phosphatase activity of three species of algae

6.4 Whole plant experiments

6.41 *Nitella flexilis*

Whole plant assays were originally carried out on *Nitella flexilis* (Section 2.65). *N. flexilis* is a delicate plant with large cells (> 1 cm in length).

Table 6.9 below compares the maximum rates of enzyme activity, using 1 μm 4-MUP substrate, of plant tips, whole above ground plant, and rhizoids measured per g of dry weight.

Table 6.9 Phosphatase activity of *Nitella flexilis* using 1 μM 4-MUP

part of *Nitella*	**max. activity (μmol g d.wt^{-1}h^{-1})**	**pH**
tips	0.52	9
whole above ground	0.19	8
rhizoids	0.04	5.5

The experimental method was modified and the assay scaled up to accommodate whole plants. Enzyme activities of the plant tips are greater than the above ground plant as a whole. The rhizoids have a lower activity than the above ground parts.

6.42 *Potamogeton obtusifolius*

To investigate whether the roots or the leaves contributed more to the phosphatase activity of the plant experiments were conducted in which all the roots and all the leaves were assayed, separately, from small plants using 1μM 4-MUP.

Table 6.10 below compares the enzyme activity, using 1 μM 4-MUP as a substrate, of leaves and roots from the same plants. As the total weight of roots and leaves were known for the plant analysed, the total phosphatase activity of the plant can be calculated.

Table 6.10 Phosphatase activity of leaves and roots (μmol g $d.wt^{-1}h^{-1}$) from the same *Potamogeton obtusifolius* plants. Substrate concentration 1 μM 4-MUP.

Plant no.	leaf activity (μmol g $d.wt^{-1}$ h^{-1})	contribution to plant from leaves (μmol $plant^{-1}$)	root activity (μmol g $d.wt^{-1}$ h^{-1})	contribution to plant from roots (μmol $plant^{-1}$)
1	0.12	0.0066	0.12	0.0019
2	0.12	0.0050	0.13	0.0016
3	0.16	0.0054	0.18	0.0008
4	0.11	0.0056	0.12	0.0022
mean	0.13	0.0056	0.14	0.0016

The activity for both roots and leaves is similar per unit dry weight, a mean of 0.13 μmol substrate hydrolysed (μmol g $d.wt^{-1}h^{-1}$) for leaves compared to a mean of 0.14 for roots. However; when the enzyme rates are estimated on a "per-plant" basis, by multiplying the activity by the total weight of either the leaves or the roots, the leaves are found to have a greater activity on account of their larger average weight than the roots.

6.6 Staining

No clear staining was achieved with the BCIP stain which indicates that there was not any hydrolysed phosphorus. Similar results were found by Milligan (1994) and Luff (1993).

7 Discussion

7.1 Nutrient inputs

The first aim of the project was to determine the significant inputs of nutrients to the Reservoir. The main inputs have been highlighted in Chapter 3, and it is likely that they are the River North Tyne receiving Butteryhaugh sewage effluent and Kielder Burn receiving the effluent from the Kielder Burn salmon hatchery. The mean values for NO_3-N, NO_2-N, NH_4-N, TFP and FRP are all higher in the main Reservoir than at USTW; however, no clear trend of decreasing nutrient concentrations away from the river has been found. There must therefore be other factors involved. Other possible sources and sinks for nutrients in the Reservoir are: other inflows, rainfall, leaf litter, the sediments, biological cycling and other internal processes.

No significant positive/negative correlations were found between monthly rainfall and nutrients measured. Rainfall can affect the level of nutrients in a water system in two ways (Section 1.23); by direct input into the water system itself and indirectly by leaching of nutrients from the catchment area. However, the lack of correlation does not rule out the possibility of rain affecting the nutrient concentration at Bakethin Reservoir. This is due to the way in which both the rainfall and the nutrients were measured. Firstly rainfall was measured daily (Northumbrian Water) at Kielder Operations Centre, which is at an altitude of approximately 200 m; heavy rainfall at higher altitudes in the catchment would not be shown by measurements at Kielder. Altitudes up to 571 m (Deadwater Fell) are found in the catchment area and it is likely that these areas have more rain. Nutrient concentrations were measured monthly, a time scale which is not sufficient to pick up all fluctuations in levels. Ideally nutrients would be measured on a smaller time scale and especially after heavy rain.

From the limited data presented on sediment nutrient concentrations (Section 4.2) it is not possible to determine if the sediments are likely to act as a source or sink for nutrients. To answer this the desorption and absorption characteristics of the sediments would have to be resolved.

7.2 Chemical and biological seasonal changes

The second aim was to investigate the seasonal changes in physical and chemical variables, especially nutrients and photosynthetic organisms at sites around the Reservoir. Sixteen months of data have been collected and the physical and chemical data are displayed graphically in Figures 4.1 to 4.8. All the parameters varied during the season and there were also differences between the sites studied. To give an indication of the spatial and temporal distribution of nutrients in the Reservoir water, the average value for each of: TFP, FRP, NO_3-N, NO_2-N, and NH_4-N were calculated firstly for each site (over the 16 months) and then for each month (using data from the 6 main Reservoir sites). Average values of TFP for the 6 sites ranged from 2.1 $\mu g\ l^{-1}$ (18/10/93) to 31.4 $\mu g\ l^{-1}$ P (14/06/94). TFP concentrations were also high in June 1993 (an average of 22.2 $\mu g\ l^{-1}$ P) Average values for each site were similar and ranged from 10.0 $\mu g\ l^{-1}$ (Site 08) to 13.1 $\mu g\ l^{-1}$ P (Site 05). Results were similar for FRP with the highest values occurring in June 1993 (10.4 $\mu g\ l^{-1}$ P) and June 1994 (20.8 $\mu g\ l^{-1}$ P) and the lowest occurring in September and October 1993 (< 1.0 $\mu g\ l^{-1}$ P). Variation between the sites was greater and ranged from 3.6 $\mu g\ l^{-1}$ for site 08 to 7.9 $\mu g\ l^{-1}$ for site 05. Values of NO_3-N averaged over the 6 sites ranged from 16.3 $\mu g\ l^{-1}$ (19/07/94) to 72.2 $\mu g\ l^{-1}$ (25/03/93) and a general pattern of higher concentrations in the winter emerges in contrast to the seasonal concentrations of TFP and FRP. Average values for each site were again similar ranging from 35.9 $\mu g\ l^{-1}$ (Site 09) to

46.9 μg l^{-1} (Site 05). Values for NO_2-N averaged over the 6 sites ranged from 0.6 μg l^{-1} (18/04/94) to 7.1 μg l^{-1} (03/06/93). Average values for each site ranged from 2.9 μg l^{-1} (Site 01A) to 3.9 μg l^{-1} (Site 03). Values of NH_4-N averaged over the 6 sites range from 16.5 μg l^{-1} (25/03/93) to 90.5 μg l^{-1} (14/06/94), but there is no clear seasonal pattern. Differences between the average values for each site were again small and ranged from 54.9 μg l^{-1} (Site 03) to 59.9 μg l^{-1} (Site 01). From the data collected; it seems that the temporal differences in nutrient concentration are greater than the spatial differences.

The N:P ratio of all the water samples collected have been calculated (Section 4.2). The ratios found indicate that P is usually the limiting nutrient rather than N. In the summer when the P levels are high (see above) N can sometimes be at a level where we infer that it might be limiting to biological growth. The sediments are spatially variable in terms of grain size, organic matter, N and P, (not investigated on a temporal scale). N:P ratios of the sediments are extremely low (Section 4.3) and indicate that N is limiting rather than P. Evidence from storage products of blue-green algae (Section 1.83) ties in with the low N found in the sediments. Polyphosphate granules were seen in *Oscillatoria* found gliding along the bottom sediments at Site 01 and this indicates an abundance of P with respect to N at this microhabitat. Also samples of *Stigeoclonium* found nearby, had long hairs indicating P limitation.

As the project ran for over a year data have been collected for the months April, May, and June in both 1993 and 1994. Significant ($P<0.01$) differences with higher values in 1994 were found for conductivity (1 site) total alkalinity (6 sites), K (2 sites), Mg (1 site) and NH_4-N (1 site). Significant decreases ($P<0.01$) with lower values found in 1994 were found for Fe (2 sites) and NO_2-N (6 sites). The differences could

possibly be connected to the flooding in May/June 1993 which was not repeated in 1994.

The phytoplankton density ranges from 17 (17/01/94) to 167 cells ml^{-1} (23/08/93), the maximum values occur in the summer (Section 5.11). Phytoplankton density is positively correlated with picoplankton density and temperature and negatively correlated with rain and NO_3-N (Section 4.33). No positive correlation was found between phytoplankton density and nutrient concentration. This may suggest that something other than nutrient levels was limiting biological growth. The phytoplankton density of Cow Green Reservoir measured from 1971 to 1975 (Atkinson 1988) is similar to that found at Bakethin and is also dominated by diatoms. Cow Green Reservoir water has certain similar properties to Bakethin, *i.e.*, during 1979 total P ranged from 7 to 30 $\mu g\ l^{-1}$ and turbidity (Secchi disc) varied from 1.15 to 1.5m. Atkinson concludes that at Cow Green Reservoir, low light levels may delay the onset of phytoplankton growth in the spring, and that throughout the year restricted light penetration in turbid water will leave many diatom cells below the euphotic zone and restrict further growth. McLellan (1971), reporting on Derwent Reservoir concluded that because of high colour, light only penetrated the water to a comparatively shallow depth and considered that high colour was probably the most important factor restricting biological growth.

The picoplankton densities in Bakethin Reservoir range from 320 (21/04/93) to 1.02 x 10^4 (23/08/93) with the maximum values occurring in the summer. Hawley (1990) determined the picoplankton density in Kielder Reservoir on two occasions (25/07/89 and 25/08/89); the maximum values found were 7.3 x 10^3 cells ml^{-1} (25/08/89), slightly lower than the maximum found at Bakethin Reservoir. Hawley also

determined the picoplankton density of a number of freshwater sites in the UK and concluded that in Kielder Reservoir the autotrophic picoplankton densities were generally low compared with densities collected in other UK lakes and from samples collected abroad. The role that picoplankton play in nutrient cycles and the effect of nutrient flux on picoplankton has yet to be elucidated. Because of their small size, positive buoyancy, photosynthetic complement and potential for rapid growth they are well adapted to cope with low nutrient concentrations (Goldman & Caron, 1985; Berman *et al.*, 1987). Experiments suggest that P-limitation can become quite severe for larger phytoplankton size classes, while cells < 2 μm may not experience P-limitation (Wehr, 1989).

Another point for discussion is that silicate, an essential nutrient for diatoms, could be limiting the growth of diatoms. Other classes of algae including those in the picoplankton size range do not require silicate for growth and are not be restricted by silicate concentration. There may have been occasions when diatom growth was limited in Bakethin Reservoir whereas other classes of algae were not. However, as silicate levels were not measured in this study this possibility cannot be elucidated further.

The high correlation found between phytoplankton and picoplankton density values could suggest that they are both being limited by the same factor or that the presence of one somehow encourages the presence of the other.

The algal data collected (87 samples in total) have been analysed using TWINSPAN. A dendrogram showing classification of the algal samples into groups has been produced (Figure 5 Chapter 5). Indicator species (generated by the TWINSPAN program) are also given. The program clearly distinguishes algal samples from Site

05, the phytoplankton from the samples collected from the other sites. The phytoplankton are classified into two end groups. Sampling date seems to be more important that sampling site in the classification of the rest of the algal samples. *Chaetophora incrassata* has been identified as an indicator species. The phosphatase activity of this species has been investigated (Chapter 6) and is discussed below (Section 7.3). The TWINSPAN program has shown differences between samples collected in the same months in 1993 and 1994. Similarly, significant differences have been found in the water chemistry of the two periods. It is likely that the differences in water chemistry and algal flora are linked.

7.3 P limitation of key organisms

7.31 Introduction

The third aim of the project was to investigate whether certain organisms are P-limited at the sites.

A certain amount of information on the general availability of nutrients can be gained from monitoring the species present in the Reservoir; all photosynthetic organisms require nutrients for growth and their presence and growth suggests that nutrients were available. In May 1994 *Nitella flexilis* was first found on the shore of the Reservoir past site 01A, over a period of approximately four weeks an extensive charophyte bed rapidly grew up but died away by September 1994. The rapid growth and decline of the underwater bed was not surprising as charophytes are known to be fast growing (Wood & Imahori, 1965), liable to form underwater meadows (Round, 1981). Ecological events such as these can give an insight into the nutrient distribution and availability in the Reservoir, indicating that nutrients were available, taken up by *Nitella flexilis* and then released back into the Reservoir (Section 1.6).

Although the disappearance of the plants was rapid it is uncertain at what time the nutrients would have been released back into the Reservoir and become available to other organisms as slow rates of release by decomposition have been reported for charophytes (Moore, 1986).

This ecological event will have also had important effects on other organisms as *Nitella flexilis* will have been a substantial, although temporary, habitat and food source for opportunistic organisms, and a potential competitor with aquatic macrophytes and certain algae.

However, monitoring changes in the flora does not give an indication of specific nutrient limitation or otherwise of the organisms. N:P ratios (Section 4.2) can indicate whether N or P is likely to be the limiting nutrient. N:P ratios were found to vary both temporally and spatially around the Reservoir and according to whether TFP or FRP is used in the calculation (see above). However, internal nutrient concentrations are a better indication of nutrient limitation as both N and P can be stored internally.

Physiological indicators focus on shifts in cell metabolism that indicate internal nutrient limitation and can be rapidly measured within a few hours of sampling (St. Amand *et al.*, 1989) Physiological indicators can be nutrient specific, in this study phosphatase activity is measured which is specific for phosphorus.

"Surface " phosphatase activity was found in all of the organisms assayed (Chapter 6). Results of the phosphatase activities found in the algae are discussed first followed by the higher plants. As the plant material was assayed straight from the field (Section 2.61) the presence of bacteria and other micro-organisms cannot be ruled out, although

material was thoroughly washed prior to assay (Section 2.65). In some cases the enzyme activity found may have been attributable to the epiphytes.

7.32 Algae

Five species of algae have been assayed to determine phosphatase activity. The rates of activity found can be compared to work by Gibson and Whitton (1986, 1987a, 1987b). They investigated the phosphatase activity of *Chaetophora* (1 species), *Draparnaldia* (1 species) and *Stigeoclonium* (30 populations) collected fresh from a number of freshwater sites in the North East of England. The substrate used was 2.7 mM pNPP so the results are not directly comparable. Table 7.1 below compares the results of Gibson and Whitton (1987a) with the results presented in Chapter 6.

Table 7.1 A comparison of phosphatase activities of Chaetophorales (μg chl a^{-1} h^{-1}). Data from Gibson and Whitton (1987a) using substrate 2.7 mM pNPP and the rest 71μM pNPP

Organism	**Activity μmol pNPP hydrolysed (μg chl a^{-1} h^{-1})**	**Author/reference**
Chaetophora (1 species)	0.038	Gibson and Whitton (1987a)
Draparnaldia (1 species)	0.027	Gibson and Whitton (1987a)
Stigeoclonium (30 populations)	0- 0.065	Gibson and Whitton (1987a)
Chaetophora (1993)	0.072	Table 6.4
Chaetophora (1994)	0.011	Table 6.5
Draparnaldia	0.009	Table 6.5
Stigeoclonium	0.047	Table 6.5

Stigeoclonium tenue (activity 0.047 μmol pNPP hydrolysed (μg chl a^{-1} h^{-1})) was in the range found by Gibson and Whitton (1987a), the *Chaetophora incrassata* collected in autumn 1993 from the northern shore had a higher activity than the species analysed

by Gibson and the *C. incrassata* from the small stream collected in 1994 had a lower activity, as did the *Draparnaldia*.

The abundance of *C. incrassata* along the northern shore (near site 03) of the Reservoir in autumn 1993 coincided with an increase in the N:P ratio of the Reservoir water. Phosphatase activity was found to be high (Table 7.1) compared to other organisms at Bakethin and when compared to work by Gibson and Whitton (1987a), and it is possible that *C. incrassata* is not as limited in growth as other organisms as it can make use of the organic P pool, giving it a competitive advantage.

The *C. incrassata* collected in May 1994 from a temporary stream entering the reservoir near Site 01 had lower phosphatase activity (Table 7.1). The N:P ratio of the water is not known. *C. incrassata* was again found on the northern shore from the end of July 1994.

The N:P ratios in July 1994 (N:TFP = 13.2, N:FRP = 46.7) were lower than July 1993, (N:TFP = 13.9, N:FRP = 62.5). They had; however, increased greatly from the previous month (June 1994). It is possible that *C. incrassata* was again making use of the organic phosphorus and that phosphatase activity if measured would have been found to be high.

The abundance and distribution appeared to have increased and it is possible that release of nutrients from the bed of *Nitella flexilis* could have effected this (Section 7.41).

7.33 Higher plants

The phosphatase data for the three species of *Potamogeton* collected and analysed in 1994 can be compared to work by Milligan (1994) on the phosphatase activities of the higher plants *Phragmites australis*, *Equisetum fluviatile* and *Typha latifolia*, which

were collected on the same sampling days but from different sites at Bakethin Reservoir. Comparable data using 1μM 4-MUP are shown in Table 7.3 below.

Table 7.2 Comparison of phosphatase activities of higher plant roots (μmol 4-MUP hydrolysed (g $d.wt^{-1}$ h^{-1})). The *Potamogeton* species are given in the order used in Table 6.6. Data are available for 4 sites for *Phragmites*, 3 sites for *Equisetum* and *Typha*.

	Average activity μmol 4-MUP hydrolysed (g $d.wt^{-1}$ h^{-1})			
Species	**Site 1**	**2**	**3**	**4**
Equisetum	0.15	0.18	0.06	
Phragmites	0.89	4.02	0.44	0.33
Typha	2.36	1.23	1.32	
Potamogeton berchtoldii	0.71			
Potamogeton obtusifolius	1.12			
Potamogeton natans	2.22			

Table 7.2 shows that the phosphatase activities of the *Potamogeton* species are within the range of the phosphatase activities of the 3 species analysed by Milligan (1994).

Higher plants are not mobile, their roots are permanently in the sediments and in the case of *Potamogeton* their leaves are permanently in the water. If the nutrient status of the Reservoir is a basic trend of P limitation in the water and N limitation in the sediments, then we would not necessarily expect phosphatase activity associated with the roots. Unless one or more of the following were true; that the key factor to trigger phosphatase activity is the internal P concentration of the plant and not the external levels in the sediment or water, or that phosphatase activity is not only a response to P limitation and may instead be a more general indication of nutrient stress. It is also possible that methods used to determine N and P levels in sediments are not representative of the N and P available to the plant.

In whole plant experiments the leaves and roots of *Potamogeton obtusifolius* were found to have similar rates of phosphatase activity per g of dry weight, but the leaves have approximately 3.5 times the enzyme activity of the roots due to their larger average dry weight. Assays on *Nitella flexilis* showed the presence of phosphatase activity on the surface of the plant 'stems' and the rhizoids, indicating the potential for hydrolysing organic P from both the water and the sediments.

7.34 Suitability of phosphatase as an ecological indicator

Phosphatase activity has been found in all organisms assayed. This suggests that the organisms may have been P-limited although it does not prove that they were. The extrapolation of laboratory results to interpret what is actually occurring in the field is always difficult. Although the material assayed was fresh from the field and had not been grown in a laboratory controlled environment the assay conditions (temperature, light and pH) may influence the organisms greatly. An advantage of using axenic lab-cultured organisms is that the possibility of the phosphatase activity measured being attributable to epiphytic micro-organisms is removed. In order to reduce the likelihood that the activity measured in this study could be attributed to micro-organisms the material was always washed thoroughly in assay medium before the assay and checked under the microscope for any micro-organisms.

No localisation of PMEase activity could be found with the BCIP stain. Milligan (1994) and Luff (1993) also reported difficulty in localising the PMEase activity. Milligan proposed that in the case of *Equisetum fluviatile*, and *Typha latifolia* this was because the roots were too pigmented and that the process may be more rewarding if

specimens had been grown *in vitro* to produce cleaner roots. The method may need to be modified to achieve results with material collected from the field.

The aims of this project (Section 1.9) have been achieved as far as possible in a 16 month project. Concluding remarks at the end of the project follow. Both the flora and the high "surface" phosphatase activity of the plants at the edge of the Reservoir indicate that they are probably growing under moderately P-limited conditions. Since the STW effluent has a high N:P ratio; this suggests that the Reservoir would be even more P-limited without the effluent. As there is no information about the release of nutrients during the initial stages of the Reservoir, it is uncertain whether the early stages of the fen community at the edge would have been P-limited. The fact that the community at the edge is apparently P-limited suggests that any change in the P status of the North Tyne might have a marked effect on the communities at the edge of the Reservoir.

It is possible that light is one factor limiting phytoplankton growth\\ at the Reservoir. The seasonal changes in water chemistry and photosynthetic organisms have been identified. The significant differences between the water chemistry and the algal flora in 1993 and 1994 suggest that the climate and management of the Reservoir does effect the chemistry and biology of the Reservoir. The algae found in the Reservoir seem to be mainly opportunistic; however, some features of the Reservoir, such as the high abundance of *Chaetophora incrassata* on the northern shore of the Reservoir in autumn, were similar for 1993 and 1994.

The results of the project are summarised below after recommendations for further work.

7.4 Recommendations for further work

The study of sediments at Bakethin Reservoir could be expanded to identify the absorption/desorption characteristics and help to determine whether the sediments act as a source or a sink for nutrients at Bakethin and if this changes on a seasonal basis.

Further research into the use of physiological indicators to determine the nutrient status of a photosynthetic organisms is recommended. Research into how the phosphatase activities measured can be more closely related to the environmental conditions is needed. Measurement of internal N and P concentration of the organisms and N and P concentrations of the sediments and water at the microsite where the organism was collected would lead to a greater understanding of the nutrient limitation of the organisms. The staining method used to attempt to localise the PMEase activity found could be modified to suit material collected fresh from the field.

SUMMARY

1. This project (25/03/93-18/07/94) investigated nutrient cycling at Bakethin Reservoir, Northumberland, England; this is a small upland water body, which serves as a feeder for the larger Kielder Reservoir located in the middle of Kielder forest.
2. The aims of the research were to: (1) determine the significant inputs of nutrient to the Reservoir and if they vary on a seasonal basis; (2) investigate the seasonal changes in chemical variables especially N and P and photosynthetic organisms at sites around the Reservoir; (3) determine whether certain organisms are P-limited at the sites.
3. The main inputs to the Reservoir have been highlighted, they are the River North Tyne, which receives Butteryhaugh Sewage Treatment Works (STW) and the Kielder Salmon hatchery.
4. Water samples were taken monthly from 6 sites around the Reservoir, Site Upstream of the Sewage Treatment Works (USTW), and bi-monthly from the Calcareous Flush. The following variables were measured: temperature, conductivity, absorbance at 420 nm, O_2, pH, total alkalinity, Na, K, Mg, Ca, Mn, Fe, NO_3-N, NO_2-N, NH_4-N, PO_4-P. In addition Secchi depth was measured at the mid-Reservoir site.
5. The mean values of NO_3-N, NO_2-N, NH_4-N and PO_4-P were all found to be higher in the main Reservoir than USTW. The mean values of NO_3-N, NO_2-N, NH_4-N and PO_4-P at Site USTW were all higher than the Calcareous Flush.
6. The ranges of NO_3-N were from < 0.1 $\mu g\ l^{-1}$ to 95.6 $\mu g\ l^{-1}$ at USTW, 5.1 to 117.8 $\mu g\ l^{-1}$-N at the main Reservoir and from < 0.1 $\mu g\ l^{-1}$ to 58.9 $\mu g\ l^{-1}$ at the Calcareous

Flush. The range of NO_2-N were from < 0.1 μg l^{-1} to 6.7 μg l^{-1} at USTW, below detection to 8.2 μg l^{-1} at the main Reservoir, and from < 0.1 μg l^{-1} to 6.5 μg l^{-1} at the Calcareous Flush. The range of NH_4-N was 15.2 to 88.2 μg l^{-1} at USTW, 15.7 to 135.5 μg l^{-1} at the main Reservoir and 15.7 to 185.9 μg l^{-1} at the Calcareous Flush.

7. The range of Total Filtrable Phosphorus (TFP) was 3.8 to 16.9 μg l^{-1} at USTW, < 1.0 μg l^{-1} to 49.7 μg l^{-1} at the main Reservoir and from < 1.0 μg l^{-1} 0.5 to 25.0 μg l^{-1} at the Calcareous Flush. The ranges of FRP found were < 1.0 μg l^{-1} to 7.7 μg l^{-1} at USTW, < 1.0 μg l^{-1} to 49.7 μg l^{-1} at the main Reservoir and < 1.0 μg l^{-1} to 10.1 μg l^{-1} at the Calcareous Flush.
8. N:P ratios were calculated for each water sample taken and suggest that P is limiting with respect to N on most occasions. Exceptions to this generally occurred in May and June; the reasons for this are explored in the discussion.
9. Sediment samples were collected once (28/05/94) from sites around the Reservoir, including those mentioned above, and analysed for grain size, organic matter, and N and P concentrations.
10. Grain size of sediment is summarised in terms of percentage gravel, sand and silt + clay. The percentage of gravel ranged from 4.8 to 49.0, the percentage of sand ranged from 5.7 to 81.7, and the percentage of silt + clay ranged from 1.8 to 91.0. Organic matter ranged from 0.5 to 55.2% by weight. Organic N concentration ranged from 3.0 to 9.1 μg l^{-1}. Total phosphorus (TP) levels ranged from 1.0 to 1082 μg l^{-1}.
11. A floristic survey of the 6 main Reservoir sites was conducted to coincide with the analysis of physical and chemical variables of the water. 210 algal taxa, 2 species

of lichen, 1 aquatic moss and 38 vascular plants were recorded. 11 of these vascular plants are typically terrestrial and were only found at the sampling sites during times of high water levels.

12. Density of phytoplankton from the mid-Reservoir site ranged from 17 (Jan 94) to 167 cells ml^{-1} (Aug 93) with the maximum values occurring in the summer. The number of taxa ranges from 3 (Oct 93, Dec 93 and Jan 94) to 19 (May 93). The maximum values occur in spring and early summer. The dominant species are *Tabellaria flocculosa* and *T. fenestrata*.

13. Density of picoplankton from the mid-Reservoir site ranged from 320 (Apr 93) to 10200 cells ml^{-1} (Aug 93). Maximum values occur in the summer, but a trend of increasing numbers during periods of minimal exchange between Bakethin Reservoir and Kielder Reservoir may also exist.

14. A computer program was written to obtain correlation coefficients in order to characterise the relationships within and between the environmental variables measured (including rain) and the quantitative biological data. Significant positive correlations were found between conductivity and total alkalinity, conductivity and Ca^{2+}, conductivity and Mg^{2+}, Ca^{2+} and Mg^{2+}, Ca^{2+} and total alkalinity, TFP and FRP, phytoplankton and picoplankton density, phytoplankton density and temperature, and optical density and Secchi depth. Significant negative correlations were found between optical density and conductivity, optical density and pH, phytoplankton density and rain.

15. TWINSPAN was used in order to characterise relationships within the semi-quantitative biological data. A dendrogram was produced showing the classification of the 87 algal samples into end groups. The phytoplankton was

classified into two groups while the main basis for classification of the rest of the samples seemed to be sample date rather than sampling site. Indicator species were automatically generated for each end group.

16. In order to give an indication of P-limitation, eight species were chosen for a preliminary study of their "surface" phosphatase activities. These were *Ulothrix zonata*, *Stigeoclonium tenue*, *Draparnaldia glomerata*. *Chaetophora incrassata*, *Nitella flexilis*, *Potamogeton berchtoldii*, *P. obtusifolius*, *P. natans*.

17. Values of enzyme activity, measured as substrate hydrolysed (μmol g d.wt $^{-1}$ h $^{-1}$), ranged from 9.6 to 480 using 100 μM 4-MUP as the substrate.

18. The data show that, in terms of enzyme activity, the following algal species are consistently ordered, regardless of substrate and substrate concentration: *Stigeoclonium tenue* > *Draparnaldia glomerata* > *Ulothrix zonata*.

19. Similarly the *Potamogeton* species are generally ordered in terms of enzyme activity: *Potamogeton natans* > *P. obtusifolius* > *P. berchtoldii*.

20. The enzyme activity of leaves and roots from the same plants of *P. obtusifolius* were investigated and found to be similar per gram of dry weight. However the contribution to the plant from the leaves is approximately 3.5 times greater than the roots on account of their larger average weight.

21. Chemical and biological features of Bakethin Reservoir are compared to nearby reservoirs. Various points are discussed including possible limits to biological growth and the most likely limiting factors at Bakethin Reservoir. The impact of Butteryhaugh STW on the nutrient regime of the Reservoir is discussed, and the effect of an increase or decrease in output is considered.

REFERENCES

Ahl T (1988) Background yield of phosphorus from drainage area and atmosphere: an empirical approach. Hydrobiologia 170: 35-44.

Allen SE, Grimshaw HN, Hunt R (1978) Chemical Analysis of Ecological Materials. Blackwell Sci. Publs., Oxford.

American Public Health Association (1989) Standard Methods for Examination of Water and Wastewater (17th edn). 1015 Fifteenth Street NW, Washington.

Andersen JM (1976) An ignition method for determination of total phosphorus in lake sediments. Water Research 10: 329-331.

Anon. (1986) The Analysis of Agricultural Materials (3rd edn). Ministry of Agriculture, Fisheries and Food, London.

Atkinson KM (1988) The initial development of net phytoplankton in Cow Green Reservoir. In: Round FE (ed.), Algae and the Aquatic Environment. Biopress, Bristol. 1-460 pp.

Berman T, Nawrocki M, Taylor GT, Karl DM (1987) Nutrient flux between bacteria, bactiverous nanoplankton protists and algae. Marine Microbial Food Webs 2: 69-82.

Berner RA (1980) Early diagenesis: A Theoretical Approach. Princetown Univ.Press 241 pp.

Bresnan EM (1993) The status of phosphorus of a calcareous flush at Bakethin Reservoir, Northumberland. M.Sc. Dissertation, University of Durham.

Causton D (1988) Introduction to Vegetation Analysis. Unwin. England.

Carvalho L (1994) Top-down control of phytoplankton in a shallow hypertrophic lake: Little Mere (England). Hydrobiologia 275/276: 53-63.

Chiaudani G, Vighi M (1974) The N:P ratio and tests with *Selenastrum* to predict eutrophication in lakes. Water Research 8: 1063-9.

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

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

Chróst RJ (1990) Micobial ectoenzymes in aquatic environments. In: Ovberbeck J, Chróst RJ (eds) Aquatic Microbial ecology: biochemical and molecular approaches. Springer 47-48 pp.

Corbridge D (1991) Introduction and background. In: Corbridge D (ed.) Phosphorus: an Outline of its Chemistry, Biochemistry and Technology (4th edn). Elesvier Scientific Publications. The Netherlands 1-43 pp.

Coston S, Holt SJ (1958) Kinetics of aerial oxidation of indolyl and some of it's halogen derivatives. Proc. R. Soc. B 148: 506-510.

Downing JA, McCauley E (1992) The nitrogen:phosphorus relationship in lakes. Limnol. Oceanogr. 37: 936-945.

Duarte CM, Kalff J (1986) Littoral slope as the predictor of the maximum biomass of submersed macrophyte communities. Limnol. Oceanogr. 31: 1072-80.

Duff SMG, Sarath G, Plaxton WC (1994) The role of acid phosphatases in plant phosphorus metabolism. Physiologia Pl. 90: 791-800.

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

Freeman JS, Rowell DL (1981) The adsorption and precipitation of phosphate onto calcite. J. Soil Sci. 32: 75-84.

Fuhs GW (1973) Cytochemical examination of blue-green algae. In: Whitton BA and Carr NG (eds) The Biology of Blue-Green Algae. Oxford, Blackwell 117-43 pp.

Gibson MT, Whitton BA (1986) Hairs in freshwater Chaetophorales - a eukaryotic parallel to hairs in blue-green algae. British Phycological Journal 32: 329-330.

Gibson MT, Whitton BA (1987a) Hairs, phosphatase activity and environmental chemistry in *Stigeoclonium*, *Chaetophora* and *Draparnaldia* (Chaetophorales). British Phycological Journal 22: 11-22.

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

Goldman JC, Caron DA (1985) Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. Deep Sea Res. 32: 899-915.

Harding JPC, Hawley GRW (1991) Use of algae for monitoring rivers in the United Kingdom. In: Whitton BA, Rott E, Friedrich G (eds) Use of Algae for Monitoring Rivers. Institut für Botanik, Innsbruck, Austria. 183-193 pp.

Hawley GRW (1990) Ecological and physiological studies on freshwater autotrophic picoplankton. Ph.D. Thesis, University of Durham.

Hawley GRW, Whitton BA (1991) Seasonal changes in chlorophyll-containing picoplankton populations of ten lakes in northern England. Internationale Revue der Gesamten. Hydrobiologie 76: 545-554.

Heathwaite AL (1993). Nitrogen cycling in surface waters and lakes. In: Burt TP, Heathwaite AL, and Trudgill ST, (eds) Nitrate: Processes, Patterns and Management. Chichester: Wiley 98-140 pp.

Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. 33: 796-822.

Holmes NTH, Whitton BA, Hargreaves JW (1978) A Coded List of 1000 Freshwater Macrophytes of the British Isles. Department of the Environment Water Data Unit. Reading.

Holt SJ, Withers RFJ (1958) An appraisal of indigogenic reactions for esterase localization. Proc. R. Soc. B 148: 510-526.

Holten H, Kamp-Nielsen L, Stuanes A, (1988) Phosphorus in soil, water and sediments: an overview. Hydrobiologia 170: 19-34.

Istanovics V, Herodek S, Szilagyi F (1989) Phosphate absorption by different sediment fractions in Lake Balaton and its protecting reservoir. Water Research 23: 1357-1366.

Jansson M (1988) Phosphatase uptake and utilisation by bacteria and algae. Hydrobiologia 170: 177-190.

Ku WC, DiGiano FA, Feng TH (1978) Factors affecting phosphate adsorption equilibria in lake sediments. Water Research 12: 1069-1074.

Lapointe BE, O'Connell J (1989) Nutrient enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda. Eutrophication of a confined, P limited marine ecosystem. Estuarine and Coastal Marine Science 28: 347-360.

Lehman JT (1980) Release and cycling of nutrients between planktonic algae and herbivores. Limnol. Oceanogr. 25: 620-632.

Luff HL (1993) A study of the root surface phosphatase activities of three species of higher plant: *Juncus effusus*, *Phragmites australis* and *Typha latifolia*. M.Sc. Dissertation, University of Durham.

Maitland PS (1978) A Coded Checklist of Animals Occurring in Freshwater in the British Isles. Edinburgh: Institute of Terrestrial Ecology.

Marker AFH, Jinks S (1982) The spectrophotometric analysis of chlorophyll a and phaeopigments in acetone, ethanol and methanol. Ergebrisse der Limnologie 16: 3-17.

McComb RB, Bowers GN, Posen S (1979) Alkaline Phosphatase. Plenum Press New York.

McLellan AG (1971) The Derwent reservoir fishery. Fish Management 2: 14-18.

Milligan AL (1994) Nutrient status of emergent macrophytes around Bakethin Reservoir, Northumberland. M.Sc. Dissertation, University of Durham.

Moore JA (1986) Charophytes of Great Britain and Ireland. BSBI Handbook no. 5 London.

Moriarty DJW, Boon PI (1989) Interactions of seagrasses with sediment and water In: Larkum WD *et al.* (eds) Biology of seagrasses. Elsevier 500-535 pp.

Murphy J, JP (1962) A modified single salt solution method for the determination of phosphate in natural waters. Analytica Chim. Acta 12: 162-176.

Nixon SW, Kelly JR, Furnas BN, Oviatt CA, Hale SS (1980) Phosphorus regeneration and the metabolism of coastal marine bottom communities. In: Tenore KK, Coull BC (eds) Marine Benthic Dynamics. University S. Carolina Press 219-242 pp.

Northumberland Wildlife Trust (1992) Bakethin Conservation area. 46 pp.

Parsons TR, Takahashi M, Hargrave B, (1984) Biological Oceanographic Processes (3rd edn). Pergaman Press, London.

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

Pieczynska E (1986) Sources and fates of detritus in the shore zone of lakes. Aquatic Botany 25: 153-166.

Redfield AC (1934) On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnson Memorial Volume (Liverpool) pp 176.

Round FE (1981) The Ecology of Algae. Cambridge University Press 460 pp.

Round FE (1991) Use of diatoms for monitoring rivers In: Whitton BA (ed.) Use of Algae for Monitoring Rivers. Studia Innsbruck 25-32 pp.

Siude W, Gude H, (1994) A comparative study on 5'-Nucleotidase (5'-Nase) and alkaline phosphatase (APA) activities in 2 lakes. Arch. Hydrobiologia 131: 211-299.

Spellerberg IF (1993) Monitoring Ecological Change. Cambridge University Press, Cambridge 215-235 pp.

Spence DHN (1982) The zonation of plants in freshwater lakes. Advances in Ecological Research 12: 37-125 pp.

Stainton MP, Capel MJ, Armstrong FAJ, (1977) The chemical analysis of freshwater. Freshwater special publication no. 25, Freshwater Institute, Manitoba.

St. Amand AL, Soranno PA, Carpenter SR (1989) Algal nutrient deficiency: Growth bioassays versus physiological indicators. Lake and Reservoir Management 5: 27-35.

Standing Committee of Analysts (1981) Methods for the analysis of Waters and Associated Materials. London: Her Majesty's Stationary Office.

Taft JL, Loftus ME, Taylor WR (1977) Phosphatase uptake from phosphomonoesters by phytoplankton in the Chesapeake Bay. Limnol. Oceanogr. 22: 1012-1021.

Taub FB (1984) (ed.) Ecosystems of the world. Volume 23 Lakes and reservoirs. Cambridge University Press, Cambridge 326 pp.

Tett P, Heaney SI, Droop MR (1985) The Redfield ratio and phytoplankton growth rate. J. mar. biol. Assoc. U.K. 65: 487-504.

Van der Molen DT, Los FJ, van Ballegoijen L, van der Vat MP (1994) Mathematical modelling as a tool for management in eutophication of shallow lakes. Hydrobiologia 275/276: 479-492.

Vollenweider RA (1968) Scientific fundamentals of the eutrophication of lakes and flowing waters with particular reference to nitrogen and phosphorus, Organisation for Economic Co-operation, Paris.

Wehr JD (1989) Experimental tests of nutrient limitation in freshwater picoplankton. Applied and Environmental Microbiology. 55: 1605-1611.

Wehr JD (1991) Nutrient and grazer-mediated effects on picoplankton and size structure in phytoplankton communities. Internationale Revue der Gesamten. Hydrobiologie 76: 643-656.

Weisse T (1988) Dynamics of autotrophic picoplankton in Lake Constance. Journal of Plankton Research 10: 1179-1188.

Wetzel RG (1983) Limnology (2nd edn). Saunders College publishing, Philadelphia 767 pp.

Wetzel RG (1988) Significance of sedimentary phosphorus to a rooted submersed macrophyte and its algal epiphytes. Aquatic Botany 32: 261-281.

Wetzel RG (1990) Limnological Analyses. Saunders College publishing, Philadelphia. 578 pp.

Whitton BA, Holmes NTH, Sinclair C (1978) A coded list of 1000 Freshwater Algae of the British Isles. Department of the Environment Water Data Unit. Reading, UK.

Whitton BA, Rott E, Friedrich G (1991) Use of Algae for Monitoring Rivers. Universität Innsbruck, Austria. 183-193 pp.

Wood RD Imahori K (1965) A revision of the Characeae. I Monograph of the Characeae II Iconograph of the Characeae. Weinheim.

APPENDICES

Appendix 1. To calculate relative centrifugal force (rcf)

$rcf = (1.12 * 10^{-5}) (rpm^2) r$

where r = radius of the centrifuge (cm) measured from the centre of the spindle to the end of the bucket rota

and rpm = revolutions per minute

Appendix 2. To calculate nutrient concentrations in sediments

$$\text{concentration in sample } (\mu g\ g^{-1}) = \frac{conc.in \cdot digest \times 25ml}{mass \cdot of \cdot sample(mg)} \times 1000$$

Appendix 3 Summary statistic of Kielder Burn Salmon hatchery effluent sampled by the NRA.

Table 3.25.1 Temperature

	upstream	effluent	downstream
average	8.89	8.76	8.11
max.	18.5	18	16.8
min	2.0	1.5	0.5
N	33	33	20
SD	4.61	4.56	4.57

Table 3.25.2 pH

	upstream	effluent	downstream
average	7.39	7.39	7.73
max.	8.27	8.19	8.48
min	6.7	6.74	6.9
N	33	33	20
SD	0.38	0.37	0.46

Table 3.25.3 Dissolved Oxygen (% sat.)

	upstream	effluent	downstream
average	96.1	94.8	99.7
max.	126	121	107

min	74	70	89
N	32	33	21
SD	9.42	9.35	4.47

Table 3.25.4 Dissolved Oxygen (mg l^{-1})

	upstream	**effluent**	**downstream**
average	11.18	11.08	12.04
max.	16.1	15	14.8
min	8.28	7.91	10.2
N	31	32	21
SD	1.63	1.58	1.06

Table 3.25.5 BOD (mg l^{-1})

	upstream	**effluent**	**downstream**
average	1.32	1.35	1.45
max.	2.4	2.6	2.5
min	0.5	0.5	0.8
N	32	31	19
SD	0.46	0.51	0.5

Table 3.25.6 Suspended solids (mg l^{-1})

	upstream	**effluent**	**downstream**
average	3.48	3.48	3
max.	30	30	16
min	1	<1	<1
N	33	33	20
SD	5.27	5.27	3.5

Table 3.25.7 Alkalinity (mg l^{-1} $CaCO_3$)

	upstream	**effluent**	**downstream**
average	73.24	80.77	66.2
max.	640	690	110
min	<10	<10	17
N	33	33	20
SD	110.0	127.85	27.1

Table 3.25.8 NH_4-N (mg l^{-1})

	upstream	effluent	downstream
average	0.085	0.218	0.123
max.	0.470	0.580	0.740
min	0.030	0.040	0.030
N	33	33	19
SD	0.104	0.174	0.169

Table 3.25.9 NO_3-N (mg l^{-1})

	upstream	effluent	downstream
average	<0.063	<0.340	<0.385
max.	1.1	0.58	0.75
min	0.05	0.04	0.01
N	33	33	19
SD	0.217	0.174	0.210

Table 3.25.10 NO_2-N (mg l^{-1})

	upstream	effluent	downstream
average	0.028	0.028	<0.019
max.	0.11	0.12	<0.03
min	0.01	0.007	<0.01
N	33	33	19
SD	0.021	0.023	0.005

SITE 01	Temp	Conductivity	OD	O2 mg/l	pH	Total alkalinity	Na	K	Mg	Ca	MnT	FeT	NO3-N	NO2-N	NH4-N	TFP	FRP
25/03/93	3.8	175.0	0.020	13.5	7.8	3.5	4.90	0.65	6.73	21.30	0.00	0.10	54.5	2.5	17.4	4.9	3.3
21/04/93	7.3	114.0	0.055	11.4	7.7	1.6	7.16	0.60	3.73	12.87	0.04	0.27	57.1	5.7	23.6	5.7	5.1
20/05/93	8.7	129.0	0.052	12.0	7.5	2.1	4.75	0.73	5.91	14.85	0.02	0.69	13.1	4.8	35.2	12.6	8.7
03/06/93	12.6	99.0	0.077	10.4	7.2	1.5	4.51	0.58	4.39	8.58	0.08	0.43	15.2	7.9	36.5	10.9	4.1
22/06/93	13.5	127.0	0.064	8.8	7.3	2.1	6.04	0.70	5.18	26.76	0.04	0.62	18.2	6.3	56.0	43.0	29.0
19/07/93	13.0	157.0	0.040	9.3	7.4	2.5	1.56	0.74	6.37	26.16	0.02	0.35	15.6	5.6	71.7	7.2	2.6
23/08/93	11.2	138.0	0.040	8.6	7.5	2.0	4.33	0.74	5.65	24.61	0.22	0.86	20.6	4.0	65.5	8.3	2.4
20/09/93	11.0	176.0	0.030	9.0	7.7	1.8	1.21	1.00	5.77	18.75	0.03	0.24	48.2	1.0	58.1	10.1	< 1.0
18/10/93	1.2	166.6	0.020	11.3	7.9	1.5	3.35	0.71	7.02	22.11	0.02	0.33	50.8	0.1	48.4	8.6	1.5
30/11/93	0.2	177.9	0.027	12.7	7.9	2.2	8.44	0.88	6.28	16.86	0.01	0.36	81.3	1.9	81.7	21.9	12.5
18/12/93	0.5	129.0	0.030	11.8	7.8	2.1	6.20	0.70	3.80	8.40	0.01	0.40	72.0	2.0	63.0	11.9	9.2
17/01/94	0.6	135.0	0.031	13.2	7.6	2.6	8.40	0.80	6.80	16.70	0.00	0.20	61.0	2.2	46.5	13.9	5.7
14/02/94	0.6	140.0	0.030	13.5	7.7	2.2	3.10	0.60	2.20	11.20	0.01	0.20	54.0	2.5	20.0	10.5	9.5
17/03/94	1.5	150.0	0.024	11.9	8.0	1.6	5.41	1.30	5.00	11.60	0.01	0.09	55.3	2.1	86.0	3.4	< 1.0
18/04/94	7.2	193.0	0.019	11.9	7.9	3.8	8.47	1.70	8.10	28.10	0.08	0.05	30.6	0.0	85.6	13.9	8.9
16/05/94	12.3	149.0	0.018	10.5	8.0	4.2	9.64	1.80	5.20	21.00	0.08	0.16	107.0	2.9	63.2	12.3	8.8
14/06/94	15.1	241.0	0.017	10.8	8.2	4.8	9.74	2.10	10.80	28.30	0.03	0.08	6.9	1.7	135.5	10.4	9.1
18/07/94	18.4	223.0	0.028	10.3	7.9	4.8	9.88	9.80	8.50	26.00	0.40	0.12	37.4	1.7	84.1	5.5	3.5
SITE 1A																	
25/03/93																	
21/04/93	7.4	112.0	0.061	10.5	7.9	1.6	8.39	0.52	4.07	11.46	0.04	0.27	42.2	5.4	15.7	4.9	2.9
20/05/93	9.9	120.0	0.056	10.9	7.3	2.0	5.18	0.65	5.44	12.65	0.02	0.23	13.1	4.2	35.2	12.6	8.7
03/06/93	11.9	114.0	0.041	6.4	6.5	1.5	6.58	0.81	5.04	12.38	0.01	0.42	16.0	6.0	28.7	26.0	8.2
22/06/93	15.3	132.0	0.059	7.9	7.3	1.8	6.20	0.71	4.94	21.43	0.01	0.30	15.0	5.8	55.0	18.9	1.9
19/07/93	12.7	158.0	0.040	8.3	7.4	2.0	2.76	3.41	6.99	9.59	0.05	0.26	14.9	6.1	96.3	7.2	2.2
23/08/93	12.3	132.0	0.040	8.3	7.5	2.3	3.08	0.72	5.23	21.97	0.23	2.10	26.5	4.0	82.0	5.0	1.8
20/09/93	10.9	149.0	0.030	10.0	7.6	1.8	3.74	0.94	6.57	8.31	0.03	0.16	50.9	1.5	76.8	< 1.0	< 1.0
18/10/93	1.2	171.0	0.020	12.3	7.9	1.7	4.42	0.70	7.29	22.09	0.02	0.04	62.0	0.1	48.4	< 1.0	< 1.0
30/11/93	0.4	158.8	0.035	13.2	7.9	1.8	7.97	0.80	6.31	16.74	0.06	0.61	86.2	1.7	78.4	16.2	9.5
18/12/93	1.0	101.0	0.030	7.4	7.6	1.5	10.40	0.60	3.70	9.00	0.01	0.80	92.0	1.5	62.0	11.2	9.2
17/01/94	0.4	135.0	0.033	13.0	7.8	2.6	15.30	0.70	2.50	21.70	0.00	0.20	88.0	1.4	54.0	11.4	5.7
14/02/94	0.6	128.0	0.040	12.8	7.9	2.4	4.70	0.80	4.00	27.60	0.01	0.30	71.0	4.6	26.0	12.7	9.2
14/03/94	2.0	130.0	0.022	12.1	8.0	2.6	4.57	1.30	4.90	15.20	0.02	0.10	55.9	2.0	68.9	2.9	0.0
18/04/94	7.2	189.0	0.017	10.8	7.6	5.0	7.21	1.60	8.20	24.10	0.20	0.12	14.9	0.0	75.2	10.2	5.6
16/05/94	12.2	142.0	0.017	10.3	7.9	5.4	10.78	1.50	5.30	15.40	0.11	0.15	36.6	2.2	47.9	12.8	7.3

14/06/94	15.3	230.0	0.018	11.2	8.2	5.4	14.29	2.80	10.60	27.50	0.05	0.08	6.2	1.6	59.4	26.7	6.6
18/07/94	18.8	214.0	0.034	10.1	8.0	4.6	12.84	2.30	8.40	22.40	0.12	0.12	24.6	1.1	104.1	7.4	2.6
SITE 03																	
25/03/93	7.8	138.0	0.031	12.2	7.59	2.2	5.20	0.69	4.94	14.90	0.00	0.17	79.5	4.2	18.0	11.0	2.6
21/04/93	8.0	94.0	0.074	11.1	7.27	1.4	11.89	0.52	3.15	9.47	0.06	0.21	34.6	6.3	23.0	8.6	2.6
20/05/93	9.9	61.0	0.096	11.1	7.3	1.6	4.04	0.45	2.33	6.29	0.03	0.42	24.5	5.2	53.2	11.0	4.0
03/06/93	12.6	97.0	0.074	10.7	7.17	1.4	5.08	0.68	4.22	9.43	0.00	0.36	30.7	7.6	34.8	9.2	3.1
22/06/93	13.8	126.0	0.056	9.0	7.46	1.8	5.43	0.71	4.71	19.41	0.01	0.21	25.0	6.0	50.0	10.8	1.5
19/07/93	12.6	159.0	0.040	8.7	7.4	2.0	1.70	0.78	6.91	23.11	0.11	0.48	20.9	5.4	68.7	6.8	1.5
23/08/93	13.5	113.0	0.050	9.0	7.38	2.0	1.90	0.74	4.29	21.00	0.17	1.27	36.8	4.2	61.0	4.2	1.3
20/09/93	12.9	132.0	0.060	9.2	7.37	1.8	1.87	0.68	4.34	20.06	0.08	0.68	54.0	2.4	62.2	3.8	1.2
18/10/93	5.6	85.5	0.085	10.9	7.23	1.5	2.00	0.56	2.32	13.52	0.10	1.00	52.0	2.7	54.9	1.9	0.0
30/11/93	1.0	140.3	0.045	11.8	7.02	1.6	8.22	0.69	5.18	13.36	0.01	0.54	48.6	2.9	81.7	18.5	7.3
18/12/93	1.9	75.0	0.050	6.0	7.32	1.7	8.20	0.60	2.20	11.00	0.02	0.10	53.0	4.1	55.3	17.7	10.7
17/01/94	1.9	81.0	0.047	12.5	7.81	1.9	12.00	0.70	1.90	13.50	0.02	0.30	55.0	5.6	42.1	14.3	4.5
14/02/94	1.6	128.0	0.050	12.9	7.87	2.0	5.30	0.30	4.20	8.10	0.01	0.10	71.0	5.0	36.0	11.8	8.5
17/03/94	2.1	125.0	0.032	11.2	7.64	1.7	5.13	1.20	3.70	8.30	0.02	0.10	54.9	2.1	81.3	0.7	< 1.0
18/04/94	7.8	99.0	0.035	11.9	7.7	3.0	7.14	1.10	3.60	9.70	0.07	0.05	27.2	1.2	54.3	18.6	4.9
16/05/94	12.6	133.0	0.029	10.3	7.85	3.6	7.70	1.10	4.80	11.60	0.07	0.09	5.1	2.6	38.5	9.9	8.8
14/06/94	15.3	198.0	0.027	11.1	7.98	4.2	9.78	1.60	8.80	23.20	0.08	0.10	32.1	2.0	91.7	31.5	19.8
18/07/94	18.5	209.0	0.027	9.2	7.92	4.2	12.01	2.50	8.10	21.90	0.10	0.11	21.3	1.0	81.3	7.8	2.2
SITE 05																	
25/03/93	6.6	138.0	0.031	13.4	7.75	2.18	5.20	0.62	4.96	4.80	0.00	0.17	78.4	4.1	15.7	12.2	2.6
21/04/93	7.7	93.0	0.064	11.3	7.25	1.1	11.89	0.56	3.04	9.00	0.09	0.40	117.8	6.0	33.2	5.3	1.8
20/05/93	11.3	57.0	0.101	11.4	7.27	1.2	3.83	0.29	2.00	5.98	0.02	0.44	25.0	7.1	16.3	12.2	5.1
22/06/93	13.9	124.0	0.055	9.3	7.44	1.8	6.16	0.60	4.74	18.81	0.02	0.26	22.3	5.2	33.4	41.2	27.2
19/07/93	16.0	160.0	0.040	8.2	7.3	1.5	2.06	0.78	5.97	15.81	0.10	0.23	18.6	6.3	93.0	5.1	2.4
23/08/93	13.6	117.0	0.050	9.6	7.35	1.6	1.52	0.67	4.17	18.62	0.03	0.58	20.6	4.5	68.5	4.0	0.9
20/09/93	11.1	131.0	0.060	9.6	7.42	1.8	1.98	0.70	3.97	14.69	0.09	0.37	34.4	2.7	61.4	6.7	< 1.0
18/10/93	5.9	85.0	0.077	9.5	7.37	1.6	8.20	0.55	2.22	12.23	0.06	0.60	42.3	3.2	60.6	0.0	< 1.0
30/11/93	1.0	136.2	0.045	10.3	7.69	2.2	7.79	0.71	5.35	13.93	0.03	0.51	40.4	2.5	94.7	13.9	6.2
18/12/93	1.0	78.0	0.050	6.5	7.8	2	8.00	0.80	2.40	21.30	0.02	0.40	61.5	3.7	82.0	12.3	10.0
17/01/94	2.4	68.0	0.064	12	7.8	2	10.00	0.60	1.50	13.70	0.02	0.30	88.1	2.0	62.1	12.6	5.7
14/02/94	1.7	107.0	0.060	13.4	7.7	1.8	4.30	0.40	0.70	10.50	0.01	0.40	86.0	4.5	41.6	16.6	6.3
17/03/94	4.1	90.0	0.042	13.1	8.18	1.2	7.17	1.20	2.70	5.50	0.02	0.10	58.6	2.6	86.0	2.9	< 1.0
18/04/94	6.9	101.0	0.047	11.5	7.51	2.8	6.79	1.10	3.70	9.70	0.09	0.11	44.1	0.9	40.1	11.1	4.0

16/05/94	12.0	131.0	0.035	10.5	8.1	3	6.93	1.40	5.00	14.00	0.07	0.16	5.1	2.6	38.5	10.4	9.6
14/06/94	14.8	190.0	0.029	10.4	7.6	3.8	10.85	4.20	8.30	21.70	0.07	0.10	29.5	2.0	91.7	49.7	49.7
18/07/94	18.3	214.0	0.028	11.7	7.99	4.2	8.08	1.90	8.00	24.30	0.11	0.11	25.0	0.9	86.9	6.4	2.2
SITE 08																	
25/03/93	8.2	137.0	0.030	13.2	8.0	2.2	5.30	0.64	4.90	12.30	0.00	0.16	75.9	4.3	15.7	10.2	2.6
21/04/93	7.6	97.0	0.062	12.0	7.4	1.1	12.46	0.06	3.00	9.08	0.07	0.13	43.7	5.5	16.9	6.2	3.3
20/05/93	10.8	57.0	0.100	12.0	7.2	0.7	4.37	0.05	2.07	5.39	0.02	0.57	26.6	7.2	53.2	16.6	6.2
03/06/93	1.7	92.0	0.093	10.2	7.2	1.4	4.42	0.65	3.77	11.00	0.00	0.44	27.3	7.0	32.0	7.1	2.8
22/06/93	14.8	122.0	0.054	9.1	7.7	1.7	5.85	0.64	4.53	18.92	0.01	0.22	20.5	8.2	48.0	10.7	1.5
19/07/93	16.1	159.0	0.040	8.9	7.4	2.0	1.79	0.81	5.44	22.91	0.08	0.43	19.4	6.3	71.1	6.1	2.4
23/08/93	14.1	110.0	0.050	8.5	7.5	1.5	2.46	0.67	4.52	17.67	0.05	0.76	30.6	3.6	74.0	2.4	<1.0
20/09/93	10.8	136.0	0.050	9.5	7.9	1.5	2.56	0.66	4.07	15.80	0.07	0.50	21.2	2.5	65.4	0.9	<1.0
18/10/93	6.3	86.0	0.065	9.3	7.3	1.5	3.92	0.50	2.37	14.35	0.06	0.80	20.0	2.6	49.2	0.0	< 1.0
30/11/93	2.1	135.2	0.054	9.9	7.8	2.2	7.85	0.75	5.10	13.53	0.06	0.89	41.1	2.8	106.8	16.6	5.1
18/12/93	1.0	71.0	0.060	6.4	7.2	0.9	6.30	0.70	1.90	11.00	0.02	0.10	61.2	2.7	69.0	13.1	10.0
17/01/94	2.2	61.0	0.063	12.6	7.4	1.5	8.20	0.60	1.60	7.50	0.00	0.40	70.5	2.1	53.0	11.4	6.5
14/02/94	1.7	107.0	0.060	13.4	7.7	2.1	5.00	0.70	2.10	14.00	0.01	0.70	75.0	4.2	21.0	10.5	6.3
17/03/94	4.3	80.0	0.040	9.1	8.0	1.4	5.87	1.20	2.60	6.00	0.03	0.10	62.4	0.0	95.5	5.6	2.9
18/04/94	7.7	97.0	0.042	11.8	7.6	2.8	10.49	1.10	3.80	9.70	0.05	0.11	22.7	0.9	40.1	10.7	6.6
16/05/94	12.7	128.0	0.035	10.0	8.2	2.0	7.58	1.30	4.90	14.30	0.07	0.16	24.6	3.2	61.3	10.9	9.2
14/06/94	17.5	190.0	0.026	10.3	8.2	4.0	13.17	1.60	7.90	21.10	0.06	0.09	41.1	2.3	72.7	36.3	33.5
18/07/94	18.3	208.0	0.028	10.7	8.0	4.8	1< 1.0	1.90	8.00	21.70	0.10	0.11	27.2	0.6	93.6	5.0	2.2
SITE 09																	
25/03/93	8.2	137.0	0.031	13.2	7.8	2.1	5.40	0.62	4.86	14.70	0.00	0.00	72.6	4.6	15.7	9.4	2.2
21/04/93	7.7	94.0	0.062	11.1	7.4	1.2	14.61	0.53	3.00	9.24	0.08	0.21	61.4	6.4	19.1	6.2	2.2
20/05/93	10.4	66.0	0.094	11.1	7.0	0.8	4.32	0.43	2.58	4.96	0.06	0.54	16.0	7.3	50.4	12.6	3.3
03/06/93	12.5	94.0	0.077	9.8	7.2	1.4	4.59	0.89	3.96	9.90	0.01	0.56	29.6	7.2	27.5	25.2	4.3
22/06/93	14.8	126.0	0.054	10.5	7.3	2.0	6.55	0.62	4.95	19.38	0.01	0.25	20.6	6.2	40.0	8.4	1.3
19/07/93	16.2	158.0	0.040	8.2	7.4	1.8	1.54	0.52	4.10	21.86	0.18	0.66	8.5	5.8	80.0	7.5	2.0
23/08/93	13.7	120.0	0.040	10.4	7.3	2.0	2.39	0.69	4.68	19.65	0.04	0.61	25.6	5.6	67.0	7.2	2.4
20/09/93	10.9	116.0	0.050	10.0	7.3	1.5	12.01	0.80	3.51	14.91	0.07	0.27	20.4	4.6	58.1	6.7	0.5
18/10/93	6.8	89.0	0.077	9.3	7.2	1.5	1.80	0.52	2.22	16.01	0.07	0.68	30.3	3.0	63.0	1.4	0.5
30/11/93	0.7	122.1	0.050	11.1	7.9	2.4	7.56	0.67	4.42	11.45	0.03	0.56	35.1	3.0	83.3	14.4	4.3
18/12/93	1.6	72.0	0.060	13.0	7.0	0.9	6.20	0.70	2.00	13.00	0.02	0.50	46.2	2.2	62.0	11.6	9.3
17/01/94	2.0	62.0	0.064	12.9	7.2	0.9	8.20	0.60	1.60	7.80	0.01	0.40	68.1	2.1	53.0	12.2	5.3
14/02/94	1.4	110.0	0.050	12.8	7.7	2.2	6.10	0.80	2.30	11.00	0.00	0.80	71.0	2.9	41.0	11.5	7.4

17/03/94	4.1	85.0	0.043	9.1	7.8	1.6	6.03	1.20	2.50	5.50	0.04	0.06	54.2	0.0	94.6	1.6	0.5
18/04/94	7.7	120.0	0.029	12.0	7.6	2.8	8.40	1.20	4.50	11.10	0.07	0.11	18.9	0.7	32.5	21.9	7.9
01/05/94	14.0	130.0	0.027	10.3	8.2	3.2	10.38	1.40	5.10	13.40	0.06	0.17	20.5	2.9	60.4	11.8	4.2
14/06/94	16.2	170.0	0.026	10.5	8.1	3.4	12.49	1.60	8.00	20.90	0.03	0.10	26.1	2.1	91.7	33.9	6.2
18/07/94	19.4	206.0	0.027	10.8	8.1	3.8	7.77	1.80	8.10	21.20	0.10	0.11	21.2	0.4	86.0	6.9	2.2
USTW																	
21/04/93	8.0	113.0	0.060	12.2	7.6	1.8	13.72	0.58	3.94	12.20	0.04	0.21	26.3	6.7	15.2	4.9	2.6
20/05/93	10.7	135.0	0.047	12.9	8.3	2.4	5.20	0.59	5.89	16.52	0.02	0.90	8.5	4.6	51.8	8.2	1.8
22/06/93	13.2	193.0	0.033	9.0	8.3	2.0	4.00	0.70	8.10	18.20	0.02	0.13	12.3	4.2	45.0	16.8	7.1
19/07/93	15.8	180.0	0.040	10.8	8.1	1.7	1.80	0.85	8.94	26.98	0.20	0.38	15.2	3.8	37.0	6.5	3.0
23/08/93	13.2	156.0	0.045	9.1	7.6	1.6	3.60	0.71	6.60	15.60	0.05	0.32	9.6	4.0	40.0	8.2	2.0
20/09/93	11.9	132.0	0.050	8.3	7.3	1.5	6.04	0.60	2.96	11.92	0.02	0.27	7.5	1.7	45.9	9.7	0.5
18/10/93	7.6	143.0	0.050	7.8	7.7	1.5	8.20	0.80	2.50	8.20	0.03	0.38	25.0	1.5	60.2	6.0	1.0
30/11/93	1.8	160.0	0.024	10.8	8.1	2.2	10.68	0.99	5.17	13.69	0.05	0.48	29.9	2.2	88.2	16.9	5.5
18/12/93	1.2	150.0	0.030	11.0	7.9	2.1	8.20	0.60	4.20	14.00	0.01	0.60	39.1	1.8	61.0	8.9	7.7
17/01/94	0.6	143.0	0.032	13.9	7.3	2.4	8.90	0.70	5.90	22.00	0.00	0.20	95.6	1.2	54.0	9.7	2.9
14/02/94	0.1	191.0	0.030	10.2	7.4	1.9	5.00	0.80	3.20	29.00	0.00	0.20	82.7	3.6	32.0	6.6	6.3
17/03/94	2.2	152.0	0.020	13.5	8.0	2.2	6.06	1.40	5.20	12.90	0.02	0.06	52.5	2.3	87.9	3.8	2.9
18/04/94	6.6	202.0	0.005	13.8	8.5	4.6	6.33	1.60	8.40	22.30	0.04	0.05	32.5	0.1	72.3	11.6	4.9
01/05/94	10.3	230.0	0.007	14.6	8.6	2.5	2.02	1.40	4.00	10.00	0.02	0.03	0.1	0.1	36.6	6.1	0.5
14/06/94	16.1	264.0	0.010	10.4	8.6	5.6	9.17	2.00	11.30	34.90	0.01	0.04	0.1	0.5	53.7	9.0	6.6
18/07/94	15.2	298.0	0.012	13.7	8.4	5.6	12.08	2.10	11.80	34.10	0.03	0.09	20.1	0.1	73.7	9.7	5.6
FLUSH																	
10/03/93	6.6	482.0	0.005	10.1	8.0	10.5	5.30	1.74	24.30	75.40	0.00	0.03					
25/03/93	13.5	475.0	0.004	10.8	8.2	11.3	5.20	1.47	23.00	71.60	0.00	0.00	16.2	1.5	15.7	1.3	< 1.
15/04/93	13.6	483.0	0.009	10.6	8.1	10.2	5.20	1.52	22.90	97.70	0.00	0.00	58.9	1.2	17.4	1.8	< 1.
21/04/93	10.5	476.0	0.002	8.9	8.3	9.9	5.00	1.50	23.20	82.00	0.01	0.00	32.0	2.0	18.2	2.8	< 1.
06/05/93	14.7	474.0	0.002	10.9	8.2	9.8	6.02	1.63	24.38	77.50	0.02	0.27	24.2	2.2	20.8	4.1	2.9
20/05/93	15.6	442.0	0.018	7.8	7.4	10.0	4.20	1.50	22.60	82.00	0.01	0.20	21.1	1.5	20.2	2.1	< 1.
03/06/93	14.0	476.0	0.049	10.4	7.2	7.0	5.84	1.56	21.60	87.50	0.00	0.30	8.5	2.8	65.6	4.1	1.5
22/06/93	16.2	483.0	0.001	8.4	8.2	9.6	1.40	1.42	24.95	99.30	0.01	0.05	17.3	3.2	25.8	8.7	4.2
08/07/93	14.0	487.0	0.001	9.9	8.2	9.5	0.86	1.25	20.85	9.05	0.01	0.10	8.2	2.0	28.0	4.2	4.0
19/07/93	13.7	492.0	0.001	8.2	8.1	9.0	0.91	1.15	25.25	94.25	0.07	0.04	0.0	1.6	35.4	3.3	2.0
05/08/93	12.2	560.0	0.001	7.8	7.8	8.0	1.61	1.27	18.00	84.25	0.02	0.17	23.5	2.0	50.8	10.2	10.1
23/08/93	11.7	465.0	0.001	10.2	7.9	8.1	0.98	1.17	33.80	120.35	0.16	0.47	32.0	1.8	62.0	10.2	10.0
02/09/93	13.0	470.0	0.001	9.8	8.1	8.3	0.60	1.20	30.00	90.00	0.08	0.45	40.0	0.8	50.8	10.1	3.6

20/09/93	17.7	493.0	0.002	9.6	8.2	8.4	0.37	1.30	25.30	77.00	0.02	0.05	46.8	0.1	45.9	< 1.	< 1.
05/10/93	11.8	490.0	0.002	9.2	8.0	8.4	0.64	1.32	6.86	101.20	0.02	0.11	37.2	0.1	80.0	9.9	< 1.
18/10/93	10.3	502.0	0.003	9.2	7.7	7.2	0.34	1.32	4.74	94.15	0.02	0.30	25.0	2.5	37.8	25.0	< 1.
08/11/93	10.6	555.0	0.004	9.6	8.2	7.8	8.25	2.15	26.30	79.00	0.05	0.06	12.0	6.5	56.0	10.1	< 1.
30/11/93	5.0	509.0	0.002	10.8	8.1	8.3	8.13	1.32	24.90	65.30	0.01	0.11	14.3	1.5	78.4	9.3	3.6
08/12/93	5.0	526.0	0.001	8.9	8.2	9.0	7.33	1.24	23.90	58.10	0.02	0.14	10.2	1.5	60.2	10.4	8.3
18/12/94	2.9	480.0	0.001	9.0	8.2	9.1	6.40	0.80	10.40	34.00	0.00	0.00	15.4	1.2	29.3	5.0	4.3
17/01/94	1.9	465.0	0.002	9.2	8.2	11.0	8.20	1.50	21.70	78.00	0.00	0.00	22.5	0.8	25.0	2.1	1.0
31/01/94	1.2	450.0	0.001	9.4	8.2	10.9	8.50	1.60	15.20	80.00	0.00	0.00	25.0	0.5	18.0	6.2	1.8
14/02/94	0.5	435.0	0.001	11.5	8.1	10.2	9.20	1.50	12.60	96.00	0.00	0.00	32.0	2.0	20.0	7.6	7.0
28/02/94	7.2	456.0	0.001	12.3	8.1	10.1	7.50	1.60	5.30	99.00	0.00	0.01	36.2	2.5	15.8	8.8	6.2
17/03/94	4.0	540.0	0.001	10.6	7.9	10.3	6.32	2.60	22.60	70.60	0.02	0.01	22.4	2.2	43.0	4.4	< 1.
24/03/94	7.0	525.0	0.001	10.5	8.3	10.6	5.28	2.30	18.20	42.30	0.03	0.01	1.4	2.5	84.1	1.6	0.5
18/04/94	9.5	490.0	0.000	10.8	8.3	12.0	8.03	2.80	23.90	75.60	0.03	0.02	0.1	0.1	35.3	21.9	3.3
29/04/94	13.8	485.0	0.001	9.2	8.2	12.5	10.70	3.10	24.20	74.40	0.03	0.02	0.1	0.1	185.9	6.1	2.3
16/05/94	16.8	480.0	0.001	9.1	8.4	10.9	21.91	3.10	20.10	43.00	0.02	0.05	7.4	0.2	47.0	5.1	4.4
30/05/94	18.0	485.0	0.001	9.4	8.3	10.5	8.86	3.40	20.30	41.70	0.02	0.03	5.5	0.3	42.0	10.0	5.6
14/06/94	20.0	492.0	0.000	9.2	8.2	9.9	18.17	2.50	23.80	79.10	0.01	0.01	1.7	0.4	49.9	20.9	7.5
27/06/94	16.9	589.0	0.001	9.6	8.3	10.2	17.00	1.90	22.00	75.00	0.04	0.01	0.0	2.5	29.9	29.5	5.5
18/07/94	17.2	481.0	0.001	9.3	8.2	10.5	16.70	3.40	22.90	70.90	0.08	0.02	25.4	0.1	87.9	4.1	3.9